

Hair Growth Stimulating and Antibacterial Activities of a Poly Herbal Hair Tonic

Karuna Sree Varicola^{1*}, Raveesha Peeriga², Vishnu Priya Cherukuri³,
Chaitanya Phanisai Raavi⁴, Yamini Durga Talaparthi¹,
and Kovida Chanumolu¹

¹KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada – 520 010, Andhra Pradesh, India

²V. V. Institute of pharmaceutical Sciences, Gudlavalleru – 521 356, Andhra Pradesh, India

³Aizen Algo Pvt. Ltd., Hyderabad – 500 081, Telangana, India

⁴Statdoc. Pvt. Ltd., Hyderabad – 500 081, Telangana, India

*Corresponding author: kspamula@gmail.com

Abstract

Healthy hair represents an individual's physical and emotional well-being. The scalp protects hair from regular wear and tear. Hair oiling protects the follicles and prevents dryness of hair by restoring moisture, sustains hair growth and gives relaxation. Herbal formulations have considerable attention because of less scope for side effects as compared to synthetic counterparts. This investigation intends to develop and assess the efficacy of hair tonics and the antibacterial activity of a polyherbal formulation prepared from the selected medicinal plants. Roots and aerial roots of *Ficus religiosa* and seeds of *Abrus precatorius* which contain valuable therapeutic constituents are selected for this work. *Abrus precatorius*, also known as 'Kunnikuru' in Malayalam, Guruvindaginja in Telugu, and Kundumani in Tamil, has been utilized for millennia in supplementary medicine. Nearly fifty different conditions, such as asthma, diabetes, diarrhoea, epilepsy, stomach issues, inflammatory diseases, infectious disorders, and sexual disorders, are treated with *Ficus religiosa* in traditional medicine. This work is primarily intended to provide a scientific base for the traditional uses of these selected plants. From this study, it is evident that the hair growth activity of 24 hours of application of poly herbal formulation on Male Wistar rats exhibited a more substantial effect compared to the standard drug Minoxidil and control

groups. Lastly, our research demonstrated that extracts from *Ficus religiosa* and *Abrus precatorius* can promote hair growth, making them useful for treating alopecia. The antibacterial potential of these extracts is on par with the industry benchmark. Research conducted by us showed that the primary components of aqueous extracts and alcohol are flavonoids, alkaloid compounds, carbohydrates, and tannins.

Keywords: *Abrus precatorius*, *Ficus religiosa*, Anti-microbial activity, Hair tonic activity.

Introduction

Incorporating plants and plant components (roots, stems, leaves, and their extracts) to cure various illnesses is known as herbal medicine. Additionally, it contributes to the enhancement of human health and well-being. Herbal remedies are often advocated by those who work in the fields of traditional medicine, Ayurveda, homoeopathy, naturopathy and herbal medicine. Herbs have long been used to treat illnesses (1,2). A person's hair is a significant physical feature that conveys their personality (3). To promote hair development, a variety of plants and extracts from them are employed (4). Alopecia, often known as hair loss or baldness, is a disorder in which a portion of the head or body loses its hair. It usually involves at least the head. The degree of hair loss varies from one part of the body to another and even throughout the body (5). By the

moment they become 35, over 40% of males are predicted to experience visible hair loss. About 65% of men experience visible loss of hair by the age of 60, and 80% by the time of 80. This rate keeps rising with age(6). Among the several forms of hair thinning, the most common is androgenetic alopecia, or male pattern baldness. It is a genetic disorder that can strike males at any age(7).

Abrus precatorius, known as kundumani in Tamil, Guruvindaginja in Telugu, and 'Kunnikuru' in Malayalam, has been utilized in complementary and alternative medicine for generations. The white species is used to produce oil that is said to be an aphrodisiac. An infusion of the leaf is used to cure fever, cough, and colds. Seeds are toxic and thus used for mitigation. Rodents have demonstrated pharmacological effects such as antioxidant, anti-inflammatory, and analgesic activity(8). When administered to Sprague-Dawley rats, a methanolic extract of *A. precatorius* seeds produces transitory changes in the pattern of the estrous cycle and prevents ovulation. When administered to guinea pigs, the methanolic extract displayed dose-dependent bronchodilator and activity (9). Approximately fifty different conditions, including asthma, diabetes, diarrhea, epilepsy, stomach issues, alopecia, inflammatory disorders, infections, and sexual disorders, are treated with it in traditional medicine(10). To stop bleeding and promote healing, *Ficus religiosa* latex can be applied to wounds and bleeding areas(11,12). The entire tree possesses a variety of features, including anti-oxidant, anti-microbial, anti-convulsant, anti-diabetic, and anti-cancer properties(13-15).

Materials and Methods

Minoxidil (Mintop Forte; Dr. Reddy's) was purchased from Appolo Pharmacy, coconut oil (Parachute) and Veet hair removal (Veet) were purchased from local retailers, and all other chemicals employed were of analytical quality.

Extraction

Airborne root tips, *Ficus religiosa* roots, and *Abrus precatorius* seeds were collected

from natural trees in the vicinity of Vijayawada. A specimen voucher was kept in our department laboratory after the plant material verification in the Botany department of Siddhartha College of Arts and Science in Vijayawada, Andhra Pradesh. These specimens were kept in airtight containers after being dried in the shade and coarsely ground with a home grinder(16). Maceration was performed on 100g of coarsely crushed plant material.

Using methanol, the crude medication is macerated for 48 hours at room temperature while being constantly stirred(17,18). After vacuum filtering the resulting mixture, the marc was extracted again using methanol for a further 12 hours. The mixture was vacuum-filtered, and the marc was left to air dry. The methanol extract from the seeds, roots, and root tips (root hairs) was obtained by combining and condensing the filtrates. The yield percentage was computed and kept for later use in a desiccator(19,20). A Soxhlet extractor was used to remove the dry marc using water. After being wrapped in Whatmann filter paper, the sample is put inside the Soxhlet device. A heating mantle heated the solvent (water), and the vapors from the solvent condensed and fell onto the packed material, removing the water-soluble components. The chamber's liquid constituents syphon into the flask. when the liquid level in the chamber reaches the highest point of the siphon tube. Once an ounce of solvent inside the siphon tube evaporates without leaving any trace, this process is repeated. After the crude extract had been concentrated and the yield percentage was determined, it was kept in a desiccator until it was needed(21). The labels for the extracts are listed here.

Plant material	Aqueous extract	Alcohol extract
<i>Abrus precatorius</i> seed	APSAqE	APSAE
<i>Ficus religiosa</i> roots	FRRAqE	FRRAE
<i>Ficus religiosa</i> root hairs	FRRHAqE	FRRHAE

Preliminary phytochemical screening

Using conventional protocols, the extracts underwent initial phytochemical screening(22).

Evaluation of antimicrobial activity(23,24)

Cup plate method

The sterile media (Table 1) were placed in sterile petri dishes after being infected at a 1% level using a 24-hour-old culture of the test organisms in question. For fifteen to twenty minutes, the medium of choice was left to settle at room temperature. Employing a sterile borer (0. 9mm), the solidified nutritious material was formed into cups(25). Utilizing a micro pipette, these cups were then filled with different dilutions of test (50 µg/ml–100 µg/ml) and standard (100 µg/ml) solutions. These petri dishes were subsequently incubated at 37°C for 2 and 48 hours, respectively, to allow bacteria to proliferate(26). The antimicrobial activity was assessed by comparing it to regular Streptomycin, and the zones of inhibition were determined in millimeters(27, 28).

Organisms used(30)

1. *Bacillus subtilis* (Gram-positive).
2. *Escherichia coli* (Gram-negative).
3. *Pseudomonas aeruginosa*.
4. *Staphylococcus aureus*.

Evaluation of hair tonic activity

Male Wistar rats weighing between 170 and 200 g were used to study the hair formation in vivo for 21 days. Rats were housed in a barrier-controlled environment with a 12-hour light-dark cycle, an ambient

temperature of 23±2°C, and a humidity range of 35–60%(31, 32). The Institutional Animal Ethics Committee protocol number KVSRSOPS/IAC/P-5/2024 examined and approved this work.

Evaluation of hair tonic activity

Hair tonic activity was evaluated following previously reported research with slight modifications(33). Six groups of six animals each were created from a total of thirty-six creatures. Veet, a commercial hair remover, was used to completely remove all of the hair from a 1 cm² region. The control group animals received a sufficient quantity of coconut oil application. The standard group received an application of 1 ml minoxidil(5%). solution. The third, fourth, fifth and sixth groups received an application of APSAqE, FRRaqE, FRRHAqE and mixed extract. After 5min application, the samples were left on the skin for 24 hours and washed off. For all the groups, care was taken to ensure that the application time for each day was approximately the same and that the time lapse between each application was consistent(34). Details of treatment are given in Table 2.

Results and Discussion

Percentage yield of extracts

$$\% \text{Yield} = \frac{\text{Weight of residue}}{\text{Weight of crude drug}} \times 100$$

The yield of aqueous extracts APSAqE, FRRaqE, FRRHAqE were found to be 10. 78%, 4. 34%, and 5. 023% respectively. The details are given in Table 3. The yield of alcohol extracts APSAE, FRRAE, FRRHAE was found to be 12. 78%, and 3. 33%8. 11% w/w respectively. APSAqE and APSAE produced the highest yield, suggesting that these extracts are rich in polar components(35). Tables 3 and 4 provide the information. The alcohol extract yield hierarchy:

APSAE >FRRHAE >FRRAE

The hierarchy of yield for aqueous extracts:

Table 1: Composition of nutrient agar media (for 1000 ml) (29)		
S. No.	Ingredients	Quantities required
1.	Beef or meat extract	3gm
2.	peptone	5gm
3.	Sodium chloride	5gm
4.	agar	20gm±0.5%
5.	Distilled water	Upto 1 litre
6.	pH	7.2±0.2

Table 2: Treatment protocol	
Group	Treatment
1	Control (coconut oil+DMSO)
2	APSAqE
3	FRRaqE
4	FRRHAqE
5	MIXED
6	MINOXIDIL

Table 3: The percentage yield of aqueous extracts		
S. No.	Name of extract	% yield
1.	APSAqE	10.78%
2.	FRRaqE	4.34%
3.	FRRHAqE	5.023%

Table 4: The percentage yield of alcohol extracts		
S. No.	Name of extract	% yield
1	APSAE	12.78%
2	FRRAE	3.33%
3	FRRHAE	8.11%

APSAqE>FRRHAqE>FRRaqE

Preliminary phytochemical screening

It is apparent through the initial phytochemical examination that *Ficus religiosa* root along with root hair extracts, in addition to *Abrus precatorius* seed extract, contain significant phytoconstituents such as carbohydrates, alkaloids, glycosides, tannins, gums, and mucilages(36, 37) (Figures 1-3). According to reports, these components have a number of therapeutic applications(38). The details of preliminary screening are given in Tables 5 & 6.

Anti-microbial activity

In comparison to streptomycin (standard) in the measured range, an aqueous extract of *Ficus religiosa* demonstrated a detectable zone of inhibition on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*(39). The Aqueous extract of *Abrus precatorius* showed no antibacterial action



Figure 1: *Abrus precatorius*



Figure 2: *Ficus religiosa*

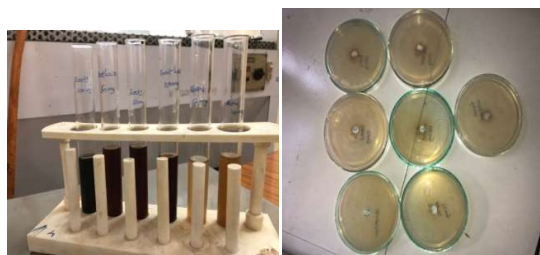


Figure 3: Anti-microbial activity screening

at the dose that was tested. Figure 4A-D and Table 7 display the extracts' antibacterial activity results.

Whenever using bacteriostatic antimicrobials, the treatment must last long enough for the host's defenses to eliminate the bacteria. Following the elimination of bacteriostatics, bacterial growth will recommence. Bacteriostatic drugs restrict the development of by obstructing the synthesis of proteins, DNA replication, and other aspects of

the metabolic activities of bacteria within cells(40). An agent that destroys bacteria is called a bactericide. Therefore, the expansion of bacteria will not resume. In this study, after

48 hours, the zone of inhibition remained the same as that of the 24-hour zone of inhibition. Hence, the extract can be confirmed to possess bactericidal activity.

Table 5: Preliminary phytochemical screening of aqueous extracts

S. No.	Components	APSAqE	FRRaqE	FRRHAqE
1	Carbohydrates	+	+	+
23	Alkaloids	+	+	+
4	Glycosides	—	—	—
5	Tannins	+	+	+
6	Flavonoids	+	+	+
7	Gums and mucilage	—	—	—

Table 6: Preliminary phytochemical screening of Alcohol extracts

S. No.	Component	APSAE	FRRAE	FRRHAE
1	Carbohydrates	+	+	+
2	Alkaloids	+	+	+
3	Glycosides	—	—	—
4	Amino acids	—	—	—
5	Tannins	+	+	+
6	Flavonoids	+	+	+
7	Gums and mucilage	+	+	+

+ = (Positive)

-- = (Negative)

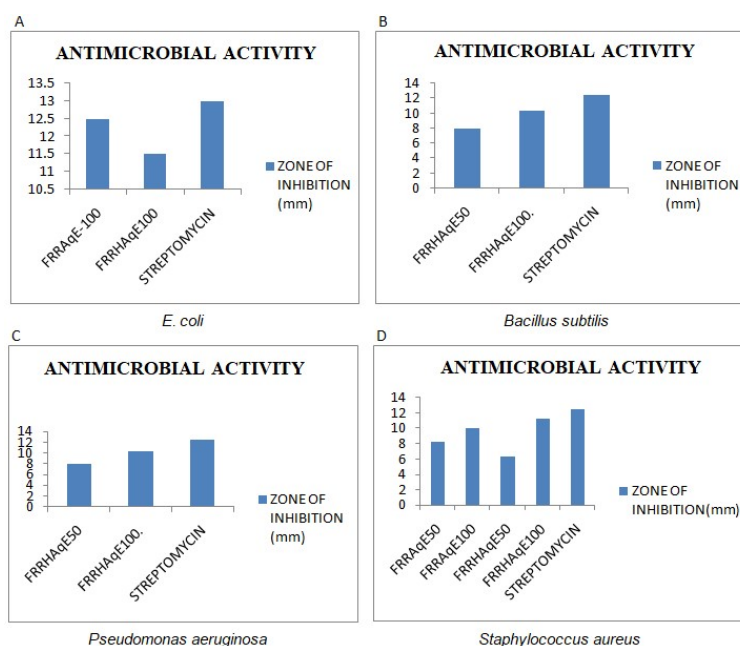


Figure 4: Anti-microbial activity

Varicola et al

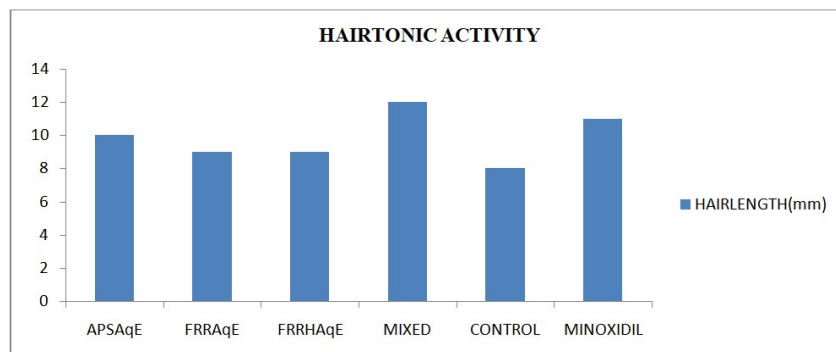


Figure 5: Hair tonic activity of aqueous extracts

Table 7: Anti-bacterial activity										
S. No.	Sample	Dose (µg/ml)	Organism							
			<i>E. coli</i>		<i>B. subtilis</i>		<i>P. aeruginosa</i>		<i>S. aureus</i>	
			Zol (mm)	Zol (mm)	Zol (mm)	Zol (mm)	Zol (mm)	Zol (mm)	Zol (mm)	Zol (mm)
			24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
1	APSAqE	50	—	—	—	—	—	—	—	—
2	APSAqE	100	—	—	—	—	—	—	—	—
3	FRRaqE	50	—	—	—	—	—	—	8.3	8.3
4	FRRaqE	100	12.5	12.5	—	—	9.2	9.2	10	10
5	FRRHAqE	50	—	—	8	8	—	—	6.3	6.3
6	FRRHAqE	100	11.5	11.5	10.4	10.4	11.5	11.5	11.3	11.3
7	STD (Streptomycin)	100	13	12	13	12.5	12.5	11	12.5	11.5
zone of inhibition is absent										

Table 8: Hair tonic activity		
S. No.	Sample	Hair length (mm)
1	APSAqE	10
2	FRRaqE	9
3	FRRHAqE	9
4	MIXED	12
5	CONTROL	8
6	MINOXIDIL	11

Parameters for Evaluating Hair tonic activity

Hair length determination

On the twenty-first day, sterile forceps were used to randomly remove hair from the rats' chosen location(41). A measurement scale was used to determine the hair length, and the findings were tallied. As compared to the regular group, the group that received mixed extract showed the longest hair. Table 8 and Figure 5 provide the specifics.

Table 9: Histological studies of tested samples

S. No.	Name of extract	Anagen phase	Telogen phase
1	APSAqE	75	25
2	FRRAqE	66	34
3	FRRHAqE	55	45
4	MIXED	85	15
5	CONTROL	40	60
6	MINOXIDIL	71	29

The following is a record of the hair tonic activity hierarchy:

MIXED>APSAqE=
 MINOXIDIL>FRRAqE>FRRHAqE>CONTROL.

Histological studies

After completion of the study period of 21 days, rat skin biopsies were prepared

for histological evaluation using formalin fixation and H&E staining". Rats from each group were anaesthetized. From the chosen area, skin specimens were taken and stored in 10% formalin(42). Slices of tissue were inserted onto glass slides. Haematoxylin and eosin were used for staining the sliced tissues, and a microscope was used to observe the hair follicular stages. The details are given in Table 9 and Figures 6-11. Hair growth has two phases i. e. telogen along with anagen phases. The stage known as anagen is when hair grows, while the telogen stage is when it rests. Hair tonic function is higher if the anagen phase level is greater than the telogen stage value(43-45). From the results of histological studies we came to know that the anagen phase value of mixed extract is more when compared to the anagen phase values of other extracts.

The order of hair tonic activity of extracts as per histological studies is as follows:

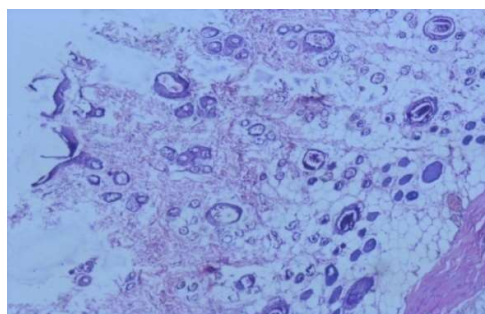


Figure 6: APSAqE

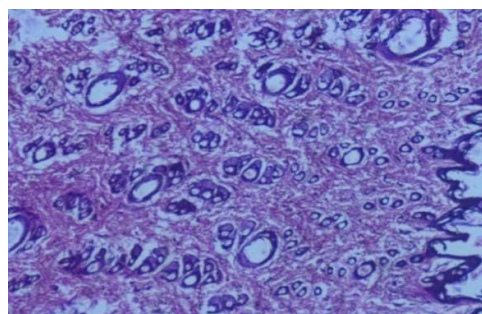


Figure 7: FRRAqE

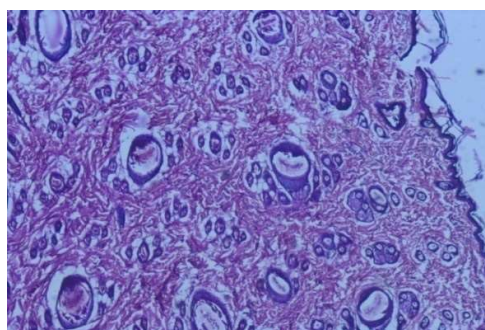


Figure 8: FRRHAqE

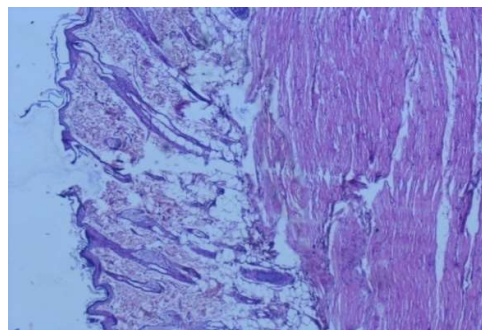


Figure 9: MIXED

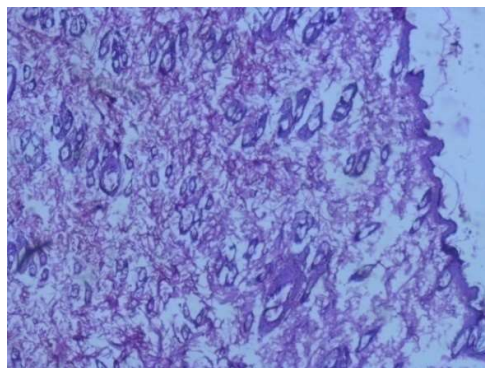


Figure 10: CONTROL

MIXED>APSAqE>MINOXIDIL>FRRHAqE>FR
 RHAqE>CONTROL

To determine how APSAqE, FRRHAqE, FRRHAqE, and combined extracts affected the development of hair, a histopathological examination of dorsal skin samples was carried out. Dorsal tissue from the skin in the group treated with mixed extract showed an upsurge in hair quantity, size, depth, and length. Histological investigation visibly validated the encouraging impact of the mixed extract on hair formation (Fig. 5). Additionally, the groups treated with mixed extract showed a spike in the anagen/telogen (A/T) ratio. It became apparent that the A/T ratio was significantly better than those using the common medication minoxidil. The group treated with mixed extract had a greater A/T ratio (5.6) than those treated with minoxidil (2.4). Compared to the control groups, the group receiving the mixed extract had significantly more hair. In contrast to the typical hair phase pattern, research demonstrated that the group receiving the mixed extract experienced an accelerated hair cycle. When combined, these results indicated that elements of the mixed extract might help hasten the growth of hair follicles.

Conclusion

Herbal hair oil not only moisturizes but also treats dry scalp and hair issues. It provides a range of essential nutrients required to

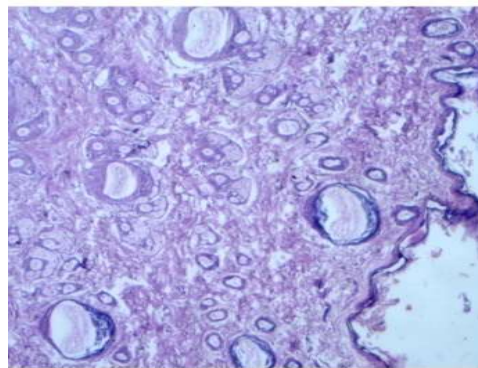


Figure 11: MINOXIDIL

maintain the regular functioning of the sebaceous glands and promote the growth of natural hair. We infer from our study that the activity of hair development of 24hrs application of *Ficus religiosa* root and root hairs and *Abrus precatorious* seed extracts exhibited substantial effect compared to standard Minoxidil and control groups. Finally, this study shows that *Ficus religiosa* and *Abrus precatorious* extracts can be used as hair growth promoters hence, they can be used in the treatment of alopecia. The theoretical antimicrobial capacity associated with these extracts was equivalent to what was traditionally employed. Our research demonstrated that the main constituents of roots, seeds, alcohol-containing root hairs, and aqueous extracts are flavonoids, alkaloids, carbohydrates, and tannins. The activities reported in this study may be attributed to these valuable phytoconstituents. Due to time constraints, we could not find out the exact mechanism by which these extracts showed positive hair tonic activity. Finding and isolating the active ingredients causing this response could lead to novel approaches to alopecia therapy. Furthermore, formulations can be created and assessed to provide a solid foundation for the findings of the research.

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Conflict of Interest

The authors declare that they do not have any conflict of interest

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