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FORMULATION AND EVALUATION OF POLYHERBAL GEL FOR PREVENTION OF HAIR FALL

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ABSTRACT

The objective of the present investigation was to prepare herbal gel formulations for prevention of hair loss. The gel formulations were prepared in carbopol 934P gel base using extracts from the leaves of *Tinospora cordifolia*, pulp from *Tamarindus indica*, seeds of *Trigonella foenum graecum* and flowers of *Hibiscus rosa sinensis*. The results of phytochemical screening suggest that most of the while flavonoids were present in the methanolic and aqueous extracts of *Tinospora cordifolia*, they were found only in the aqueous extracts of the other plant materials. The extracts were mixed in varying proportions with the gelling agent to prepare the hair gel formulations. The formulations were evaluated for physicochemical properties. For all formulations, the pH value lies within the 5.86 to 6.75 which is within range compatible with the pH of the skin (4.5 to 7.4). The viscosity of the formulations ranged from 35.7 cp to 38.6 cp whereas the spreadability was from 24.2 to 48.4 mm. The rheological behavior of the formulations exhibited pseudoplastic flow as indicated by shear thinning. The antifungal activity of the polyherbal gels was evaluated against *Candida albicans* using disc diffusion method. All the gel formulations were able to exert antifungal action as witnessed by the zone of inhibition in the culture plate.

KEYWORDS: Polyherbal gel, antifungal, *Tinospora cordifolia, Hibiscus rosasinensis, Tamarindus indica.*

INTRODUCTION

Both men and women can develop androgenetic alopecia (AGA), a non-scarring alopecia. In men and women who are genetically susceptible, it is characterised by a gradual miniaturisation of hair follicles with a distinctive pattern distribution.[1] Both sexes experience it the most frequently.[2-4] Around 80% of Caucasian males over the age of 70 are affected by it, and it typically manifests itself in the third and fourth decades, affecting 30% to 50% of men by the age of 50. More than 30% of adults and 12% of children, according to data from the National Institute of Health, use treatments other than traditional ones. There is an unmet need for medicines that produce satisfying, long-lasting effects in the treatment of alopecia. In an effort to locate secure, organic, and effective hair restoration treatments, patients frequently turn to complementary and alternative medicine (CAM). A multimodal strategy is necessary for treating hair loss, and using CAM may have additional advantages. As hormones, antioxidants, immune modulators, enzyme cofactors, and antioxidants, vitamins and trace minerals are essential for the hair follicle cycle and maintain homeostasis. India is a treasure trove of healing plants. Herbs are utilised for more than just health treatment; they may also be used to make a variety of cosmetics and to enhance physical beauty. Numerous herbs and herbal remedies are said to promote hair growth in the traditional system of medicine, but their usage is restricted due to a lack of reliable scientific evidence. *Hibiscus rosa sinensis*[5], *Tamarindus indica*[6], *Trigonella foenum-graecum*[7] and *Tinospora cordifolia*[8] have all been linked to reduced hair loss or increased hair volume. Therefore, it was intended to create a polyherbal formulation with the aforementioned plant extracts that would be beneficial in reducing hair loss and encouraging hair growth.

MATERIAL AND METHODS

Collection of plant material

Hibiscus rosa sinensis (HR) flower, *Trigonella foenum graecum* (TFG) seeds, *Tamarandus indicus* (TI) fruit, *and Tinospora cordifolia* (TC) leaves were all obtained from various sources. The botany department at Safia Science College in Bhopal verified all of the plant material. The plant material was cleaned of any trash and extraneous items and processed into a fine powder using a blender running at a low speed. Until usage, the powdered substance was kept in airtight flasks.

Extraction of herbal constituents

Following each other, petroleum ether, methanol, and water were used to remove the powdered plant material. A paper thimble containing 100 g of the powdered substance was filled and placed in the soxhlet extractor.

To fill the flask connected to the extractor, 150 mL of solvent was poured down the thimble. As seen by the colourless solvent in the extractor's syphon tube, the solvent was heated to extract the contents until the extraction was complete. The flask was removed from the extractor, and a rotating vacuum evaporator was used to evaporate the solvent. The resinous residue that was left behind was dried and put in an airtight container for storage. All three solvents were employed to extract the same marc.

Phytochemical Screening[9,10]

The plant extracts were subjected to phytochemical analysis to detect the presence of various phytoconstituents by chemical test such as Molish, fehling solutions, benedict solutions (Carbohydrate); Libermann-Buchard (Steroids); ferric chloride, Gelatin solution test(Tannins); Keller-killani test (Glycoside); wagners, dragendroff (Alkaloids); Hemolysis test (Saponine) and Xanthoprotien test (Proteins).

Test for alkaloids

Small portion of the extract was stirred with a few drops of dilute hydrochloric acid (HCl) and then filtered. The filtrate of resulting solution was then analyzed with various reagents.

- ***Wagner's test:*** A few drops of Wagner's reagent were added to few ml of plant extract sample along the sides of test tube.
- ***Dragendroff's Test:*** A few drops of Dragendroff's reagent were added to 1 ml of the each extract sample.

Glycosides

Saponin glycosides

- ***Froth test:*** 1 ml solution of the extract in water was placed in a test tube and shaken vigourously.

Anthraquinone glycosides

- ***Borntrager's test:*** The extract was boiled with 1.0 ml of dilute sulphuric acid in a test tube for 5 minand filtered while hot. The filtrate was cooled and shaken with an equal volume of dichloromethane and the lower layer (dichlormethane) was separated and shaken with half its volume of dilute ammonia.

Cardiac glycosides

- ***Kedde's test:*** The extract was *e*xtracted with chloroform and evaporated to dryness. One drop of 90% alcohol and 2 drops of 2% 3, 5-dinitro benzoic acid (3, 5-dinitro benzene carboxylic acid Kedde's reagent) in 90% alcohol are added to the above residue. The solution is made alkaline with 20% sodium hydroxide solution.
- ***Keller killiani test (Test for deoxy sugars):*** The extract was *e*xtracted with chloroform and evaporated to dryness. To the residue was added 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride. The solution was transferred to a test tube and 0.5 ml of conc.sulphuric acid was added along the wall of the test tube.

Tannins and phenolic compounds

- ***Gelatin test:*** To the extract was added 1% gelatin solution containing 10% sodium chloride.
- ***Ferric chloride test:*** To the extract was added a freshly prepared solution of ferric chloride.
- ***Vanillin hydrochloride test:*** Test solution of the extract was treated with few drops of vanillin hydrochloride reagent.
- ***Alkaline reagent test:*** Test solution of the extract was treated with sodium hydroxide solution.

Flavonoids

- ***Shinoda test:*** To the test solution of the extract, few fragments of magnesium ribbon were added and conc. hydrochloric acid was mixed drop wise to it.
- ***Zinc hydrochloride reduction test:*** To the test solution a mixture of zinc dust and conc. hydrochloric acid was added.
- ***Alkaline reagent test:*** To the test solution a few drops of sodium hydroxide solution was added. Later if colour appeared, a few drops of conc. HCl were added to it.

Proteins and amino acids

- ***Millons test*:** Test solution of the extract was allowed to react with 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid).
- ***Ninhydrin test:*** The solution of extract was boiled with 0.2% solution of ninhydrin.

Steriods and triterpenoids

- ***Salkowski test:*** The extract was dissolved in chloroform and a few drops of conc. sulphuric acid were added to it. The mixture was shaken well and allowed to stand for some time.

Test for carbohydrates

- ***Molisch Test:*** To the extract was added a few drops of Molisch reagent and concentrated sulfuric acid was flown down the test tube and was observed for formation of purple color.

Formulation of hair gel

The accurately weighed quantity of the ingredients (Table 1) was dispersed in purified water with constant stirring and the solution was heated to 50°C. The amount of carbopol 934P as per formula was added to the solution under continuous stirring while maintaining the temperature at 50°C to ensure no air entrapment. The dispersion of the gelling agent was neutralized using triethanolamine solution to neutral pH and the stirring was continued to obtain a gel.[11]

Table 1: Batch formula for polyherbal hair gel.

Ingredient	Batch formula for 100g gel					
	PHG1	PHG2	PHG3	PHG4	PHG5	PHG6
TCME	10%	10%	10%	10%	10%	10%
TCAE	5%	10%	5%	10%	5%	10%
TIAE	15%	15%	20%	20%	25%	25%
TFGAE	5%	5%	5%	5%	5%	5%
HRAE	10%	10%	10%	10%	10%	10%
Carbopol 934	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Triethanolamine (mL)	qs	qs	qs	qs	qs	qs
Water (mL)	qs 100 mL	qs 100 mL	qs 100 mL	qs 100 mL	qs 100 mL	qs 100 mL

qs – Quantity sufficient; TCME – *Tinospora cordifolia* methanolic extract; TCAE – *Tinospora cordifolia* aqueous extract; TIAE – *Tamarindus indica* aqueous extract; TFGAE – *Trigonella foenum graecum* aqueous extract; HRAE – *Hibiscus rosa sinensis* aqueous extract; qs – quantity sufficient.

Evaluation of gel

Homogeneity

After the gels were firmly set in the container, the homogeneity of each gel formulation was checked visually. They were scrutinised for appearance and any aggregates that might have been present.

Grittiness

A light microscope was used to inspect each formulation for the presence of particulate materials. The requirement for a good gel formulation is satisfied by the lack of particles.

pH determination

100 cc of distilled water were mixed with 1 gramme of gel, and the mixture was let to stand for 2 hours. Each formulation's final solution's pH was assessed using a digital pH metre in triplicate, and average values were computed.

Viscosity

Using a Brookfield Viscometer, the viscosity of the produced gel was measured. The spindle no. 64 was used to rotate the gels at 20 rpm, and the corresponding dial reading was recorded to determine the viscosity values. Centipoises (cp) were used to represent the viscosity.

Rheological Study

By rotating the spindle no. 64 at 10, 20, 40, 60, 80, and 100 rpm for 15 minutes, the gel formulations were subjected to shear stress (rpm), and the viscosity in centipoise was calculated.

Spreadability

The Arvouet-Grand Method was used to assess the gels' spreadability.[12] Briefly, two horizontal 20 X 20 cm plates were used to press 1 g of the gel between them. For 1 minute, a weight of 125 g was placed on the upper plate, and the gel's spreading diameter was measured. Formulation spreadability was assessed three times, with the average value being calculated.

Antifungal activity

Potato Dextrose Agar

Ready to use potato dextrose agar powder was used for preparing the medium for study of fungus/yeast.

Pre-poured nutrient agar plates that are about 3 mm thick were inoculated by swabbing on the agar's surface with a few drops of the fungus (Candida albicans). The disc diffusion method was used to test the antifungal activity.[13] To achieve a concentration of 50 g/mL, extracts were dissolved in DMSO. Using a cork borer (10mm), wells were created in the agar plate at equal intervals, and 200 L of the fractions were added to each one. To promote fungi development, the plates were incubated for 72 hours at 37 0.1°C. The millimeter-scale zone of inhibition surrounding each well was measured, and its average diameter was computed. The gold standard was ketoconazole.

RESULTS AND DISCUSSION

Plant material

The herbal components from *Tinospora cordifolia* leaves, *Tamarindus indica* fruit pulp, *Trigonella foenum graecum* seeds, and *Hibiscus rosa sinensis* flowers (Figure 1) that have been previously documented to play a function in hair loss prevention or hair thickening were isolated and employed. The plant material was gathered from the neighbourhood or from local vendors and was verified by Saifa Science College in Bhopal.

Figure 1 (A) *Tinospora cordifolia* leaves (B) *Tamarindus indica* fruit (C) *Trigonella foenum graecum* seeds (D) *Hibiscus rosa sinesis* flower.

Extraction of phytoconstituents

Successive solvent extraction of pants yielded extracts and the yield of the extracts are presented below (Figure 2).

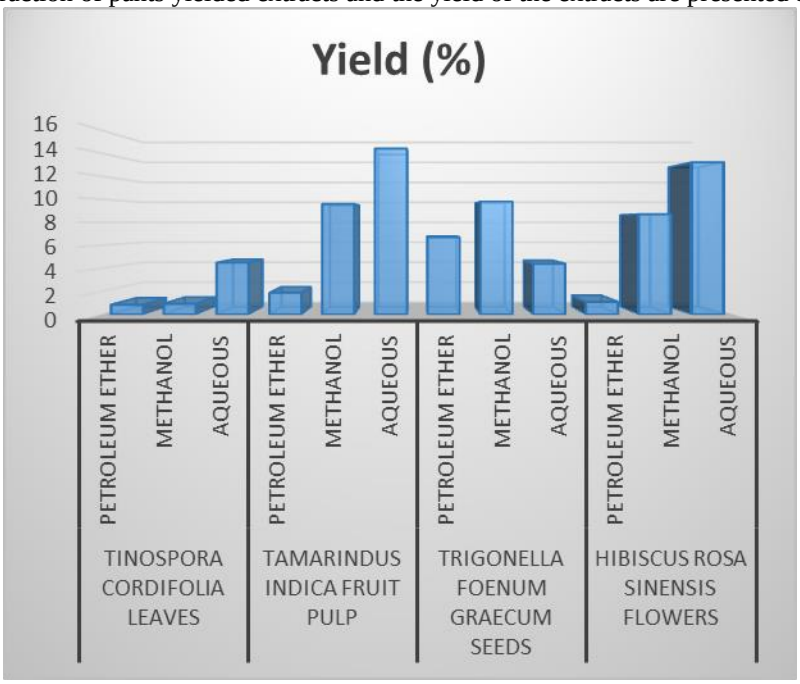

Figure 2 Extraction yield in various solvents.

Phytochemical Screening of extracts

According to the findings of phytochemical screening, the majority of flavonoids were only discovered in the aqueous extracts of the other plant materials, even though they were present in the methanolic and aqueous extracts of Tinospora cordifolia. In the past, it has been documented that natural extracts with flavonoids have been utilised to prevent hair loss and encourage hair growth.[14–20] According to these research, flavonoid-rich extracts were employed to create a variety of gel formulations to stop hair loss. The gelling foundation for the extracts was carbopol 934P.

Three extracts with various extract concentrations and 1.0% carbopol 934P as the gelling agent were used to create the gel formulation. The drug pH, viscosity, rheology, spreadability, and antifungal effect (*in vitro*) of the produced gels were assessed.

Table 2 displays the findings for pH, viscosity, homogeneity, and spreadability. The pH range for all formulations is 5.86 to 6.75, which is suitable with the skin's pH range of 4.5 to 7.4. The outcomes also suggest that the gels' low viscosity may be advantageous for skin distribution applications.

Table 2: pH, viscosity, homogeneity and spreadability of polyherbal gels.

Formulation No.	pH	Viscosity (cp)	Spreadability (mm)	Homogeneity
PHG1	6.32	35.7	48.4	Homogenous and non-gritty
PHG2	6.39	36.1	43.2	Homogenous and non-gritty
PHG3	6.75	36.9	36.8	Homogenous and non-gritty
PHG4	5.86	37.4	31.3	Homogenous and non-gritty
PHG5	5.99	38.1	28.5	Homogenous and non-gritty
PHG6	6.53	38.6	24.2	Homogenous and non-gritty

After the gels were put in the containers, the homogeneity of each gel was assessed visually. With the naked eye, the gels seemed to be homogeneous and uniform. Gels were then examined under a light microscope to see the particle inside of them. Nothing could be seen under a light microscope. This assessment confirmed that the gels were made consistently. Additionally, there were no grit particles.

The different amounts of extract in the gels could be the cause of the different viscosities of the gels. The compositions' viscosities ranged from 35.7 cp to 38.6 cp. It was obvious that an increase in extract concentration had a considerable impact on the formulation's viscosity.

The Arvouet-Grand Method, which uses the spread diameter under the experimental conditions as a gauge of the gel's stiffness or fluidity, was employed to determine the spreadability. If the spread diameter of the gel is 50 mm or less, it is regarded as semi-stiff, and if it is greater than 50 mm but less than 70 mm, it is regarded as fluid. The formulations were determined to be semi-stiff in character and acceptable for topical application based on the findings.

Shear thinning, a sign of pseudoplastic flow, demonstrated the formulations' rheological behaviour (Figure 3). This suggests that the gel formulations would have great syringeability or might be easily extrudable from the tubes in which they were contained.

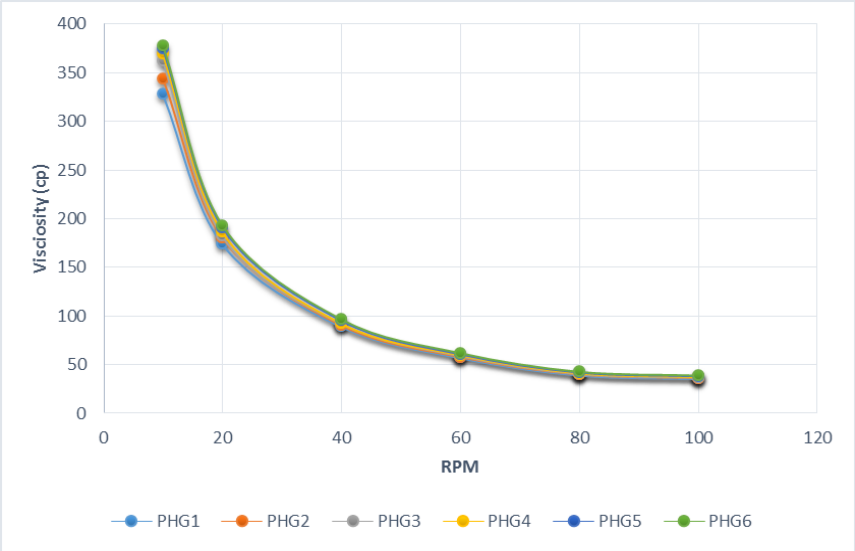

Figure 3 Effect of shear on viscosity of gel.

Antifungal action of the polyherbal gels

The antifungal activity of the polyherbal gels was evaluated against *Candida albicans* using disc diffusion method. All the gel formulations were able to exert antifungal action as witnessed by the zone of inhibition in the culture plate (Figure 4).

The results revealed that formulations with higher amount of the extracts were able to produced better antifungal activities. *Candida albicans* was selected as the fungal strain for screening as it has been reported that scalp yeast infections are caused by *Candida* sp. And lead to hair loss, coupled with other symptoms like itching and burning of the scalp.[21] A similar procedure to evaluate the hair fall prevention action of herbal gel has been previously reported.[22]

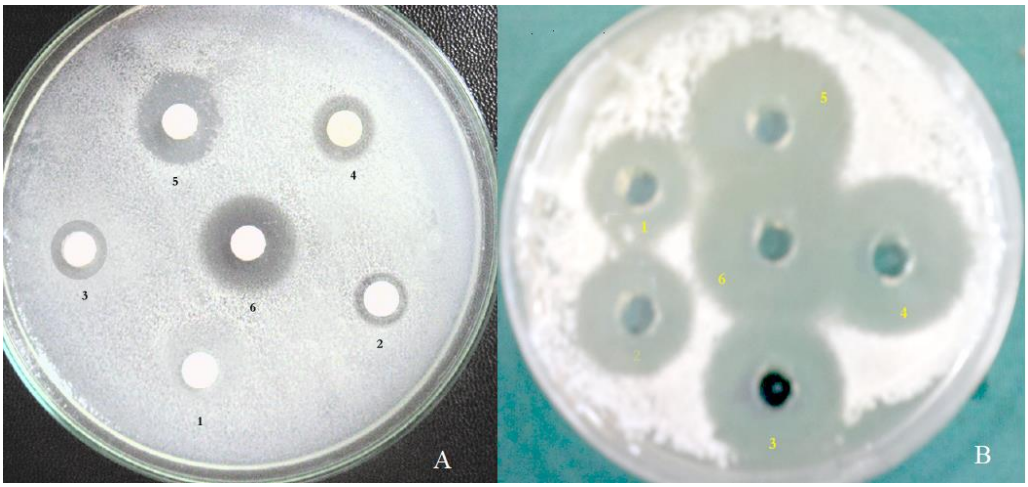

Figure 4 Zone of inhibition exhibited by gel (A) Disc diffusion method (B) Cup Plate method.

CONCLUSION

In the present investigation polyherbal hair gel formulations were prepared using carbopol 934P gel base mixed with flavonoid rich extracts from four different plant materials. Previous studies have linked flavonoids to prevention of hair fall. In the next phase of the studies, the effect of the oils on hair fall prevention, hair growth, improvement in hair thickness and other follicle regeneration parameters would be studied. The present study has revealed that all the formulations were suitable for application on scalp and would be helpful in fighting fungal infections and preventing hair loss.

REFERENCES

1. Blume-Peytavi U, Blumeyer A, Tosti A, Finner A, Marmol V, Trakatelli M, et al. European Consensus Group. S1 guideline for diagnostic evaluation in androgenetic alopecia in men, women and adolescents. Br J Dermatol, 2011; 164(1): 5–15. doi: 10.1111/j.1365-2133.2010.10011.x
2. Paik JH, Yoon JB, Sim WY, Kim BS, Kim NI. The prevalence and types of androgenetic alopecia in Korean men and women. Br J Dermatol, 2001; 145(1): 95-99.
3. Tosti A, Piraccini BM. Androgenetic alopecia. Int J Dermatol, 1999; 38 Suppl 1: 1–7.
4. Paus R, Olsen EA, Messenger AG. Hair growth disorders. In: Fitzpatrick, Dermatology in General Medicine, Ed Panamericana, 2009: 225-243.
5. Sarje SK, Narwade S, Thakur M, Ghiware NB. Pharmacognostic and Pharmacological review on herbal plant: Hibiscus rosa sinensis LinnInternational Journal of ChemTech Research, 2019; 12(4): 266-270.
6. Ritika. Trigonella foenum-graecum L.: A review of its ethnobotany, pharmacology and phytochemistry. International Journal of Advance Research in Science and Engineering, 2016; 5(9): 192-204.
7. Resny AR, Indulekha VC, Binitha Raj RV. A critical Ayurvedic Literary review of the plant Amleeka. International Ayurvedic Medical Journal, 2018; 2(3): 1060-1067.
8. Saeed M, Naveed M, Leskovec J, Ali kamboh A, Kakar I, Ullah K, Ahmad F, Sharif M, Javaid A, Rauf M., Abd El-Hack ME, Abdel-Latif MA, Chao S. Using Guduchi (Tinospora cordifolia) as an eco-friendly feed supplement in human and poultry nutrition. Poultry Science, 2020; 99: 801-811.
9. Kushwah A, Jain S. Evaluation of anti-inflammatory activity of leaf extract fractions of Alangium salvifolium. Journal of Pharmacology and Biomedicine, 2021; 5(4): 466-472.
10. Tiwari P, Joshi A, Dubey BK. Total phenolic content, flavonoid concentration, antimicrobial and insecticidal screening of aqueous extracts of Annona squamosa (seeds), Azadirachta indica (leaves) and Lavandula angustifolia (flower). Journal of Pharmacology and Biomedicine, 2017; 1(1): 30-43.
11. Vaishya MK, Sharma V, Rai JP. Tazarotene loaded niosomal gel for effective acne treatment. Journal of Pharmacology and Biomedicine, 2021; 5(1): 235-241.
12. Borse VA, Gangude AB, Deore AB. Formulation and evaluation of antibacterial topical gel of Doxycycline Hyclate, neem oil and tea tree oil.

Indian Journal of Pharmaceutical Education and Research, 2020; 54(1): 206-212.
13. Manandhar S, Luitel S, Dahal RK. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. J Tropical Med, 2019, Article ID 1895340. Available from https://doi.org/10.1155/2019/1895340
14. Orhevba BA, Sunmonu MO, Iwunze HI. Extraction and Characterization of Moringa oleifera Seed Oil. Research and Reviews: Journal of Food and Dairy Technology, 2013; 1(1): 22-27.
15. Ji Kyung yeop Kim Geun-hoe. The hair growth compositions containing flavonoids. Korean Patent No. KR101208736B1. 2010.
16. Rambwawasvika H, Dzomba P, Gwatidzo L. Hair Growth Promoting Effect of Dicerocaryum senecioides Phytochemicals. International Journal of Medicinal Chemistry, 2019; 7105834. Doi: 10.1155/2019/7105834
17. Taguchi N, Yuriguchi M, Honma T, Hata T, Kamiya E, Kobayashi A, Kitai R, Aoki H, Kunisada T. The effect of flavonoids on regenerated hair follicles with pigmentation. Journal of Dermatological Science, 2017; 86(2): E63. Doi: 10.1016/j.jdermsci.2017.02.18
18. Cheon HI, Bae S, Ahn KJ. Flavonoid Silibinin Increases Hair-Inductive Property Via Akt and Wnt/β-Catenin Signaling Activation in 3-Dimensional-Spheroid Cultured Human Dermal Papilla Cells. *J. Microbiol. Biotechnol*, 2019; 29: 321–329. doi: 10.4014/jmb.1810.10050.
19. Takahashi T, Ishino A, Arai T, Hamada C, Nakazawa Y, Iwabuchi T, Tajima M. Improvement of androgenetic alopecia with topical Sophora flavescens Aiton extract, and identification of the two active compounds in the extract that stimulate proliferation of human hair keratinocytes. *Clin. Exp. Dermatol*, 2016; 41: 302–307. doi: 10.1111/ced.12753.
20. Riccio G, Sommella E, Badolati N, Salviati E, Bottone S, Campiglia P, Dentice M, Tenore GC, Stornaiuolo M, Novellino E. Annurca Apple Polyphenols Protect Murine Hair Follicles from Taxane Induced Dystrophy and Hijacks Polyunsaturated Fatty Acid Metabolism toward β-Oxidation. *Nutrients*, 2018; 10: 1808. doi: 10.3390/nu10111808.
21. https://www.healthieruny.com/resources/can-candida-cause-hair-loss
22. Ramakrishna S, Gopikrishna UV. Formulation and evaluation of herbal hair gel. Scholars International Journal of Traditional and Complementary Medicine, 2022; 5(2): 28-32.