Effects of Herbal Hair Oil on Promoting Hair Growth in Wistar Albino Rats

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Herbal Hair oil, Virgin Coconut Oil, Flax Seed Oil, Wistar Albino Rats

ABSTRACT
The study investigates effect of VCO and FSO on hair growth activity on Wistar albino rats. The herbs used in formulating VCO and FSO have some medicinal properties which act in reducing inflammation and stress. The formulations were made by mixing two parts.one made up of herb in oil by simple maceration and second one of dried hydroalcoholic extract of herbs. The formulations were then applied on Wistar albino rats whose dorsal side had been shaved. Both VCO and FSO showed promising hair growth activity. The VCO displayed much faster growth of hair than marketed formulation. The herbs remained the same in both formulations. So, it can be concluded that VCO has a better extraction of herbs and VCO is a much better formulation. Apart from this marketed formulation was in fact a less effective than VCO. Hair growth activity showed an incrementally response over a period of 4 weeks and histopathological studies corroborate with the fact.

1. INTRODUCTION

Since ancient times, hair oils have been utilised all over the world, although it is still unclear how exactly they affect the hair and scalp. They are typically affordable and easily accessible. Many different types of oils have been used, and new ones are being developed daily. The majority of hair oils primarily serve as emollients, but their distinct properties indicate that they have an even greater effect than that. The many kinds of hair oils and their potential advantages for hair are the main topics of this essay. Dermatologists must understand the effects of hair oils and how to use them. (Mysore & Arghya, 2022).
Virgin coconut oil (VCO) is a one of the types of processed edible oil which is used in various functions and is made from grown coconuts. It is a colourless oil and is extracted by using the hot and cold process. The active components of VCO are mainly contributed as a result of by lauric acid, its primary content. VCO has demonstrated its anticancer, antimicrobial, analgesic, and anti-inflammatory and antipyretic properties. Because of these herbal properties, VCO has gained the wider attention among the pharmaceutical field. The best property of VCO is antioxidant property, the reason behind lies because of its phenolic compounds and medium chain fatty acids. These compounds lead to one of the beneficial compounds used to prevent and treat the oxidative stress induced neurological disorders like stress, depression and Alzheimer's disease. Oral dietary supplementation of VCO is simple and economical and safer especially when taken daily. It is beneficial for cardiovascular, pulmonary, dermatological, reproductive, and skeletal health. It is also used for application in to the skin as a moisturizer in the children. Hence, the avid exploration of antioxidant property as well as trying to

maximise the beneficial effects of VCO in various health conditions will be valuable. (Rao et al., 2024), (Shamsuzzaman et al., 1997).

This study demonstrates that the combined evaluation of hair thickness and cohesive force following oil application can effectively determine the degree of oil penetration into the hair. The study demonstrated the beneficial effect of oil penetration on hair strength. (Sureka et al., 2022) This study demonstrates that the combined evaluation of hair thickness and cohesive force following oil application can effectively determine the degree of oil penetration into the hair. The study demonstrated the beneficial effect of oil penetration on hair strength. (Sureka et al., 2022) Current work provides evidence of compromised hand skin barrier with ABHS daily usage. Overnight VCO application helps prepare the skin for next day alcohol use. Based on the findings, a regimen of overnight VCO application on hands as a natural prophylactic is recommended (Saraogi et al., 2021) Hair breakage is a multifaceted phenomenon with various contributing factors. This study identifies inconsistencies in mean diameter along hair length as a precursor to hair strength. The weakest link in hair is defined by intrinsic flaws that are preceded by surface imperfections. (Kaushik et al., 2020) The Zingiberaceae family includes the herbaceous plant Curcuma aromatica (CA). It possesses anti- inflammatory and anti-oxidant qualities. This study sought to determine how extraction techniques and solvents affected CA rhizomes. (Wuttikul & Sainakham, 2022)

Flax (Linum usitatissimum) seeds yield a nonabsorbable fibre utilised as a laxative and applied topically for the treatment of numerous dermatological diseases. Flaxseed oil comprises alpha-linolenic acid (ALA), which is largely metabolised into the omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) within the body. Flaxseed oil elevates the ALA concentration in breast milk, it does not enhance the DHA level. Maternal consumption of preformed DHA, predominantly sourced from seafood or marine oils, is necessary to enhance the DHA concentration in breast milk.

The findings indicated that the ethanolic extract of flax seeds considerably ($p < 0.05$) diminished prostate gland weight, prostate index, blood levels of PAS, testosterone, and 5-alpha reductase enzyme in rats with BPH, while enhancing the tissue shape of the prostate (Alhussien et al., 2024)

Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are recognised for their protective effects against many metabolic diseases. Given the concerning rise in chronic illness prevalence, consumer interest and demand for natural dietary sources of n-3 PUFAs are escalating swiftly. Seed oils from chia (Salvia hispanica), flax (Linum usitatissimum), and garden cress (Lepidium sativum) are now generally recognised for their ability to enhance dietary α-linolenic acid (ALA) levels. (Mechchate et al., 2021)

Mustard oil (MS) and flaxseed oil (FS) are abundant in unsaturated fatty acids, including oleic acid, alpha-linolenic acid, and linoleic acid, as well as several antioxidants. These oils facilitate hydration, protect the skin from free radical damage, and reduce the visibility of fine lines, wrinkles, and dullness, while also enhancing collagen production, essential for sustaining firm, youthful skin. The majority of the studies focused solely on the bactericidal efficacy of these oils on the skin. (Ismail et al., 2024)

Linseed (Linum usitatissimum L.), one of the most ancient oilseed crops, is regarded as a superfood. Its abundant alpha-linolenic acid, a vital omega-3 fatty acid, together with proteins and vitamins, makes it significant in the food, pharmaceutical, and personal care product industries. The oil's antioxidant and anti-inflammatory characteristics, together with other phenolic components, have enhanced its application, especially in the skincare sector. Linseed oil and mucilage derived from its seeds provide cost-effective alternatives for a range of beauty items and other vital applications. Moreover, these seeds, as a source of dietary fibre, aid in

weight management and help avert related disorders. This chapter examines the application of linseed in cosmetics and personal care products, highlighting various products and their industrial importance. (Sarma et al., 2024)
These ancient grains possess a substantial concentration of consumable and emollient oils, digestible proteins abundant in key amino acids for vegetarian and vegan diets, and vital polyunsaturated fatty acids, including alpha-linolenic acid. (Bakowska-Barczak et al., 2020)

Table 1 Name, part and concentration of herbs used

S.No	Name of Herbs	Part of herbs used	Herbs concentration in VCO (w/v)
1.	*Asparagus racemosus*	root powder	5%
2.	*Nardostachys jatamansi*	root powder	5%
3.	*Rubia cordifolia*	root powder	5%
4.	*Bacopa monnieri*	leaves Powder	5%
5.	*Eclipta alba*	leaves powder	5%
6.	*Cyperus rotundus*	root powder	5%
7.	*Curcuma longa*	rhizome powder	5%
8.	*Trigonella foenum*	seeds and leaves powder	5%
9.	*Glycyrrhiza glabra*	rhizome powder	5%
10.	*Withania somnifera*	root powder	5%

2. MATERIALS AND METHODS

2.1 Preparation of herbal hair oil

The given method was used for Preparation of herbal hair oil for both VCO and FSO (Tang et al., 2021)

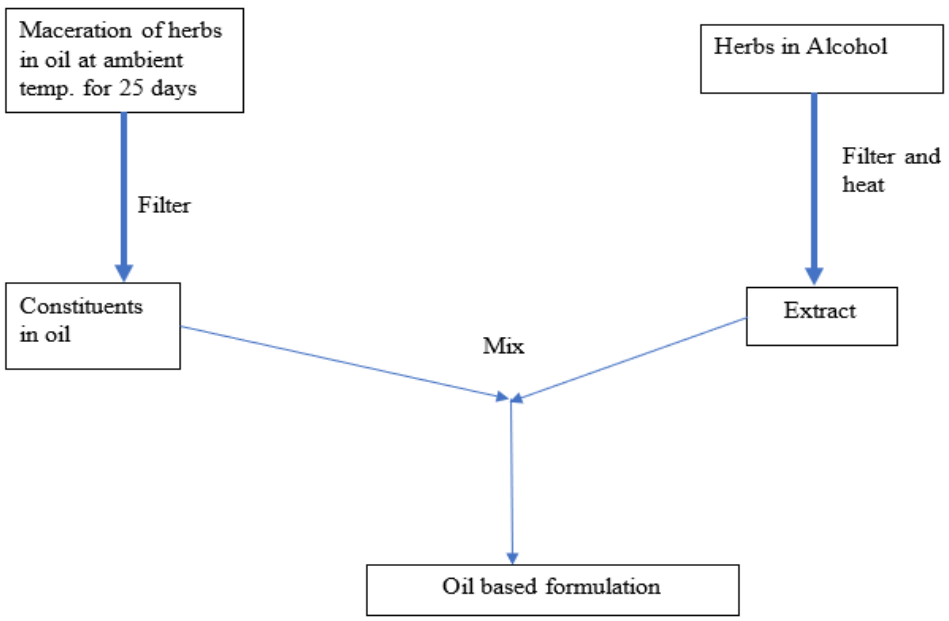

Figure 1 Method of Preparation of herbal hair oil formulation

2.2 *In-vivo* studies on VCO and FSO based formulations

Five-week-old male Wistar Albino Rats were arranged and kept so that they get used to their surroundings for 7 days with food and water ad libitum. The temperature was 24 ± 1°C to adapt, the circadian rhythm was of 12 hrs of light and dark alternatively, with relative humidity of 50 ± 5% relative humidity. The male Wistar Albino Rats were clean shaven on the dorsal side by help of clippers. By performing this act all Wistar albino rats will become synchronized in hair cycle. All will have telogen phase demonstrating pink colour of skin. After this process animals were randomly distributed into 4 groups of 6 animals, each based on different topical concentrations: Control, Marketed Formulation-Kesh Kanti, Formulation-I and Formulation-II. (Ismail et al., 2024)

Table 2 Description of groups (*in-vivo* studies)

Groups(s)	Description
Group-I	Control
Group-II	Marketed Formulation
Group-III	Formulation-I (Virgin Coconut oil based)
Group-IV	Formulation-II (Flax Seed oil based)

2.3 Hair growth evaluation and observation:

To quantify the hair growth in each and every distinct animal group made, clear pictures of the dorsal shaven area of animals would be taken at fixed regular intervals of week one, two, three and four. This process has to begin immediately after topical application of oil is done. Then hair growth score was recorded and further.

Table 3 Relation of % Hair growth and score recorded (*in-vivo* studies)

S. No	Score	% Hair Growth
1.	0	no hair growth
2.	1	less than 20%
3.	2	20% to less than 40%
4.	3	40% to less than 60%
5.	4	60% to less than 80%
6.	5	80% to 100%

Table 4 Hair Growth Score Format

Group(s)	Hair growth score
Group-I	
Group-II	
Group-III	
Group-IV	

Second list of parameters include frequent of water change, habit of food intakes, increase in body weight or food capacity

Gain in body mass or increase in food efficiency, and any transpose in trajectory of weight change is noted. Once a week the reading of weight measurement was recorded between 9:00-10:00 AM.

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Table 5 Body weight of different groups of animals

Group(s)	Body weight of animals
Gr-I	
Gr-II	
Gr-III	
Gr-IV	

2.4 Histopathological analysis

For histopathological analysis, after the rats are euthanized with diethyl ether. Skin tissue samples of the shaven area are extracted. The order of sacrifice of Rats is regular intervals of 7 days. Thus, the samples collected would be fixed in 10% buffered formalin for one day, this step is followed by embedding using paraffin wax. Basic histology was dyed by hematoxylin-eosin (H&E) staining techniques, and the parameter mentioned were observed by microscopy. (Ismail et al., 2024)

The slides were then studied for different aspects.

3. RESULTS AND ANALYSIS

3.1 *In-vivo* studies on VCO and FSO based formulations

Hair Growth Observation at different time durations:

CONTROL | MARKETED FORMULATION | FORMULATION I | FORMULATION II

Figure 2 Photos demonstrating Wistar Albino Rats at initiation of animal study (Week 0)

Control | Marketed Formulation | Formulation I | Formulation II

Figure 3 Photos demonstrating Wistar Albino Rats after Week One of animal study (Week 1)

Control | Marketed Formulation | Formulation I | Formulation II

Figure 4 Photos demonstrating Wistar Albino Rats after Week Two of animal study (Week 2)

Control | Marketed Formulation | Formulation I | Formulation II

Figure 5 Photos demonstrating Wistar Albino Rats after Week Three of animal study (Week 3)

Control | Marketed Formulation | Formulation I | Formulation II

Figure 6 Photos demonstrating Wistar Albino Rats at end of animal study (Week 4)

Table 6 Hair Growth Score at end of week 4 of animal study

Group(s)	Hair growth Score				
	At start	Week 1	Week 2	Week 3	Week 4
Group-I	5	1	2	3	4
Group-II	5	1	2	3	4
Group-III	5	1	2	4	5
Group-IV	5	1	2	3	4

3.2 Body Weight Change

Table 7 Body weight average throughout the study

Group(s)	Body weight of animals (g)
Group-I	155
Group-II	164
Group-III	157
Group-IV	173

Visually changes in colour of skin is used in evaluation of Hair growth, this is earmarked by movement of telogen phase to anagen phase. This is also indicated by the fact that telogen phase is bright pink in colour while anagen phase is greyish-black. The change in colour of dorsal skin as mentioned above indicates hair growth. The faster the change, the rapid the hair growth. It is more prominent in week 2 rather than week.

The formulation which performs the above changes in colour faster is said to have better potent effect in hair growth.

There are some other parameters such as hair follicle depth, its number and substantial positive outcome in dermal thickness.

The four-week topical application and Histological analysis inferred that hair oil induced very plush and heightened hair growth and fueled the elongation of hair follicles from skin layers i.e. dermis to subcutis marketed formulation. At week four the results showed that the hair follicles of hair oil formulation were in the anagen stage. Some moderate increase of epidermal thickness in hair oil formulation was observed. At second week of the study, it displayed more hair growth of VCO than FSO. At week 4, VCO had more growth than FSO and marketed formulation both.

Second list of parameters include frequent of water change, habit of food intakes, increase in body weight or food capacity

Gain in body mass or increase in food efficiency, and any transpose in trajectory of weight change is noted. The group was marked higher than the other groups but did not show major change.

3.3 Histopathological Analysis

The histopathological slides made were as follows-

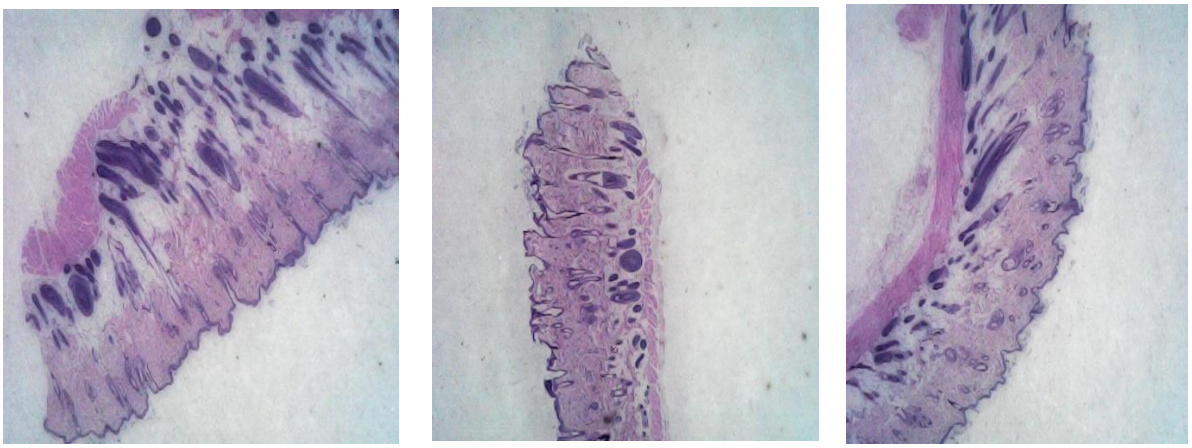

Figure 7 Microscopic histopathological view of Control group

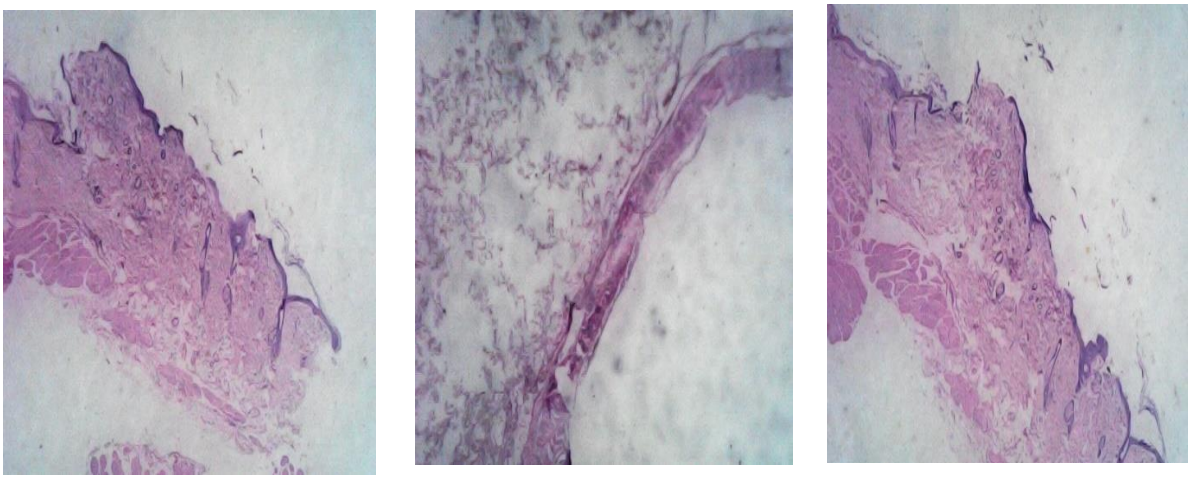

Figure 8 Microscopic histopathological view of Marketed Formulation group

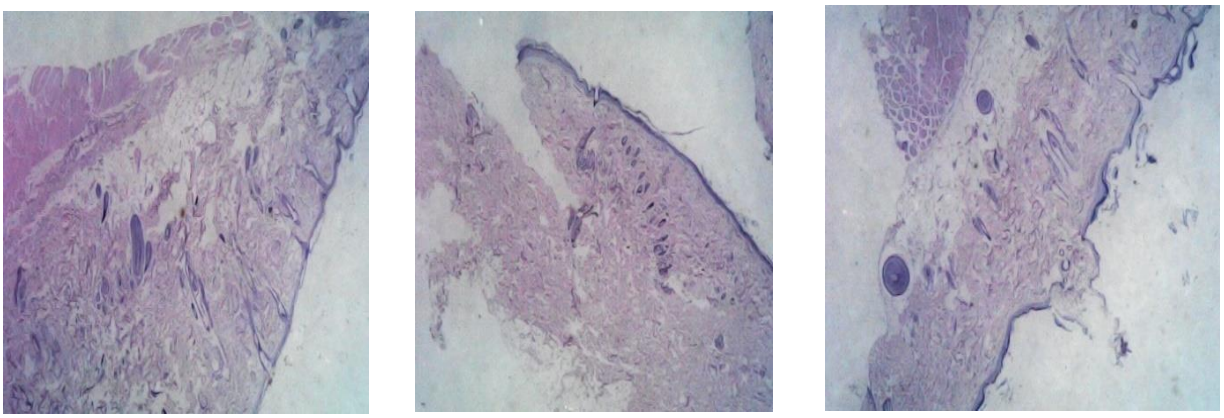

Figure 9 Microscopic histopathological view of Formulation I group

Figure 10 Microscopic histopathological view of Formulation II group

4. CONCLUSION

The study indicated that herbal hair formulations hold a key to hair growth activity without any potential side effects. The herbs used definitely promote hair growth activity but the oil used for extraction holds a key element also. In this research it focused on the type of hair oil used but also the method of preparation of hair oil by using two different methods, maceration and extraction by hydroalcoholic extract. The VCO and FSO obtained were put to animal study on Wistar Albino Rats. The study demonstrated that both VCO and FSO are both good oils from natural sources and can act as good base oil for herbal hair oil. The histopathological studies indicated that hair growth activity is seen at different stages of hair growth. The future studies can be done by using modifying different herbs used and changed the percentage of herbs in herbal hair oil.

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