Journal homepage www.jzs.univsul.edu.iq
Journal of Zankoy Sulaimani
Part-A- (Pure and Applied Sciences)

The Effects of Traditional Kurdistan Plant Extracts on Rat Hair Growth in vivo

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Article info

Original:1 July 2016
Revised:10 July 2016
Accepted: 15 July 2016
Published online:
20 December 2017

Key Words: *Hair growth, in vivo study, plant extracts, Wistar rats.*

Abstract

Pathologies of hair growth can be psychologically distressing but they are poorly controlled. Hormones and paracrine factors regulate the hair follicle and its associated glands. However, our understanding of their mechanisms is limited, restricting the development of new treatments for hair disorders. Therefore better treatments for hair loss disorders are required. Some plant extracts are believed to have effect on hair growth. Few local plants in Kurdistan region are used traditionally as stimulators of human hair growth, but their effects on hair growth scientifically has not been studied yet. Therefore, the aim of this study was to investigate the actual effects of those local plant extracts used as a traditional herbal treatment for hair loss, using *in vivo* rat model (Wistar-Bratislava rats); and to compare their effectiveness with the best medical treatment available (Minoxidil).

The effects of extracts from Myrtus (*Myrtus communis*), Galls (*Quercus infectoria*), Oak (*Quercus aegilops*) both separately and mixed at the recommended concentrations were compared with the medical treatment for hair loss and a negative control group. Shaved backs of Wistar rats (6 weeks old) were treated daily for 42 days (six groups, n=6 per group), and the degree of their effectiveness was observed and compared with each other and with both positive and negative controls. Results show that the mixture of the three plants extracts and Minoxidil have similar significant hair growth promotion effect compared to other groups. Therefore, extracts from Myrtus, Galls and Oak stimulate rodent pelage follicles *in vivo* suggesting they can be used as promoter of hair growth in human.

Introduction

Hair follicle is one of the key features of mammals including human, and it put forth a broad variety of roles including physical shield, thermoregulation, interactions socially and sensory related activities [1]. The ability of hair follicle to grow, change cycles and produce color are making hair follicle a popular model for research, especially for to researchers in biology and medicine fields, and a enormous industry that provides scientists who are willing to influence these parameters [2].The hair follicle consists of numerous diverse populations of cells including cells of mesodermal or ectodermal origin and neural crest which are separate in their function, location as well as protein and gene expression uniqueness [1, 2]. Additionally, the hair follicle is a distinctively vibrant mini-organ which go through nonstop cycling during adult time at which all the parts of its development are repeated [3].

In mammals, including human beings, hairs are cyclically shed and re-grown rapidly enabling it to renew itself and has the ability to cycle to produce a new hair and discard the old one [4]. During the hair follicle

growth cycle, the higher portion of the hair follicle does not have a cyclic pattern, but the lower follicle does. The hair follicle growth cycle (Figure 1) has three stages. Anagen, or the growth phase, has different lengths depending on the area of the body and the species. In catagen, the regression phase, the dermal papilla cells become inactive and the hair bulb becomes keratinised and moves up in the skin and forms a club hair, which rests in the hair follicle during telogen. At the end of telogen, or resting phase, the cells of the dermal papilla became activated again and with related keratinocytes they travel downwards in to dermis, forming a fresh hair bulb and lower follicle.

In exogen, the hair club fibre is shed from the follicle gradually [5]. A cluster of unique mesenchymal cells, situated in a construction called the dermal papilla, mainly organize the development and the exclusive cycling activities of hair follicle [6].

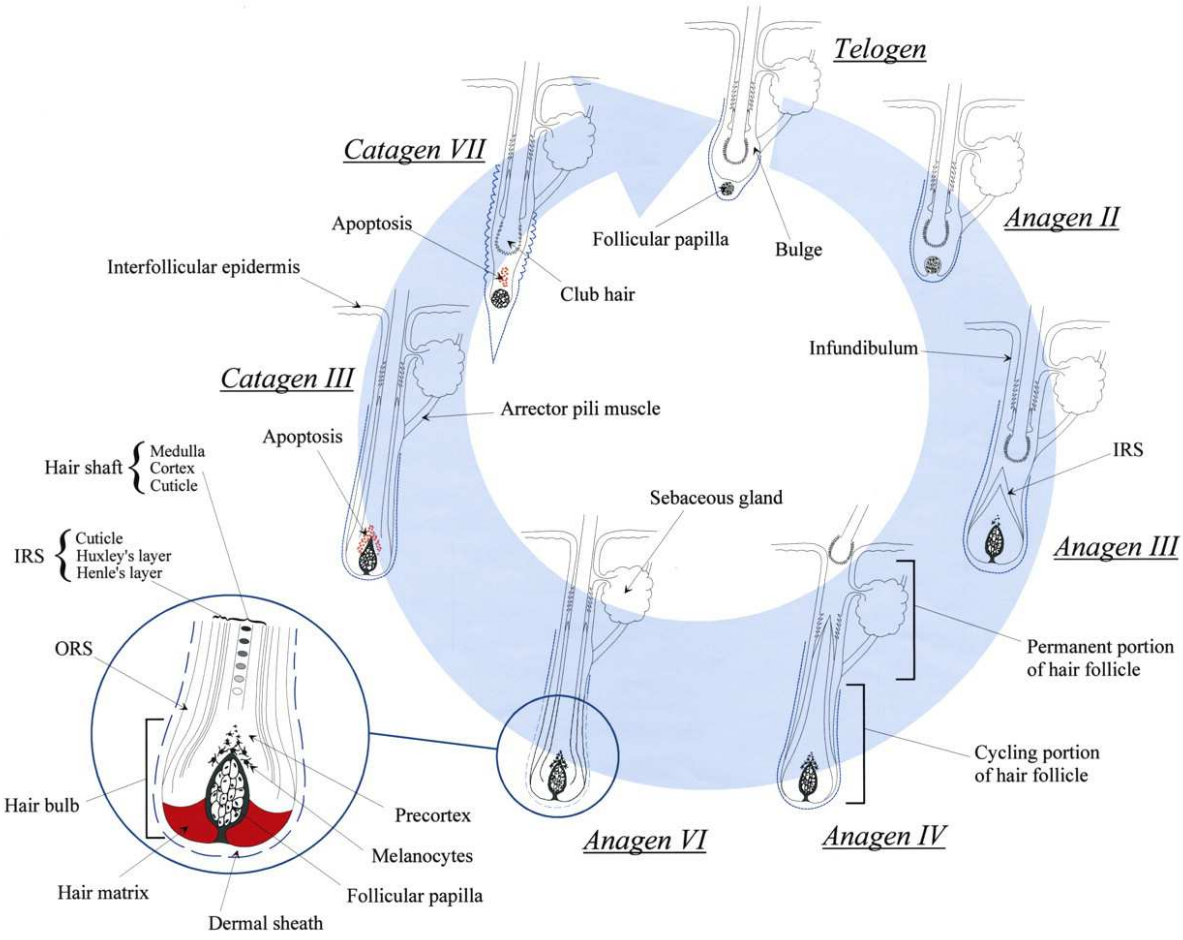

Figure 1: The mammalian hair cycle [4].

Although pathologies of hair growth such as hair loss (alopecia) or excessive hair growth (hirsutism) are not physically painful or life threatening they do cause significant psychological distress. Several studies have reported the negative effects of hair loss in men and women [7-9]. Hair disorders are poorly controlled due to the weak understanding of normal and abnormal hair follicle function and how established treatments work. The unsolved medical problems increase demand for fresh methods in producing efficient treatments. This is therefore the focus of this study.

Minoxidil is the most preferable topical therapy for hair loss [10, 11] which works through ATP-sensitive potassium (K(ATP) channels in dermal papilla [12]. Traditionally extracts of local Myrtus (*Myrtus communis*), Galls (*Quercus infectoria*) and Oak (*Quercus aegilops*) have been used for promoting hair growth. The objective of this study is to examine the influence of extracts from Myrtus, Galls of *Quercus infectoria* and Oak separately and mixed on rodent hair growth *in vivo*, compared with the most effective medical treatment available, Minoxidil.

Materials and Methods

The efficacy of local plant extracts was examined for their possible role on hair growth by *in vivo* technique. Male rats (Wistar-Bratislava albino rats aged 6 weeks, weighing 250~300 g) were randomly distributed into 6 groups to avoid any sibling bias and housed in groups of 6 with standard diet food pellets and water available at all time. Initially, the rats were partially anesthetized and dorsal hairs were removed externally by shaving (¬ 2cm width x 6 cm length) using an electric trimmer to reveal the pink skin. From the next day, termed day 0, each Wistar rat, except control group, was treated topically for 20 days with either the 5% extracts of Myrtus, Galls, Oak, or combination of all 3 and Minoxidil (5%); 6 rats were used for each condition. Dried Myrtus, Galls and Oak nuts were grinded and aqueous extract of the powder was prepared using (60%) ethanol by stirring 0.5 g powder into 10 mL of the solvent for 2 h, followed by centrifugation at 2000 g for 10 min.

The appropriate solution (¬100µl) was rubbed gently into the dorsal skin of each rat daily; new gloves were worn for each treatment type. Hair growth was recorded daily for each animal for 42 days, and dorsal photographs were taken at day 0, 7, 14, 21, 28, 35, and 42. The first day when visible hair growth could be seen was defined as the first day of anagen (the active growth phase of hair follicles) that subsequently increased and progressed to visible white hairs, was recorded for each animal. To calculate mean values, the first day of growth was designated as day 43 for any rat showing no hair growth signs by the last day. The day when the shaved dorsal area was fully covered with new hairs, i.e., there was no pink skin remaining and no visible difference in hair length to the adjacent unshaved areas, was also recorded.

Statistical analysis

All the values were expressed as mean ± standard error of mean; and data used for the statistical analysis were found to be normally distributed (Shapiro-Wilk normality test). Statistical analysis of data was performed by using two-sample t-test. A value of ($p<0.05$) was considered to be statistically significant. The Statistical Package 16.0 for Windows (SPSS Inc., Chicago, IL, USA) software was used for statistical analysis.

Results

Generally, the results show that topical treatment of 5% extracts of mixture from Myrtus, Galls and Oak promoted hair follicle growth similar to Minoxidil in rats.

The effects of local plant extracts on rodent pelage hair growth were assessed to determine whether those three plants which traditionally used for promoting hair growth could stimulate rat hair follicles *in vivo*, particularly when supplied through the skin directly, in comparison with negative control and the most effective medical treatment available, Minoxidil (positive control).

After shaving (under partial anesthesia, using inhalation anesthesia method of pure chloroform) the dorsal skin of all rats remained pink, confirming that the hair follicles were in telogen (Figure 2). Negative control animals which didn't receive any treatment as the plant extracts were dissolved in distilled water, showed few skin changes until about day 28. In contrast, the extracts of mixture from Myrtus, Galls and Oak, as well as Minoxidil application for 20 days significantly ($P<0.001$) advanced the next hair cycle at 5% concentration, with new hair growth clearly visible around day 14 in most of the rats (Figure 3). The first sign of anagen occurred at 29.0 ±2.11 day (mean±SE) in negative control animals, while 5% Myrtus extract brought this forward to 21.3±1.15 day ($P>0.001$), 5% Galls extracts to 20.5±2.3 day ($P>0.001$), 5% Oak extracts to 23.2±2.87 day ($P>0.001$) and both 5% mixture (Myrtus, Galls and Oak) and Minoxidil even earlier to 13.1±2.2 day and 13±1.7 day respectively ($P<0.001$; mean±SE) (Figure 3A). This advancement of anagen extended across the dorsal area, despite treatment stopping after 21 days. Although 18% of negative control rats had completed anagen in all their dorsal follicles after 6 weeks, but the 5% mixture (Myrtus, Galls and Oak) and Minoxidil clearly exhibited anagen over the whole area, and this increased to 77 and 80% .

Overall, 5% mixture (Myrtus, Galls and Oak) extracts and Minoxidil had significant effects on promoting hair growth in rats in vivo, with little difference between them (Figure 3B). The Myrtus, Galls and Oak extracts alone stimulated more follicle in to growth phase but their effect was much less than when combined (mixture).

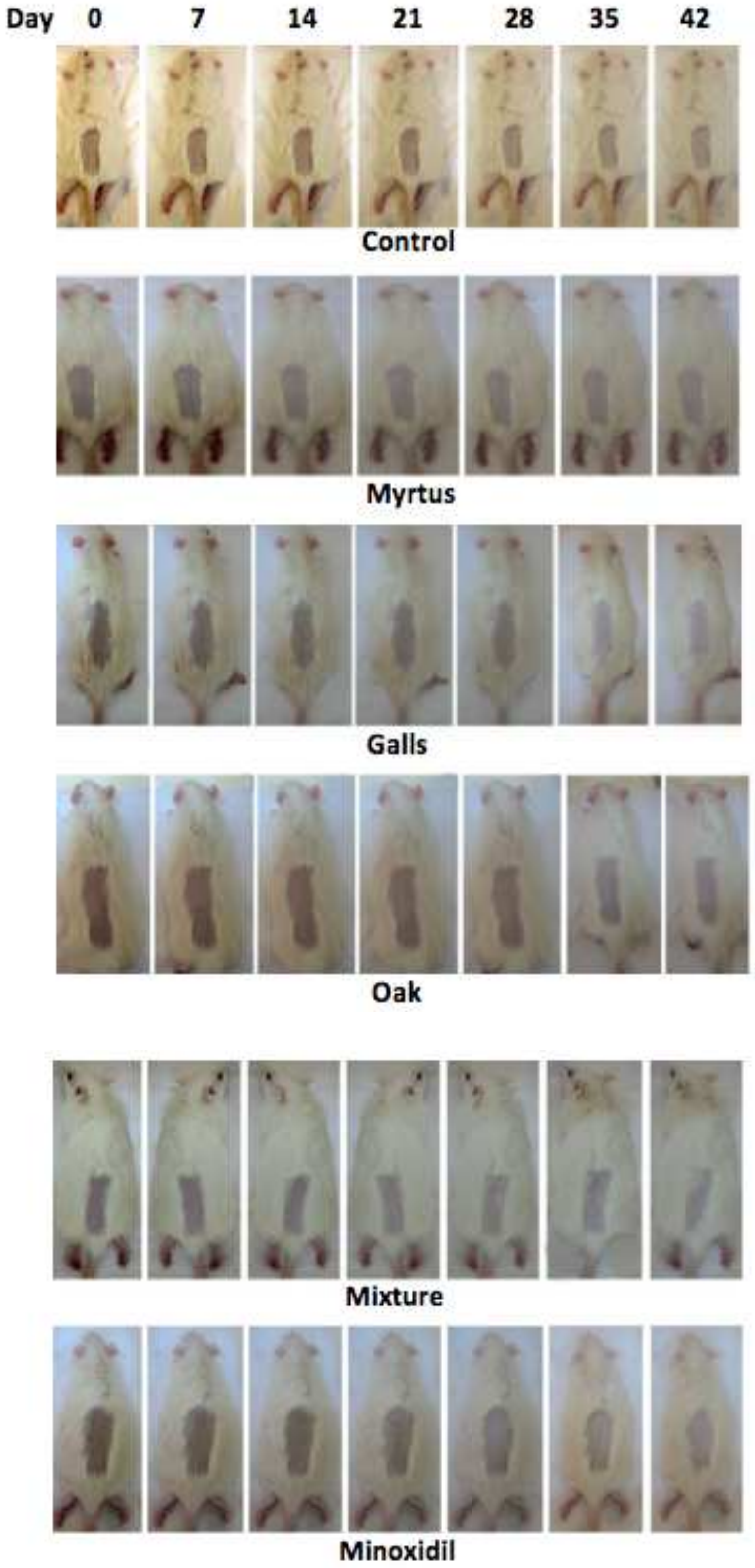

Figure 2. Topical application of 5% extracts of Myrtus, Galls, Oak, mixture of all three plants and Minoxidil promoted hair growth in rats *in vivo*. The treatments at 5% were applied topically to male rats for 20 days stimulated resting (telogen) follicles to start to grow and enter the next hair growth cycle (anagen). Pink shaved skin on back of the rats

which was visible in day 0 photographs confirms telogen status of the hair follicles. Growing hairs are initially seen as whitening skin until white hair projects outside the skin (as seen in sequential photographs of individual animals). Toward the end of the experiment, 5% extracts of mixture (Myrtus, Galls and Oak) and Minoxidil had caused the most amount of hair growth in the rats compared to control.

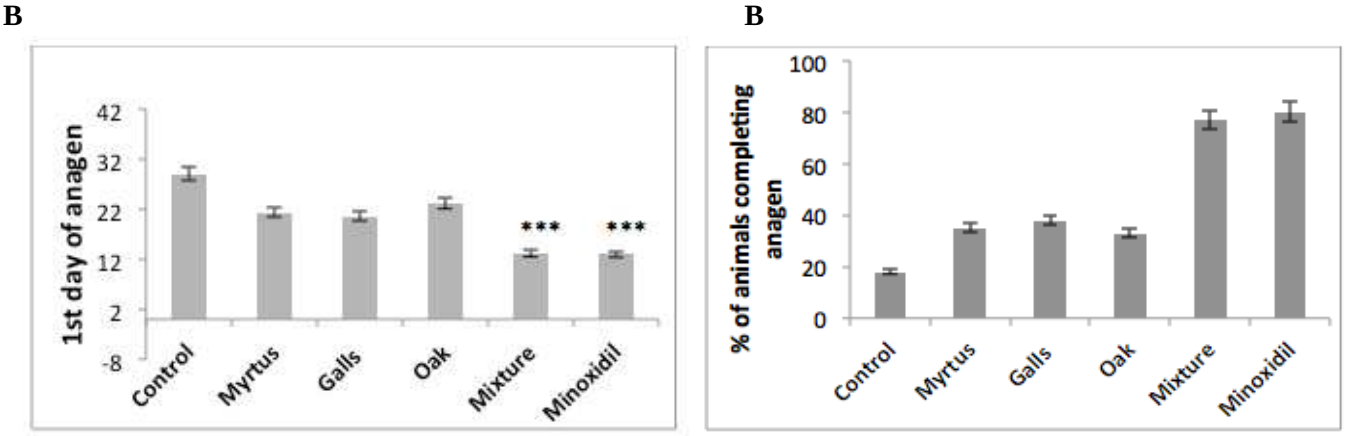

Figure 3. The topical application of 5% extracts of mixture (Myrtus, Galls and Oak) and Minoxidil significantly (P<0.001) advanced the first day at which anagen was visible, compared to the control alone (graph A) and increased the percentage of animals in which all follicles on the back had grown at day 42 (graph B). n=6 rats/treatment; ***p<0.001 vs. control.

Discussion

Extracts of local plants have been used traditionally to promote hair growth, as it was believed that those plants extracts stimulate hair growth. Among the plants commonly used as herbal remedies for hair loss are local species of Myrtus (*Myrtus communis*), Galls (*Quercus infectoria*) and Oak (*Quercus aegilops*). However none of those local plant extracts were tested scientifically. Although many plant extracts have been tested for their antimicrobial activity, but very few were studied for their hair growth promoting effect [13]. An ethnobotanical survey was carried out in the Hawraman region, southern Kurdistan, Iraq, identified these plant species in the region and reported that these plants are currently utilized in various traditional uses, including medicines [14].

Male pattern hair loss or androgenetic alopecia can distresses self-confidence directly, affecting people's value of life [7]. Therefore, hair loss is a major psychosocial symptom that merits a great deal cost on curing. Androgenetic alopecia is regarded as gradual alteration of bulky terminal hair follicles on scalp in to smaller, finer, and superficial vellus hair having a smaller anagen [15]. Although dutasteride, Minoxidil and finasteride as well as other artificial curative materials are mainly utilized for alopecia healing, their unfavorable outcomes support searching for different effective remedy agent with a narrow offshoot such as herbs [16].

Therefore, the objective of this study was to examine the effects of local plant extracts (separately and mixed) used by people traditionally as herbal treatment for hair loss, on rodent pelage hair *in vivo*, in comparison with negative control and the most effective medical treatment available, Minoxidil (positive control). The results of this study show that topical treatment of 5% extracts of mixture from Myrtus, Galls and Oak promoted hair follicle growth similar to Minoxidil in rats *in vivo.*

The shaved dorsal skin of all rats on day 0 showed pink color confirming that the hair follicles were in telogen (Figure 2). Negative control animals which didn't receive any treatment showed few skin changes until about day 28. In contrast, the 5% extracts of mixture from Myrtus, Galls and Oak, as well as Minoxidil application for 20 days significantly (P<0.001) advanced the next hair cycle at, with new hair growth clearly visible around day 14 in most of the rats (Figure 3 A). This advancement of anagen extended across the dorsal area, despite treatment stopping after 21 days. Only 18% of negative control rats completed anagen in all their dorsal follicles after 6 weeks, but the 5% mixture (Myrtus, Galls and Oak) and Minoxidil clearly

exhibited anagen over the whole area, and this increased to 77 and 80% (Figure 3 B). Indicating that mixture of Myrtus, Galls and Oak extracts at 5% can stimulate hair growth *in vivo.*

Myrtus communis of the Mediterranean has been shown to have anti-inflammatory effect and also reported to influence hair growth [17]. For many centuries, different fractions of *Myrtus communis* such as its leaves, fruits and berries were utilized widely as a traditional remedy. The plant was consumed habitually for curing diseases such as ulcer, diarrhea, infection, hemorrhoid, skin and lung diseases. Studies of experimental and clinical nature propose that it has a wider range of therapeutic and pharmacological effects for example anticancer, antioxidative, antiviral, anti-diabetic, neuroprotective, hepatoprotective, antifungal and antibacterial [18]. However, not much of its effects reported on hair growth.

One of the vital remedies utilized in Unani System of Medicine since olden Greece era is *Myrtus communis Linn* or general Myrtle belonging to Myrtaceae Family. This medicine is known as Aas and its berries recognized under the name of Habb-ul-Aas. The leaves, berries and vital oil of this plant products are often used for a range of diseases, therefore, it is frequently grown for its useful products [19].

Galls is a species of oak, growing galls which have been conventionally applied as cure for disorders in Asia for centuries. Extracts of local Galls of *Quercus infectoria* is another herbal remedy traditionally used as a promoter of hair growth, antibacterial and antifungal [20] and for helping wound healing [21]. A study by [22] observed new hairs on mice treated with *Quercus infectoria.*

Several studies carried out for determination of the effects of different plants extracts in animal studies, using rodents [22]. In order to seek a natural extract for curing hair loss disorders, a study established that the components of dehydrated root of Sophoraflavescens have great effects on enhancing hair growth. The local application of Sophoraflavescens components on to the dorsal side of mice C57BL/6 has enabled the premature transformation of telogen stage in to anagen stage [23].

A different research carried out to observe the effectiveness of essential oil extracted from seeds of plant known as *Zizyphus jujuba* on changing growth of hair using *in vivo* animal study model, in which the oil had been used at various concentrations on the bald skin of mice BALB/c and observed for three weeks. Following the three weeks of application, the treated mice had showed significant increase in the hair length compared to the control group of mice [24].

Conclusion

Overall, 5% mixture of local Myrtus, Galls and Oak extracts and Minoxidil had significant effects on promoting hair growth in rats *in vivo*, with only little difference between them. This result suggest that the effects of local Myrtus, Galls and Oak extracts on the stimulation of hair growth could be arbitrated via changing the regulatory growth factors concentration in the cells of dermal papilla inside hair follicle. Additional analysis of the effects of these plant extracts on the molecular mechanisms and cellular signaling of hair follicle may lead to the development of new hair growing products.

Acknowledgement

The authors express sincere thanks to the staff of Biology Department at Faculty of Sciences and also to KISSR Institute for supporting this work and also extend appreciation to researchers at the animal house.

References

[1] Schneider, M.R., R. Schmidt-Ullrich, and R. Paus, "*The hair follicle as a dynamic miniorgan*". Curr Biol, Vol. 19, No.3, pp R132-142. (2009).

[2] Paus, R. and G. Cotsarelis, *"The biology of hair follicles*". N Engl J Med, Vol. 341, No. 7, pp 491-497. (1999).

[3] Fuchs, E., et al., *"At the roots of a never-ending cycle"*. Dev Cell, Vol. 1, No. 1, pp 13-25. (2001).

[4] Panteleyev, A.A., C.A. Jahoda, and A.M. Christiano, *"Hair follicle predetermination*". J Cell Sci, Vol. 114, (Pt 19), pp 3419-3431.(2001).

[5] Higgins, C.A., et al., "*Exogen involves gradual release of the hair club fibre in the vibrissa follicle model"*, Exp Dermatol, Vol. 18, No. 9, pp 793-795.(2009).

[6] Higgins, C.A., et al., "*Microenvironmental reprogramming by three-dimensional culture enables dermal papilla cells to induce de novo human hair-follicle growth"*. Proc Natl Acad Sci U S A, Vol.110, NO. 49, pp 19679-19688. (2013).

[7] Girman, C.J., et al., *"Effects of self-perceived hair loss in a community sample of men"*. Dermatology, Vol. 197, No. 3, pp 223-229. (1998).

[8] Cash, T.F., *"The psychosocial consequences of androgenetic alopecia: a review of the research literature"*. Br J Dermatol, Vol. 141, No. 3, pp 398-405. (1999).

[9] Girman, C.J., et al., *"Patient-perceived importance of negative effects of androgenetic alopecia in women"*. J Womens Health Gend Based Med, Vol. 8, No. 8, pp 1091-1095. (1999).

[10] Hordinsky, M.K., *"Current Treatments for Alopecia Areata"*. J Investig Dermatol Symp Proc, Vol. 17, No. 2, pp 44-46. (2015).

[11] Vary, J.C., Jr., "*Selected Disorders of Skin Appendages-Acne, Alopecia, Hyperhidrosis"*. Med Clin North Am, Vol. 55, No. 6, pp 1195-1211. (2015).

[12] Shorter, K., et al., "*Human hair follicles contain two forms of ATP-sensitive potassium channels, only one of which is sensitive to minoxidil"*. FASEB J, Vol. 22, No. 6, pp 1725-1736. (2009).

[13] Erdogrul, Ö.T., *Antibacterial activities of some plant extracts used in folk medicine.* Pharmaceutical Biology, Vol. 40, No. 4, pp 269-273. (2002).

[14] Ahmad, S.A. and A.A. Askari, *"Ethnobotany of the Hawraman Region of Kurdistan Iraq"*. Harvard Papers in Botany, Vol. 20, No. 1, pp. 85-89. (2015).

[15] Randall, V.A., *"Androgens and human hair growth"*. Clin Endocrinol (Oxf), Vol. 40, No. 4, pp 439-457. (1994).

[16] Lourith, N. and M. Kanlayavattanakul, "*Hair loss and herbs for treatment"*. Journal of cosmetic dermatology, Vol. 12, No. 3, pp 210-222.(2013).

[17] Chalchat, J.-C., R.-P. Garry, and A. Michet, *"Essential oils of myrtle (Myrtus communis L.) of the Mediterranean littoral"*. Journal of essential oil Research, Vol. 10, No.6, pp 613-617. (1998).

[18] Alipour, G., S. Dashti, and H. Hosseinzadeh, "*Review of pharmacological effects of Myrtus communis L and its active constituents"*. Phytotherapy Research, Vol. 28, No. 8, pp 1125-1136. (2014).

[19] Sumbul, S., et al., "*Myrtus communis Linn. A review"*. Indian J Nat Prod Resour, Vol. 2, No.4, pp 395-402. (2011).

[20] Digrak, M., et al., *"Antibacterial and antifungal effects of various commercial plant extracts"*. Pharmaceutical Biology, Vol. 37, No. 3, pp 216-220.(1999).

[21] Jayaprasad, B., D. Thamayandhi, and P. Sharavanan, *"Traditionally using antidiabetic medicinal plants in Tamil Nadu"*. IJRPB, Vol. 2, No. 1, pp 1-8. (2012).

[22] Sharma, B., P. Kumar, and S.C. Joshi, *"Topical treatment of dermatophytic lesion on mice (Mus musculus) model"*. Indian journal of microbiology, Vol. 51, No. 2, pp 217-222.(2011).

[23] Roh, S.-S., et al., *"The hair growth promoting effect of Sophora flavescens extract and its molecular regulation"*. Journal of dermatological science, Vol. 30, No. 1, pp 43-49.(2002).

[24] YOON, J.I., S.M. AL-REZA, AND S.C. KANG, *"Hair growth promoting effect of Zizyphus jujuba essential oil"*. Food and chemical toxicology,Vol. 48, No. 5, pp. 1350-1354. (2010).