



## Hair growth promoting effect of *Zizyphus jujuba* essential oil

Jung In Yoon<sup>a,1</sup>, Sharif M. Al-Reza<sup>a,b,1</sup>, Sun Chul Kang<sup>a,\*</sup>

<sup>a</sup> Department of Biotechnology, Daegu University, Kyongsan, Kyungbuk 712-714, Republic of Korea

<sup>b</sup> Department of Applied Chemistry and Chemical Technology, Islamic University, Kushtia 7003, Bangladesh

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### ABSTRACT

This study was undertaken to examine the efficacy of essential oil from seeds of *Zizyphus jujuba* for its potential role on hair growth by *in vivo* method. Essential oil was applied at different concentrations (0.1%, 1% and 10%) over the shaved skin onto the backs of BALB/c mice and monitored for 21 days. After 21 days, mice treated with 1% and 10% of oil produced a greater effect on the length of hair which were measured to be 9.96 and 10.02 mm, respectively, as compared to the control (8.94 mm). We measured the weight of hair/cm<sup>2</sup> area of dorsal skin and also evaluated hair thickness and hair follicles microscopically after plucking the hair immediately from the shaved area of mice and found the best results for 1% of essential oil-treated mice. From this study, it is concluded that *Z. jujuba* essential oil possesses hair growth promoting activity.

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### 1. Introduction

Hair is one of the vital parts of the body derived from ectoderm of the skin, is protective appendages on the body and considered accessory structure of the integument along with sebaceous glands, sweat glands and nails (Ebling, 1987). They are also known as epidermal derivatives as they originate from the epidermis during embryological development. Hair is an important of the overall appeal of the human body (Cash, 2001; Stough et al., 2005). Many people suffer from hair loss or hair thinning, despite the development of several medical treatments. Therefore, it is important to develop novel therapies that prevent hair loss and enhance hair growth. In this respect, alternative medicine has attracted interest. Although it has not yet been incorporated into mainstream of medical care, due to limited scientific evidence and incomplete knowledge of the mechanisms involved, alternative medicine has become an increasingly attractive approach worldwide (Bhaumik et al., 2000). The search for treatment results into few drugs of synthetic origin, but side effects associated with them can not be neglected. Herbal cosmetics are now emerged as the appropriate solution to the current problem, natural products are fancy in cosmetics and about 1000 kinds of plant extract have been examined with respect to hair growth and still it is a fast growing segment

with a vast scope of manifold expansion in coming years (Rathi et al., 2008).

In recent years, the study of hair growth promoting agents has greatly advanced. Several animal models, beginning with the C3H mouse (Hattori and Ogawa, 1983) have been used experimentally to evaluate the extent of hair growth. Newer techniques for evaluation based on cell cultures, such as dermal papillae cells, hair matrix cells and outer root sheath cells (ORSCs) have been developed to analyse quantitatively the extent of hair growth *in vitro* (Arase et al., 1991; Fujie et al., 1993). A unique feature of hair growth is its cyclicity (anagen, catagen, and telogen phases). These cyclic changes involve rapid remodeling of both the epithelial and dermal components of the hair follicles (Stenn et al., 1996; Paus and Cot-sarelis, 1999). The dermal papilla (DP), which is the main mesenchymal component, is located at the deepest end of the hair follicle, and is thought to play essential roles in the induction of new hair follicles and the maintenance of hair growth (Oliver, 1970; Jahoda and Reynolds, 1992).

*Zizyphus jujuba* is a thorny rhamnaceous plant that is widely distributed in Europe and Southeastern Asia. Fruits of this plant are edible and different parts of *Z. jujuba* possess multiple medicinal properties such as antifertility, analgesic, and antidiabetes (Ambasta, 1986; Erenmemisoglu et al., 1995). The local tribal people use the bark mixture of *Z. jujuba* to prevent the pregnancy (Souleles and Shammis, 1998). However, there are few scientific studies about the effect of *Z. jujuba* seeds. In traditional medicine, the seed of *Z. jujuba* has been used for its action on insomnia and anxiety (Lee, 1986). As reported by Kim (2002), *Z. jujuba* seeds were effective on the improvement of the blood glucose, lipid compositions in serum of dietary hyperlipidemic rats. In particular, *Z. jujuba*

\* Corresponding author. Address: Department of Biotechnology, College of Engineering, Daegu University, Kyongsan, Kyungbuk 712-714, Republic of Korea. Tel.: +82 53 850 6553; fax: +82 53 850 6559.

E-mail address: [sckang@daegu.ac.kr](mailto:sckang@daegu.ac.kr) (S.C. Kang).

<sup>1</sup> First and second author equally contributed in this work.

seeds were more effective as a therapeutic regimen for the control of metabolic derangements in adult disease.

Previously, we reported the chemical composition, antioxidant and antimicrobial activities of the essential oil and organic extracts of *Z. jujuba* seeds against a diverse range of bacterial *in vitro* conditions (Al-Reza et al., 2009, 2010). However, there is no report available on hair growth promoting activity of the essential oil from seeds of *Z. jujuba*. Therefore, the present study was focused on the scientific investigation of the hair growth potential of the essential oil from seeds of *Z. jujuba*.

## 2. Materials and methods

### 2.1. Plant material

The seeds of *Z. jujuba* were collected from the local area of Kyongsan, Republic of Korea, in August 2008. Seeds were cleaned, dried and ground. Initially the seeds were identified by morphological features and in-house data base by Prof. Man Kyu Huh. A voucher specimen number was deposited in the Herbarium of the College of Engineering, Department of Biotechnology, Daegu University, Republic of Korea.

### 2.2. Isolation of the essential oil

About 250 g ground seeds of *Z. jujuba* were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and preserved in a sealed vial at 4 °C until further analysis.

### 2.3. Experimental animals

Five-week-old female BALB/c mice (18–20 g) were purchased from Orient Bio Inc. (Seoul, South Korea). The animals were kept in polypropylene cages (three mice per cage) and maintained on a standard laboratory diet and water *ad libitum*. They were housed in an air-conditioned room with 12:12 h light and dark cycle at least 7 day prior to experiment. The room temperature (about 23 °C) and humidity (about 60%) were controlled automatically.

### 2.4. Hair growth activity in vivo

The mice were divided into five groups of three mice each. A 4 cm<sup>2</sup> area of the hair from dorsal portion of all the mice was shaved with electric hair clippers. Ten microliters of the prepared oil and the hydrocortisone were applied to the denuded area of the respective groups once a day and a control group received no treatment. This treatment was continued for 7 days. The hair regrowth at 7, 14 and 21 day after beginning of topical application was observed visually and recorded.

### 2.5. Hair length determination

Hairs were plucked randomly from the shaved area of all mice on 7, 14, and 21 day after beginning the treatment. The length of 10 hairs was measured and the average length was determined. The results are expressed as the mean length  $\pm$  S.D. of 10 hairs.

### 2.6. Hair weight determination

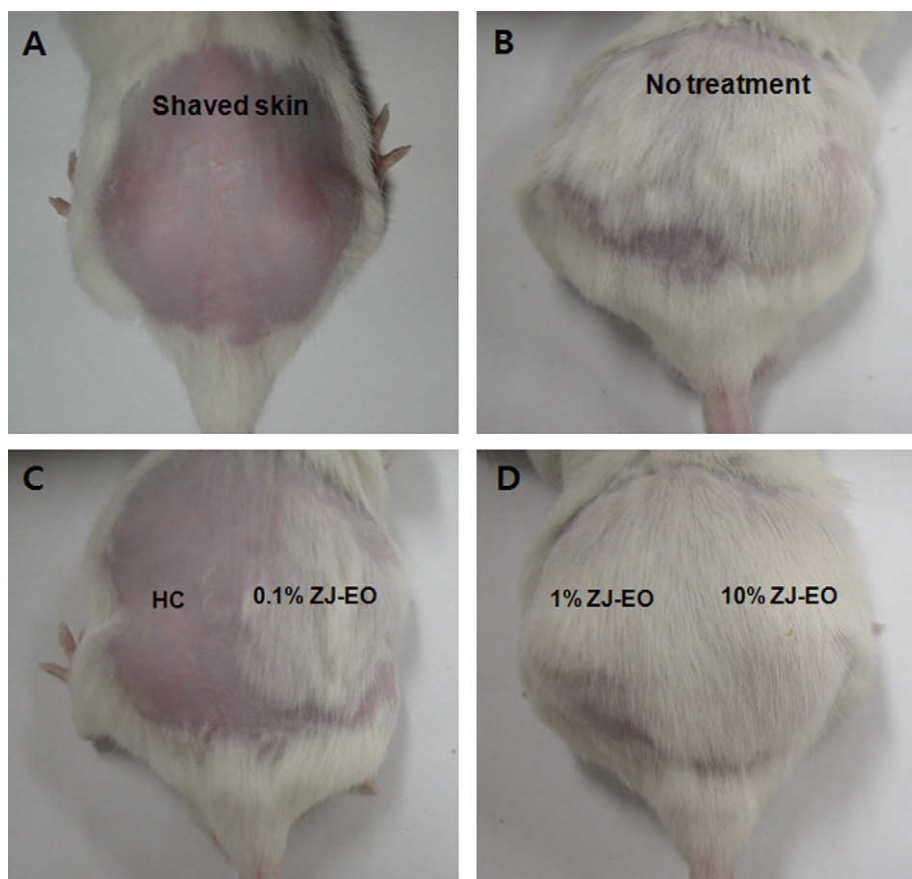
After 21 days, the mice were sacrificed by cervical dislocation. A 1 cm<sup>2</sup> area of dorsal skin with hair and without hair was cut from all the mice of each group and weighed with the analytical balance. After measuring, hair weight was calculated by subtracting skin weight from skin with hair weight.

### 2.7. Measurement of hair thicknesses and area of hair follicles

Hair thicknesses and hair follicles were evaluated by microscopic photograph (magnification  $\times 400$ ) after plucked the hair immediately from the shaved area of all mice on Day 21. The width of hair and area of hair follicle were calculated from the photographs and expressed as mean  $\pm$  S.D. of 10 vibrissal hairs for each group.

### 2.8. Statistical analysis

Each experiment was run in triplicate, and mean values were calculated. A Student's *t*-test was computed for the statistical significance of the results.



**Fig. 1.** Hair growth promoting effect of the *Z. jujuba* essential oil in BALB/c mice after 21 days. A. initially shaved skin of mice; B. no treatment; C. treated with HC (hydrocortisone) (left) and 0.1% ZJ-EO *Zizyphus jujuba* essential oil (right); and D. treated with 1% ZJ-EO (left) and 10% ZJ-EO (right).

### 3. Results

#### 3.1. In vivo hair growth effect

It was observed that the hair growth initiated from the shaved area at the end of the course in all the groups, except hydrocortisone treated group. As the results shown in Fig. 1, the whole denuded area of oil-treated mice has been covered at the end of third week, in comparison to the control. Moreover, in 1% and 10% of essential oil-treated groups, the hair was looking sparse than control group.

#### 3.2. Measuring of hair length

As the results shown in Fig. 2, mice treated with 1% and 10% of oil produced a greater effect on the hair growth when compared to other groups. It was found that the length of hair for 1% and 10% oil-treated groups were 9.96 and 10.02 mm, respectively, compared to the control (8.94 mm) and 0.1% oil-treated group (9.22 mm) at the end of the experiment. On the other hand, hydrocortisone did not show any effect on hair growth.

#### 3.3. Measuring of hair weight

The weight of newly grown hairs in all the test groups were measured, and compared with that in the control group. It was found that weight of hair was highest for 1% oil-treated mice. The weight of hair was measured to be 53, 57 and 54 mg/cm<sup>2</sup> area of dorsal skin for 0.1%, 1% and 10% of essential oil-treated mice, respectively; while it was found 50 mg/cm<sup>2</sup> area of dorsal skin for the control group (Fig. 3).

#### 3.4. Measuring of hair thickness and area of hair follicle

As shown in Fig. 4, hair thicknesses were measured on the total length of hair at different points from the hair follicle using a highly magnified microscopic photograph of each hair (hair length from follicle: 0–5 cm, point 1; 5–10 cm, point 2; 10–15 cm, point 3). It was found that thickness varied with increasing the length from the follicle. The hair thickness of all treated groups was nearly same as control group at point 1, whereas, the hair thicknesses of all oil-treated groups were markedly increased at point 2 as compare to the control. The hair thickness of 0.1%, 1% and 10% oil-trea-

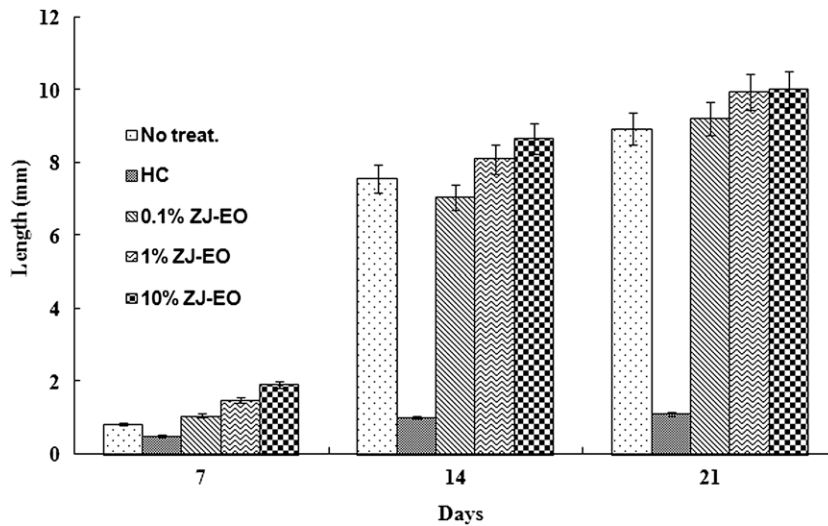


Fig. 2. Length of hair at different time intervals after beginning the treatment of *Zizyphus jujuba* essential oil. ZJ-EO: *Zizyphus jujuba* essential oil; HC: hydrocortisone.

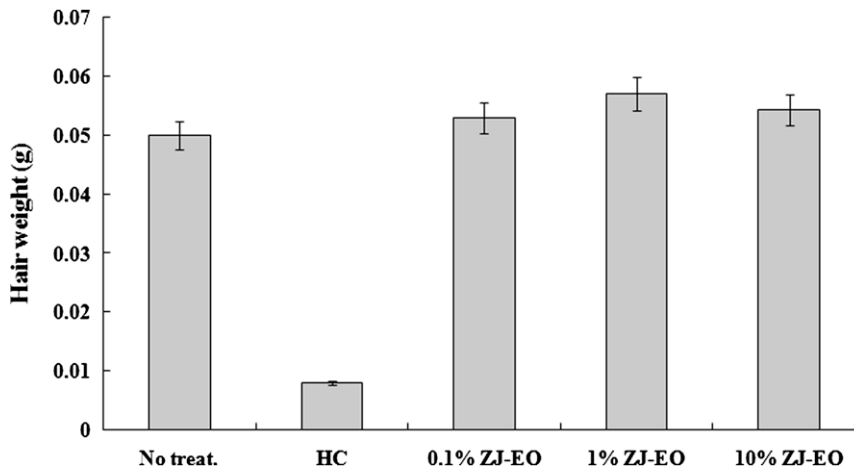


Fig. 3. Weight of hair per cm<sup>2</sup> area of dorsal skin at different conc. of *Zizyphus jujuba* essential oil after 21 days. ZJ-EO: *Zizyphus jujuba* essential oil; HC: hydrocortisone.

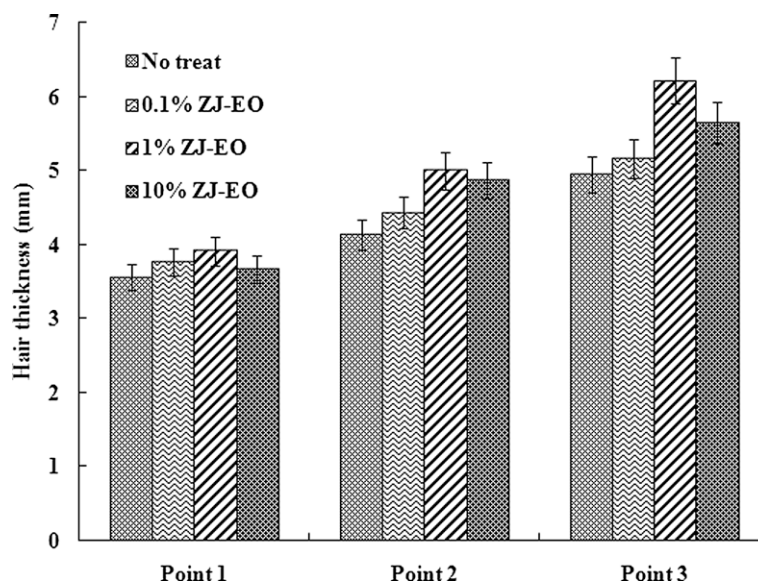


Fig. 4. Effect of different conc. of *Zizyphus jujuba* essential oil on hair thickness at different points of hair length after 21 days, (hair length from follicle: 0–5 cm, point 1; 5–10 cm, point 2; 10–15 cm, point 3) ZJ-EO: *Zizyphus jujuba* essential oil.

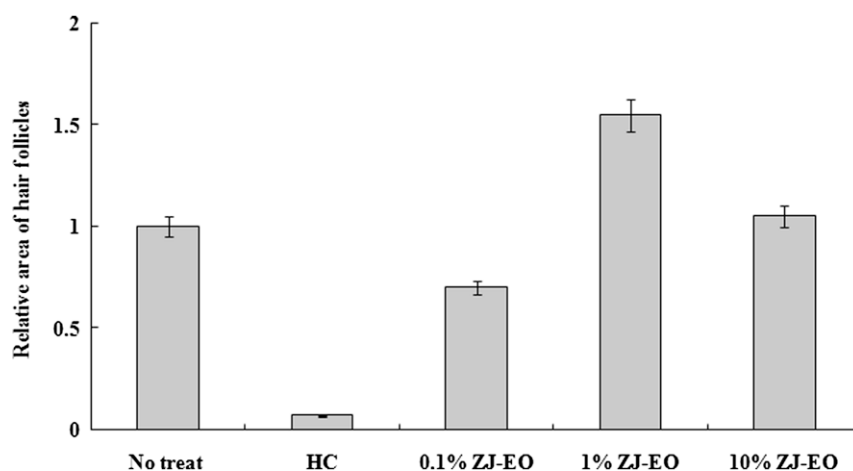


Fig. 5. Relative area of hair follicles at different conc. of *Zizyphus jujuba* essential oil after 21 days. ZJ-EO: *Zizyphus jujuba* essential oil; HC: hydrocortisone.

ted groups was found to be 4.4, 5 and 4.8 mm, respectively, as compared to the control (4.1 mm) at point 2. On the other hand, the hair thickness of 0.1%, 1% and 10% oil-treated groups was found to be 5.1, 6.2 and 5.6 mm, respectively, as compared to the control (4.9 mm) at point 3.

The relative area of hair follicles was illustrated in Fig. 5. We found that the area of hair follicles was higher for both 1% and 10% oil-treated groups with respect to control group. On the other hand, hydrocortisone and 0.1% oil-treated groups did not show potent effect on the hair follicle area.

#### 4. Discussion

Products that claim to be useful for treating hair loss target a steadily growing, multi billion dollar market worldwide. In consequence, few other life sciences areas sport as many patents – often with sweeping claims – as the ever-expanding circus of certified, potential or dubious ‘anti-hair loss’ agents. And yet, pharmaceutical hair loss management still occurs within a clinical theatre of Dickensian proportions: ‘great expectations’-contrasted by plenty of disappointed hopes (Bandaranayake and Mirmirani, 2004).

Much of that disappointment appears to result from unrealistic expectations, ill-targeted (and therefore inefficient) drug therapy and insufficient industrial interest in dissecting the basic mechanisms by which hair loss occurs and by which human hair growth-promoters exert their effects.

Despite the plethora of patented and advertised ‘anti-hair loss’ agents, convincing evidence-based medicine still is the exception rather than the rule in this field, and just two FDA approved ‘hair loss drugs’ (the dihydrotestosterone-suppressing 5 $\alpha$ -reductase inhibitor, finasteride and the antihypertensive potassium channel opener, minoxidil) supremely rule in current clinical practice (Price, 1999). Given the widely underestimated psychological burden that hair loss inflicts upon affected patients, and the limited, transient and somewhat unpredictable efficacy of finasteride and minoxidil in hair loss management, more and better pharmacological treatment options are urgently needed (Paus, 2006).

Attempts have been focused to discover effective materials for hair loss treatment. For instance, procyanthocyanidins extracted from grape seeds were reported to induce hair growth (Takahashi et al., 1998), and extract of *Sophora flavescens* has been reported to promote hair growth (Roh et al., 2002). In another study, Uno and Kurata (1993) reported that the topical application of fuzzy rat

with minoxidil, diazoxide and copper peptide produced a conversion of short vellus hairs to long terminal hairs and an enlargement of the follicular size with prolongation of anagen phase by enhancing the rate of cell proliferation. Jain et al. (2006) investigated the hair growth activity of almond oil, til oil and coconut oil preparation containing ariel part of *Z. jujuba*, *Cuscuta reflexa*, *Citrus burgamia*, *Lagenaria sicararia*, *Hibiscus rosasinesis* and *Allieum cepa*, seed of *Trigonella foinum graceum* fruits of *Embellica officinalis*.

Plant essential oils are plant secondary metabolites containing a mixture of fatty acids. Considering the experimental results by Liang and Liao (1997), it is expected that several fatty acids, e.g. palmitic, oleic, linoleic, linolenic and arachidonic acids, and a mixture of these acids show a significant anti-androgenic effect owing to their testosterone 5 $\alpha$ -reductase inhibitory activity. Seeds of *Sesamun indicum* L. (Pedaliaceae) which contain a considerable amount of fatty acids have been prescribed for the treatment of hair growth in Chinese medicinal herbals (Li, 1992).

The present study is focused on the scientific investigation of the hair growth potential of *Z. jujuba* essential oil in a dose-dependent manner. The shaved skins of BALB/c mice were treated with the topical application of *Z. jujuba* essential oil and hydrocortisone for 7 days and observed the hair growth promoting effect for 21 days. The hair growth initiated significantly higher from 1% to 10% of essential oil-treated denuded area at the end of treatment, in comparison to the control (Fig. 1). This explains the presence of greater number of hair follicles in the anagen phase of the hair growth cycle in 1% and 10% of essential oil-treated groups. This was again confirmed by measuring the hair length on 7, 14 and 21 day after beginning the experiment (Fig. 2).

To further investigate the hair growth promoting effect, we measured the weight of hair/cm<sup>2</sup> area of dorsal skin. As shown in Fig. 3, the hair weight of essential oil-treated mice was found higher than control group. However, treatment with hydrocortisone did not show any potent effect on hair growth. We also evaluated the hair thickness and relative area of hair follicles microscopically. One percent and ten percent essential oil-treated mice exhibited a more substantial effect in both these assay compared to control and other groups as shown in Figs. 4 and 5.

Looking at the increasing popularity of the herbal drugs in hair care, it should be worthwhile to take up systemic investigation on the efficacy of these drugs and their preparation. However, the exact mechanism of stimulation of hair growth was not known. Finally, this study concluded that the essential oil from seeds of *Z. jujuba* possesses hair growth promoting activity and it is suggested that essential oil from seeds of *Z. jujuba* could be included as a constituent in the hair growth promoting agent originated from a natural resource.

#### Conflict of interest statement

The authors declare that there are no conflicts of interest.

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