



Amla Oil, a Pharmaceutical Product Based on Traditional Knowledge for Hair Loss Treatment

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Abstract

Background and objectives: Iranian traditional medicine (ITM) resources have recommended different formulations for overcoming hair loss which is a beauty concern worldwide; one of these recommendations is amla oil which has been mentioned to be considerably effective. In the present study, amla oil has been formulated according to ITM and the quality control assessment of the product has been fulfilled. **Methods:** *Phyllanthus emblica* L. fruits (amla) and *Myrtus communis* L. leaves (myrtle) were provided from local market and their quality was evaluated according to pharmacopeia. The aqueous extract of myrtle was obtained by 24 h maceration; then amla was extracted in myrtle aqueous extract for 48 h. The mixture was filtered and sesame oil was added to the filtrate and heated till all water was evaporated. The resulting oil was assessed for viscosity, density, total phenolics and total tannins contents and acid and saponification values; the microbial content was also evaluated. **Results:** Amla oil was yellow in color with sesame odor. The acid value, viscosity, density, total phenolics and total tannins as pyrogallol were found to be 3.03 mg KOH/g, 102.8 cp, 0.966 g/mL, 0.2 and 0.05%, respectively. The product saponification value was zero and no microbial contamination was detected. **Conclusion:** The product showed proper physicochemical and microbial quality and could be suggested for treatment of hair loss after passing clinical trial.

Keywords: amla; hair loss; Iranian traditional medicine; *Phyllanthus emblica*; *Myrtus communis*

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Introduction

Hair loss is a beauty concern with different aetiologies. One important factor for male pattern hair loss is dihydro testosterone (DHT). Testosterone is converted to DHT by 5- α -reductase enzyme which is inhibited by finasteride, a medication used in male pattern hair loss. Usually hair loss is started from temples or forehead in men while in women it is often started from crown of the head. Minoxidil, a vasodilator drug, is used as a treatment for male pattern hair loss; however the condition will restart after cutting off the medication [1]. In Iranian traditional medicine (ITM), different

reasons have been mentioned as the causes of hair loss. In "Canon of medicine" it is stated that the disease may happen due to defects in the hair follicle or the material needed for hair growth [2]. Various remedies have been mentioned in ITM books for treatment of hair loss. For example, in "Teb-e-Akbari" amla oil, myrobalan fruit, oak and other astringent agents in combination with myrtle oil have been recommended [3]. Fruits of amla have been mentioned for preparation of amla oil. Aghili Khorasani, an ITM scholar, has suggested a formulation containing myrtle leaves or seeds, amla and sesame oil for treatment of

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hair loss [4]. This formulation has few ingredients, is easy to prepare and includes two components (*Phyllanthus emblica* and *Myrtus communis*) that have been suggested many times in different ITM resources for treatment of hair loss.

Phyllanthus emblica is well known for its nutritive qualities and contains variety of chemical constituents including tannins, music acid, amino acids, alkaloids, flavonoid glycosides, phenolics glycosides and terpenoids [5-7]. The plant has been reported to have antioxidant, anti-inflammatory, anti-cancer, adaptogenic, anti-diabetic, nootropic, antimicrobial and immunomodulatory potential [8]. According to Indian ancient history, amla is believed to be the first tree to be grown in the universe which shows the importance of the plant in traditional medicine of India [9]. According to ITM, amla has cold and dry temperament and acts as an astringent and tonic agent for brain, stomach, eye, etc. It is used for prevention of hair fall and darkening the hair [10].

Different parts of *Myrtus communis* (leaves, fruits, branches) have been used extensively in folk medicine for a long time. The astringent, tonic and antiseptic characteristics of the leaves justify its use for healing wounds or disorders of the digestive and urinary systems. The oil is antiseptic and anti-catarrrhal and is therefore used to treat chest ailments [11]. The plant contains many biologically active compounds such as essential oil, flavonoids, coumarins and tannins [12-14]. According to ITM, it has cold and dry temperament and is used in different ailments especially for hair fall and prevention of hair whitening [10]. Regarding the hair tonic effects of amla and myrtle and recommendations of ITM, in this investigation, amla oil containing amla and myrtle was formulated and its physicochemical characteristics were determined.

Material and Methods

Plant material

Phyllanthus emblica L. fruits (amla) and *Myrtus communis* L. leaves (myrtle) were provided from Tehran herbal market and were authenticated at the Herabrium of Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. The voucher numbers were 450 and 449 HMS for *Phyllanthus emblica* and *Myrtus communis*, respectively.

Quality control of amla and myrtle

Loss on drying, foreign matter, total ash, acid insoluble ash, aqueous extractable value, ethanol extractable value, amount of essential oil, total phenolics and total tannins contents were evaluated according to pharmacopeia [15].

Total phenolics and tannins contents as pyrogallol

Amla powder (1 g) was extracted with water (25 mL, 30 min), the volume was adjusted to 25 mL and filtered; two mL of this solution was further adjusted to 25 mL and kept for the experiments.

For preparing pyrogallol standard solutions, first, the stock solution was prepared (0.5 mg/mL) and serial dilutions were provided accordingly (0.25, 0.125, 0.0625, 0.0312 mg/mL).

Then, one mL of Folin-Ciocalteu reagent and 10 mL water were added to two mL of the sample/standard solution in a 25 mL volumetric flask and the volume was adjusted using sodium carbonate 29%. Thirty min later, the absorbance were recorded at 760 nm. The amount of total phenolics was calculated using calibration curve of pyrogallol, accordingly.

In order to determine total tannins content, 100 mg of hyde powder was added to ten mL of the sample solution; one hour later, the mixture was filtered. The above mentioned procedure was performed on the filtrate. The difference between the two absorbance was correlated to the tannin content of the sample which can be measured using pyrogallol calibration curve [15].

Amla oil preparation

According to ITM reference "Qrabani-e-kabir", for preparing the oil, amla should be macerated in juice of myrtle seed or leaves (1:2 amla: myrtle) and then sesam oil would be added to the extract and boiled till the water is evaporated [4]. Since fresh myrtle was not accessible and considering 50% water content of myrtle, the equivalent dry plant was used in the formulation.

For selection of the best solvent: herb ratio in extraction of myrtle, different solvent: ratios including 1:10, 1:15 and 1:20 were examined. Moreover, maceration times 24, 48 and 72 h were tried as well. Finally, Seventy five g of myrtle leaves was ground and extracted with 1500 mL water during 24 h of shaking and filtered afterwards. Then, Seventy five g of amla fruits were ground and mixed with the myrtle extract and kept for 48 h with continuous shaking. The

mixture was filtered afterwards. Sesame oil was added to the extract in equal volume and the mixture was heated until all water was evaporated; the final oil was centrifuged.

Quality control assessments of the oil

Density was measured using a pycnometer. Viscosity was evaluated using a DV2 RV viscometer (Brookfield, USA) with spindle No. 2, 25.7 torque, rpm 100. The total viable count and evaluations for specific microorganisms was carried out according to WHO guide lines [16].

Acid and saponification value

Acid value shows the amount of potassium hydroxide (mg) needed to neutralize the free fatty acids in one g of sample while the amount needed to neutralize the free fatty acids and saponificate the esters is regarded as the saponification value. These values were measured according to previous references [17,18].

Total phenolics and tannins contents of the oil

In order to determine total phenolics and tannins contents of the oil, 25 mL methanol was added to 100 g oil and shaken for 2 h. Then the mixture was kept in freezer for 4 h and methanol phase was separated. The volume was adjusted to 25 mL and this solution was used for phenolics and tannins determination using the above mentioned methods [15,19].

Results and Discussion

The results of quality control assessments of the plants material has been presented in table 1. Total phenolics and tannin contents have been calculated regarding the pyrogallol calibration curve and the related formula $Y=5.514X+0.0839$, $R^2=0.995$.

Among the different herb: solvent ratios tried for extraction of myrtle, ratio 1:20 was selected as the best method because other ratios produced a low amount of extract and most of the solvent was retained in the leaves. Different extraction times were examined as well and 24 h maceration duration was found to be the best. Longer times produced a sticky extract. It seemed that longer extraction times decomposed the plant components. The characteristics of the oil have been shown in table 2. Microbial evaluation of the oil was in agreement with requirements [16]. Hair loss is not a life threatening disease but it affects social relationships in any age, sex or race.

The risk factors include genetics, androgen hormones, insufficient blood circulation in hair follicles, psychological disorders, infections, toxic materials, chemotherapy, etc. [1].

Table 1. physicochemical analysis of amla and myrtle

Test	Amla (%)	Myrtle (%)
Total ash	3.13±0.20	-*
Sulfates ash	-	4.60±0.41
Water soluble ash	-	1.92±0.15
Acid insoluble ash	-	0.19±0.01
Loss on drying	6.21±0.41	4.76±0.42
Foreign matter	4.50±0.12	11.50±0.95
Water extractive content	51.22±2.20	-
Ethanol extractive content	18.40±1.52	-
Essential oil	-	1.50±0.00
Total phenolics content	5.70±0.46	-
Total tannins content	2.70±0.34	-

*The test has not been performed on the plant.

Table 2. physicochemical analysis of amla oil

Test	Result
Appearance	Yellow with sesame odor
Viscosity	102.8 cp
Density	0.966 g/mL
Acid value	3.03 mg KOH/g
Saponification value	0.0 mg KOH/g
Total phenolics content	0.2±0.04 %
Total tannin content	0.05±0.006 %

There are some synthetic and anti-androgen medicines for treatment of hair loss but they are either not effective or produce side effects. On the other hand, some herbal products have proved to be effective with fewer side effects.

Amla has been known as a hair tonic to strengthen and promote the hair growth and for preventing premature hair greying [20-21]. It has nutritional value and contains high amounts of tannins which are astringent agents. This property may affect the hair follicles and prevent hair loss. This plant is used in various hair shampoos and hair oils for promoting hair growth. It is a source of minerals which are also necessary for hair growth [8].

Myrtle has been used as a hair tonic agent in French and Persian folk medicines. The leaves contain essential oil, tannins and flavonoids such as quercetin, catechin and coumarins [11]. The effect of the plant on the hair growth has been studied in different investigations. In a study conducted by Bureau et al., the effect of applying a blend of 100% essential plant oils containing myrtle combined with low electromagnetic pulses on hair growth in treatment of androgenetic alopecia was assessed. Using the oils alone prevented hair loss and occasionally induced hair growth by two mechanisms: first, favoring nutritive intake of the hair papilla cells due to the

stimulation of the microcirculation and second, regulating the function of sebaceous glands [22]. Considering the effect of amla and myrtle in hair loss, it seems that their oil will also be useful in hair loss. In the present study, amla oil has been formulated according to ITM; the resulting product was yellow in colour with sesame odor. Odor is important in pharmaceutical products regarding patients' acceptance; moreover, the density and viscosity and acid value of the oil was also acceptable (acid value acceptable range is 1-4 for herbal oils) [17]. High acid value is an indication of hydrolysis of triglycerides which shows unsuitable conditions during production or storage concerning temperature and humidity [23]. Hydrolysis of triglycerides results in unpleasant odor of the products. The acid value and the smell of the present amla oil could be the approval of its quality. Along with being a parameter for quality control evaluation, the saponification value is also of importance regarding industrial application of the oil. High saponification value demonstrates the presence of medium to long chain fatty acid triglycerides [24-26]. The saponification value of the present amla oil suggested that the oil was not saponifiable. The oil contains poly phenolics and tannins. Poly phenols are known for diverse biological activities of amla and myrtle especially antioxidant activity which is the cause of the herbs usage as anti-aging agents [8,11]. Tannins are polyphenolic components as well which are usually extracted with polar solvents such as water but during oil preparation using traditional method which aqueous extract is boiled in oil, the polar components will be trapped in the oil, therefore the traditional oil will contain tannins as was found for amla oil. Regarding the astringent effect of tannins, it seems that the most effective compounds in the anti-hair loss effect of the amla oil are the tannins. In conclusion, the formulated oil presented suitable physicochemical and microbial quality and could be suggested for hair loss treatment after clinical trial are performed for the product.

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Author contributions

Homa Hajimehdipoor and Saeed Mohammadi-

Motamed designed and supervised the study; Nasim Nikmanesh performed the experiments; Homa Hajimehdipoor analyzed the data.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

ITM: Iranian traditional medicine; DHT: dihydro testosterone; WHO: world health organization