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Product development and comparison of physio-chemical parameters of traditional drug recipe mentioned in *Thalpathe pillium* for hair growth

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Abstract
Thalpathe pillium is the series of books, which rewrite the formulae in Ola leaves to protect ancient medicines for future generations. This series contains many remedies for various diseases including hair care and for hair growth. This recipe containing coconut milk (*Cocos nucifera*), *Ixora coccinea* and *Eclipta alba.* The objective of this study was to develop this formula as value added product which mentioned in *Thalpathe pillium* and compare with its freeze-dried form using basic chemical analysis. According to the results, pH values of both forms are nearly similar. The moisture content of the natural form is higher than the freeze dryer form. Total ash value, water soluble ash value and acid insoluble ash values are less in freeze dryer form than the formula. Both forms of test samples shown similar TLC profiles revealed that those samples contained similar chemical constituents that can induce biological effects. Had given same peeks. Average R*f* values are nearly similar. So present study concludes natural form of hair growth promoter and the freeze dryer form of hair growth promoter has mostly similar chemical constitute.

Keywords: Chemical analysis, freeze dried, hair growth, *Thalpate Pillium*

1. Introduction
Sri Lanka has its own indigenous system of medicine. This system has been practiced for many centuries in the island nation. The Sri Lankan traditional medical system (*Deshiya Chikitsa*) is a combination of the Traditional medicine, Ayurveda, Siddha and Unani medicine. These systems use mainly plant base herbal preparations to treat diseases. Same time traditional systems of medicine have a vast literature, mainly in the form of manuscripts. The ancient physicians like king Buddhadasa hand over their knowledge of medicine generation to generation over a period of 3000 years and these generations used Ola leaves to write their knowledge to preserve it. Ola leaf is a palm leaf used for writing traditional manuscripts and in fortunetelling (horoscopes) in Southern India and Sri Lanka. The leaves are from the talipot tree, a type of palm, and fortunes are written on them and read by fortune tellers [1]. *Thalpathe pillium* is the series of books, which rewrite the formulae in Ola leaves to protect ancient medicines for future generations. This series contains many natural remedies for many diseases, including hair care remedies. Among all these remedies one specific formula is mentioned in *Thalpathe pillium*-01 for use only for hair growing. Itcontains fruit pulp of *Cocos nucifera* name as coconut milk, Flowers of *Ixora coccinea (Sinhalese name: Rathmal)*, Whole plant of *Eclipta alba (Sinhalese name: Kikirindiya)* as main ingredients [2].
Decreasing natural hair growth is a more prominent problem today among the people. Poor nutrition, chemicals using for hair coloring, and hair remodeling, using heavy chemical contain shampoo and conditioners, stress, some medicines cause to enhance this dilemma. Natural remedies can be used to prevent these critical conditions. But the problem is to difficulty in collecting those natural herbs and preparing this formula is not that easy with today's busy lifestyle in the society. Hence product development using natural remedies is more practical to this competitive lifestyle. Therefore, this study was conducted todevelop hair growth promoter, to develop its powder as value added productusing the freeze drying method, and to compare basic physiochemical parameters of prepared drug and the developed freeze dried product.

2. Materials and methods
2.1 Authentication of plants
The plant specimens for the proposed study *Eclipta alba* and coconut were collected from paddy fields and other cultivated fields around Gampola, in Kandy district, Sri Lanka in month

April 2018. *Ixora coccinea* was collected from home garden in Galgamuwa. Kurunagala district, Sri Lanka in month of April, 2018. The herbarium of these plants were identified and authenticated by National herbarium, Peradeniya, Kandy, Sri Lanka (6/01/H/03).

2.2 Preparation of drug

Whole plant of *Eclipta Alba* and flowers of *Ixora coccinea* were washed with tap water to remove unwanted foreign materials like soil and dust. After, washed the plant materials were shade dried at room temperature for 4 days and then coarsely powdered by using a high-pressure grinder (Sumeet/ serial no: 101). The powdered plant materials were sieved using a mesh (no 40) and stored in an airtight container separately. For making coconut milk, add 1 part of water to the 2 parts of grinded coconut and squeeze the coconut milk from it. Then took 30g of each powdered drug and added 30 ml of coconut milk separately and mix them well. Then kept these mixtures under the room temperature for 7 days (Figure 1 and Figure 2).

Fig 1: Appearance of the mixture Day1

Fig 2: Appearance of the mixture Day 7

2.3 Preparation of freeze dried form of the herbal product

After 7 days the settled mixture of the formula was freeze dried using the freeze dryer (Labconco 130644529J) and convert it at a -30^0 C temperature under the stranded vacuum pressure for 48 hours obtained the powdered form of the product (Figure 3).

Fig 3: Freeze dried powdered sample of the product

2.4 Determination of physiochemical parameters

Physiochemical parameters are very important in determine the quality of herbal drug preparations [3].

2.4.1 Determination of pH value

The pH value is taken to signify the alkalinity or acidity of an aqueous solution. It is defined as logarithm of the reciprocal of hydrogen in concentration of the known solution. The pH of particular concentration of an aqueous solution of the sample is often used as one of the parameters. The prepared drug (1g) of the sample and freeze-dried powdered sample were weighted separately and mixed each drug sample in 5ml of distilled water. These solutions were kept aside for a period of 2 hours with shaking it intermittently. Then these solutions were filtered and the pH values was noted with the help of the digital pH meter.

2.4.2 Determination of loss of drying

Loss of drying was determined by taking 3g of accurately weighed each sample, in a dried petridish (Tarred evaporating dish) and drying them an oven at 105^0 C till obtained a constant weight. The percentages were calculated on the basis of air dried samples.

2.4.3 Determination of total ash

Accurately weighed 2g of two samples were incinerated in a crucible at a temperature 500-600^0 C in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of ash values were calculated with reference to the air dried samples [4].

2.4.4 Determination of water soluble ash

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450^0C in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water soluble ash. The percentage of water soluble ash were calculated with reference to the air dried powdered samples separately [6].

2.4.5 Thin Layer Chromatography

Measured 1g of both samples and add 10 ml of dichloromethane in separate beakers and kept both beakers on the shaker in 15 min and shake well and filtered. The obtained extracts were further concentrated and were loaded on a glass column (20x1 cm) using silica gel (200-400#) as stationary phase.

The fractions were collected by gradient elution using toluene and chloroform as mobile phase. Fractions (each of 1ml) were collected and monitored simultaneously on a TLC plate using silica gel as a stationary phase and toluene: chloroform (3:1) as mobile phase [7].

3. Results

3.1. Physiochemical parameters

Determination of basic physiochemical parameters of prepared drug and its freeze dried powder is given in Table 1. The pH values of both samples were almost same (5.36 and 5.37). Moisture content was more in aqueous form than freeze dried sample (5.333% and 5.2%). Total ash value was higher in aqueous form (0.75%) than the freeze dried sample (0.7%). Acid insoluble ash and water soluble ash vales were also higher (0.933%, 0.7%) than the freeze dried sample (0.5%, 0.1%).

Table 1: Basic physical chemical parameters of aqueous form and freeze dried form

Parameter	Aqueous form	Freeze dried form
pH value	5.36	5.37
Moisture content	5.533%w/w	5.200%w/w
Total ash	0.75%w/w	0.7%w/w
Acid insoluble ash	0.933%w/w	0.5%w/w
Water soluble ash	0.7%w/w	0.1%w/w

3.2 Thin Layer Chromatography

Identification of chemical markers in aqueous form for the hair growth formula and its freeze dried form using Thin Layer Chromatography (TLC) were given below Fig. 1and Fig. 2. The average R*f* values were 0.48 and 0.49 respectively.

Fig 4: Thin Layer Chromatogram of aqueous form

Fig 5: Thin Layer Chromatogramoffreeze dried form

4. Discussion

The present study compared the aqueous form of hair growth promoter and the powdered form made using the freeze drying method. For the comparison: pH value, total Ash value, acid insoluble ash value, water soluble ash value, moisture content, TLC were determined.

Ash values are helpful in determining the quality and purity of the crude drugs in powder form. The ash or residue yielded by an organic chemical compound is as a rule, tomeasure of the amount of inorganic matters present as an impurity. Acid insoluble ash value and water soluble ash values are also more important to compare the purity of both forms of powders. The ash value aqueous form is 0.75%. Ash value of freeze dried form is 0.70%. An ash value of freeze dried form is less than the aqueous form.

Determination of pH is defined as the reciprocal of the common algorithms of hydrogen ion activity, which is the product of hydrogen ion concentration and the activity coefficient. Conventionally it is used as a scale of hydrogen ion concentration of a sample solution. The pH value of aqueous form was 5.36, and the pH value of freeze dried form is 5.37 this results revealed that both forms have nearly similar pH values.

Moisture content is one of the most commonly measured properties of samples. It is important for labelling and legal requirements, economic purposes, microbial stability, etc. there are legal limits to the maximum or minimum amount of water that must be present in certain types of sample. And also the propensity of microorganisms to grow in a sample depends on their water content. For this reason, many samples are dried below some critical moisture content. Mostly the texture, appearance and stability of samples depend on the amount of water it contains. The Moisture content of the prepared drug was 5.533% and moisture content of freeze dried form was 5.200%. So, the moisture content of the aqueous form is little higher than freeze dried form.

Thin-layer chromatography (TLC) is a chromatographic technique used to separate non-volatile mixtures. It can be used to monitor the progress of a reaction, identify the compounds present in a given mixture, and determine the purity of a substance. This is a good method for identifying the plant material and its phyto-chemical constitutes which responsible for induce biological activities. For more quantitative analysis High Performance Thin-layer chromatography (HPTLC) can be used. That is also most important for identification of plant materials. Thin-layer chromatographic studies given eluted 5 spots, so this can be considered as a reference for the quality of the preparations. Average R*f* value of aqueous form was 0.48 and average R*f* value of freeze dried form was o.49. So, both R*f* values are nearly similar.

5. Conclusion

The present study concludes that aaqueous form for hair growth promoter and freeze dried form of hair growth promoter has the same chemical constitutes. Moisture content of freeze dryer form is less than aqueous form. Because of that the freeze dried form has more stability than the aqueous form. Ash values also less in developed product than aqueous form. Therefore, it indicates freeze dried form is more pure than aqueous form. Consequently, this study will be help to develop a novel drug product for easy application without modify its chemical composition.

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