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Orchid callus extract: a sustainable and natural active to promote hair growth

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ABSTRACT

Background: Tissue culture is an important tool that can help to overcome many of the limitations associated with traditional plant cultivation methods. By adopting tissue culture techniques, it is possible to produce high-quality plant based raw materials in a more sustainable and environment friendly way. By cultivating calluses of *Dendrobium spp.* under controlled conditions, it is possible to obtain a consistent and high-quality supply of bioactive compounds that can be harvested and purified. This approach may also reduce the need for traditional harvesting methods that can damage natural habitats and ecosystems. **Aims:** Evaluating the efficacy of orchid callus extract as a sustainable active that can be deployed in the personal care industry as a possible hair growth promoting ingredients **Methods:** Six different callus extracts were prepared and tested on *in vitro* hair growth assays using goat and human hair follicles. Selected extracts were further validated on the proliferation profile of human hair follicle stem cells. **Results:** This study provides evidence that orchid callus extracts have a positive impact on stimulating the growth of hair follicles and improving the proliferation capacity of hair follicle stem cells. **Conclusion:** The findings of this study suggest that orchid callus extracts may have potential applications in hair care formulations. The use of sustainable and natural sources of bioactive compounds, such as orchid callus extract, may offer a more environment friendly alternative to traditional ingredients and may help to meet the growing demand for natural and sustainable personal care products.

Keywords: Orchid callus extract, Hair growth, Sustainable active, Plant tissue culture.

INTRODUCTION

The personal care industry has faced increasing scrutiny in recent years regarding the origin and impact of its ingredients and manufacturing techniques. As consumers become more conscious of environmental and ethical concerns, there is a growing demand for cosmetics that are sustainable, environment friendly, and ethically sourced. Plant tissue culture, offers a sustainable solution that can support and drive the cosmetic industry towards more sustainable growth [1].

The availability of plant-based materials can be limited due to seasonal changes, weather conditions, storage capacity, and cultivation requirements, posing challenges for industries reliant upon them, such as cosmetics and pharmaceuticals. Plant tissue culture offers advantages over traditional methods of propagation, including faster growth rates, higher yields, consistent quality, and the ability to produce disease-free plants. Additionally, it enables the production of plant materials that are otherwise difficult or impossible to obtain through traditional means [2].

We identified Dendrobium spp. as a promising source of sustainable bioactives for the personal care industry, known for their reparative and protective properties in Asian cultures. Extracts from Orchidaceae plants, including Dendrobium spp., have shown potential cosmetic applications, such as moisturizing and anti-aging effects [3].

An alternative to culturing whole plant is the utilization of plant calluses, which require significantly less time to grow in comparison to full-sized plants. Research has demonstrated that extracts derived from plant calluses are equally potent, and in some cases, even more potent than those obtained from mature plants [4].

Plant tissue culture facilitates the cultivation of calluses under sterile and standardized conditions independent of season and other environmental restraints [5-7]. It is possible to initiate the plant callus from nearly any part of the plant, which is called an explant. From the cut surface of the explant a colorless mass of cells emerges which is called a callus. The callus is a mass of undifferentiated cells that is commonly produced in response to a wound reaction [8,9].

In this study, 6 different extracts were derived from orchid callus extracts. Goat hair follicles were initially used to shortlist these extracts, providing initial insights into their potential effectiveness. Subsequently, the shortlisted actives were tested on human hair follicles to obtain more relevant and reliable data. To further substantiate the hair growth-promoting activity of these ingredients, they were tested on human hair follicle stem cells (hFSC), which are responsible for regenerating new follicles with each cycle and re-epithelializing the epidermis during wound repair [10].

The study provides evidence of the positive impact of orchid callus extracts on stimulating hair follicle growth and improving the proliferation capacity of hair follicle stem cells. The article describes different extraction methodologies from callus and demonstrates the impact of these extracts on hair-promoting efficacy.

MATERIAL AND METHODS

Extraction of Orchid Callus

Orchid callus (leaf origin) was purchased from Growmore Biotech Limited, Hosur, Tamil Nadu, India. Plant calluses were initially ground in a blender to make a fine paste. Thereafter 0.3% (w/w) of sodium benzoate (preservative, Sigma Cat No. V800367) and 0.2% (w/w) of L-ascorbic acid (anti-oxidant, Sigma Cat No. A4403) were added to the plant callus paste. This paste was then taken forward for further processing:

1. Dry Preparation *via* Lyophilization,
2. Liquid Preparation *via* Aqueous Formulation
3. Solvent Extractions

Dry Preparation *via* Lyophilization:

The paste was taken in freeze-drying flask and was frozen using liquid nitrogen and acetone mixture. While cooling, the flask was continuously rotated in order to make an equally distributed layer of the frozen paste on the wall of the flask. Once the paste was frozen the flask was connected to a lyophilizer for freeze drying. Next day (approx. 18 hours) the flask was disconnected and a freeze dried fluffy light greenish powder was obtained which was stored at -20 ºC in an air-tight container.

Liquid Preparation *via* Aqueous Formulation:

The following components were mixed and blended on a magnetic stirrer at 300 rpm for 12 hours. at room temperature

Stem cell (callus) paste: 9%

Soy lecithin (lecilite, food grade): 0.14%

Glycerin (Sigma Cat No. G2289): 0.4%;

Phenoxyethanol (Sigma Cat No. 77699): 1.4%;

Xanthan Gum (Sigma Cat No. G1253): 0.5%

Deionized Water: 88%

The turbid aqueous liquid so obtained was stored at -20 ºC in an air-tight container for bio-evaluation.

Solvent Extracts:

Successive extraction with Dichloromethane (C) (s d fine-chem CAS No. 7509-2) followed by Methanol (D) (s d fine-chem CAS No. 67-56-1): 50g of paste was taken into a beaker and 250ml of Dichloromethane (DCM) was added gradually. The mixture was stirred continuously for a minimum of 12 hours. Thereafter, the solution was filtered using Whatman filter paper. The filtrate was evaporated under reduced pressure to yield the DCM extract. The residue of the first filtrate was further extracted with 250 ml of methanol in similar fashion as described above to get successive methanolic extract of the callus.

Successive extraction with Methanol (E) followed by Dichloromethane (F):

50 g of paste was taken into a beaker and 250 ml of methanol was added gradually. The mixture was stirred continuously for a minimum of 12 hours. Thereafter, the solution was filtered using Whatman filter paper. The filtrate was evaporated under reduced pressure to yield the methanolic extract. The residue of the first filtrate was further extracted with 250 ml of DCM in similar fashion as described above to get successive DCM extract of the callus.

All these six extracts were dissolved in DMSO (Sigma, Cat No. D8418) and taken forward for *in vitro* assays.

In vitro hair growth assay (goat/human)

Goat skin was obtained from a butcher shop with prior signed consent, ensuring that the goats were sacrificed solely for culinary purposes and not for experimental study. Tissue was cut into smaller pieces using scalpel and washed vigorously in 2x antibiotic solution (Gibco, Cat. No. 15240). Thereafter, the tissue chunks were digested using 3% collagenase (sigma, Cat. No.- C2674) for 3 hours at 37 ºC in a humidified incubator. Following digestion, hair was plucked using forceps by pulling it in the direction of their growth and stored in William's E medium (Invitrogen, CA, USA). The follicles were trimmed close to the root, rinsed in PBS and used for culturing. In case of human hair follilces after securing the due consent from healthy donors, human hair follicles were plucked from the scalp using a standardized protocol by a dermatologist at MSCR Pvt. Ltd., Bangalore. Hair follicles were collected and maintained in William's E medium (Invitrogen, CA, USA) till use. Following PBS (Sigma) wash, the hair follicles were trimmed to about 1cm from the bulb using scissors without harming the root. Hair follicles with an intact bulb and root sheath (RS) were selected for this study. The hair follilces were inoculated singly in wells of 24-well plates with William's E medium. For each treatment a set of 6-10 hair follicles (for statistical significance) were taken. For testing the effect of callus extracts on hair growth, the various extracts were added in the William's E medium without supplements (Veh. Ctr) at 10 µg/ml and medium was changed every alternate day. Extracts that showed promising results on goat hair follicles were solely tested on human hair follicles. At the start of the experiment (day 0) and end of the experiment (day 6) pictures of the hair follicles were taken under a light microscope (Leica DMI 4000B) using a 5x objective. The length of the hair follicles was subsequently measured using the Leica Q Win Image processing and analysis software V.3.5.0. The growth of each hair follicle was measured by subtracting the length on day six from the length on day zero. The difference in length for the treatment set is averaged and taken as growth (in micrometers). Average and standard errors were calculated.

Cell culture

Human hair follicle stem cells (hHFSC) were purchased from Celprogen (San Pedro CA, USA) and expanded in human hair follicle stem cell expansion medium supplemented with serum and antibiotics (Celprogen Cat No. M36007-08ES) in a standard cell culture incubator. Cells were cultured in ECM pre-coated tissue culture flasks. Cell stocks were frozen at P6 in liquid Nitrogen.

WST-I assay

A WST-I assay was conducted to assess the metabolic activity of human hair follicle stem cells (hHFSC) exposed to varying concentrations of orchid callus extracts over different time intervals

(24 hours, 48 hours and 72 hours). hHFSC were seeded in triplicates in 96-well plates at a density of 3000 cells/cm^2 in human hair follicle stem cell expansion medium containing serum (Celprogen Cat No. M36007-08E). Following overnight incubation, the cells were treated with the respective concentrations of callus extract in serum-free medium and incubated for 24 hours, 48 hours, and 72 hours. Subsequently, the WST-1 reagent (Clontech, Cat. No. 630118) was added to the cells in serum-free medium at a 1:10 ratio and incubated for 3 hours in a cell culture incubator. Absorbance measurement were then taken at 460 nm using a plate reader to evaluate the metabolic activity of the cells.

HPLC analysis of orchid callus extracts

Chromatography For Liquid paste and solvent extracts:

Preparation of Mobile phase:

Mobile phase- A: 0.2% Trifluoroacetic acid in water

Mobile phase- B: Methanol

Preparation of Diluent: Mix Methanol and Dichloromethane in the ratio of 50:50 v/v

Chromatographic conditions:

Column : Lichrospher RP-e (18), 250*4.6 mm, 5 µm

Flow : 1.0 mL/min

Pump mode : Gradient

Injection Volume : 20 µL

Detector : DAD

Column Temperature : 40 ºC

Run time : 55 mins

Elution Type : Gradient

Preparation of Sample solutions:

Add around 6 mL of diluent to 100 mg of extract sample pre-weighed in a 10.0 mL volumetric flask. This was dissolved by sonication and made up to 10.0 mL with diluent. This solution was filtered through 0.45 µ membrane filter and 20 µL of solution so obtained was injected in to HPLC and the chromatogram was recorded (Fig. 5 -9).

Statistical analysis

Data are expressed as mean standard deviation. Statistical analysis was performed using the unpaired Student's t-test. The results were taken to be significant at a probability level of $p<0.05$. For all experiments, a minimum of two independent experimental runs were performed**.**

RESULTS

Stimulatory effects of orchid callus extract on hair follicle growth

In this study, six different types of orchid callus extracts (A-F) were evaluated for their potential to promote hair growth using an *in vitro* goat-hair model. These extracts included lyophilized (A), aqueous (B), dichloromethane (C), successive methanol (D), methanol (E), and successive dichloromethane (F) extracts. The goat hair follicles were cultured in Williams E medium without supplements in the presence of each extract at a concentration of 10 µg/ml for a duration of 6 days. The length of the hair follicles was measured at days 0 and 6, and the difference in length was used to assess hair growth. The results revealed that extracts C, D, and F demonstrated 1.7-fold, 2.4-fold, and 1.9-fold higher hair growth, respectively, compared to the control (refer Fig. 2). Conversely, extracts A and B exhibited only marginal improvements in growth, while extract E led to a decrease in hair growth to 0.9-fold compared to the control. Additionally, the HPLC fingerprint analysis of extracts C, D, and F revealed a distinct peak at approximately 46.8 minute, likely associated with a non-polar molecule that may contribute to the observed hair growth promotion. Subsequently, extracts D and F were further validated using *an in vitro* hair growth assay with human hair follicles, where D and F have shown a 11- and 3.9-fold higher growth than the vehicle control respectively (see Fig. 3). These findings suggest that certain orchid callus extracts, particularly D and F, have the potential to significantly enhance hair growth, warranting further investigation into their specific bioactive components and mechanisms of action.

Stimulatory effects of orchid callus extract on human hair follicle stem cell proliferation

Following the *in vitro* hair growth assays, extracts D and F were examined for their effects on human hair follicle stem cell (hHFSC) proliferation. To conduct the study, hHFSC were seeded in 96-well plates and exposed to various orchid extracts in serum-free medium. Subsequently, the cells were cultured for 24, 48, and 72 hours, after which a WST-1 assay was performed to evaluate cell viability and proliferation. Extract D refers to the successive methanol orchid callus extract, while extract F denotes the successive dichloromethane orchid callus extract.

At a concentration of 10 µg/ml, extracts D and F did not significantly influence the proliferation of hHFSC compared to untreated cells. When hHFSC were cultured in medium without fetal bovine serum (FBS), no further cell growth was observed after 24 hours, with a slight decrease in absorbance likely due to the transition to serum-free medium. However, at higher concentrations of 50 and 100 µg/ml, both extracts D and F demonstrated enhanced cell proliferation compared to untreated cells (see Fig. 4). These findings align with the *in vitro* hair growth results obtained with goat hair follicles, where both extracts at a concentration of 10 µg/ml promoted hair growth compared to the vehicle control. Furthermore, these results were corroborated as extract D exhibited significant hair growth in the *in vitro* hair growth assay using human hair follicles compared to the vehicle control, while extract F showed directionally better hair growth.

Although the aqueous extract of orchid callus did not show improved hair growth potential over the vehicle control in the *in vitro* hair growth assay using goat hair follicles, it may still be worth exploring for potential skin benefits due to the absence of a solvent used in its production and its economic viability.

DISCUSSION

Plant stem cell extracts have become popular in the personal care industry due to their potential benefits for skin health and appearance. Using plant cell culture to produce calluses offers several advantages, as callus cells resemble plant stem cells found in the meristem region, possessing the ability to differentiate and give rise to different parts of the plant. Commercially available plant stem cell extracts are commonly derived from plant calluses, [11,12] known to produce a variety of bioactive compounds, including alkaloids, phenolics, flavonoids, terpenoids, and other specialized substances.[13-16].

Plant callus extracts can demonstrate varying levels of activity compared to extracts from the adult plant. For instance, Ravinder Singh observed increased antimicrobial inhibitory activities in callus extracts compared to those from adult plants.[17] This difference may be attributed to the undifferentiated nature of callus cells, which have the potential to produce a variety of secondary metabolites, contributing to their bioactivity.

Moreover, plant callus extract is increasingly utilized in hair care products due to its potential benefits for hair health and growth. For example, the 3HC hair stimulation complex derived from grape callus extract has been reported to improve hair follicle lifespan and reduce hair loss.[18]

The production of plant cell cultures in controlled environments reduces the need for pesticides, herbicides, and other agrochemicals used in traditional agriculture, minimizing the release of harmful substances into the environment and reducing the overall ecological footprint of the manufacturing process. Additionally, the use of plant cell cultures enables independent production regardless of location and season, ensuring a reliable and continuous supply of products for the market. Growing plant cell cultures in tissue culture labs also requires less energy compared to cultivating and maintaining whole plants in large agricultural fields.[12]

Previously, orchid callus extracts have been linked to promoting skin rejuvenation, soothing the skin, and providing moisturization. However, there is no existing evidence of its benefits for hair care. The Dendrobium spp. used in this study is a hybrid variety of Dendrobium, combining beauty and fragrance through its parentage of Compactum and Halawa beauty orchids.[19,20]

In this study, orchid callus extracts have been demonstrated to enhance the growth of goat and human hair follicles *in vitro*. This reliable test showcases the ability of these extracts to promote hair growth. Furthermore, the extracts have been found to increase the cellular proliferation of human hair follicle stem cells, which play a critical role in hair growth. Thus, by stimulating these hair follicle stem cells, it may be possible to induce hair growth.[21] Considering these *in vitro* assays collectively, it becomes evident that orchid extracts have the potential to improve hair growth.

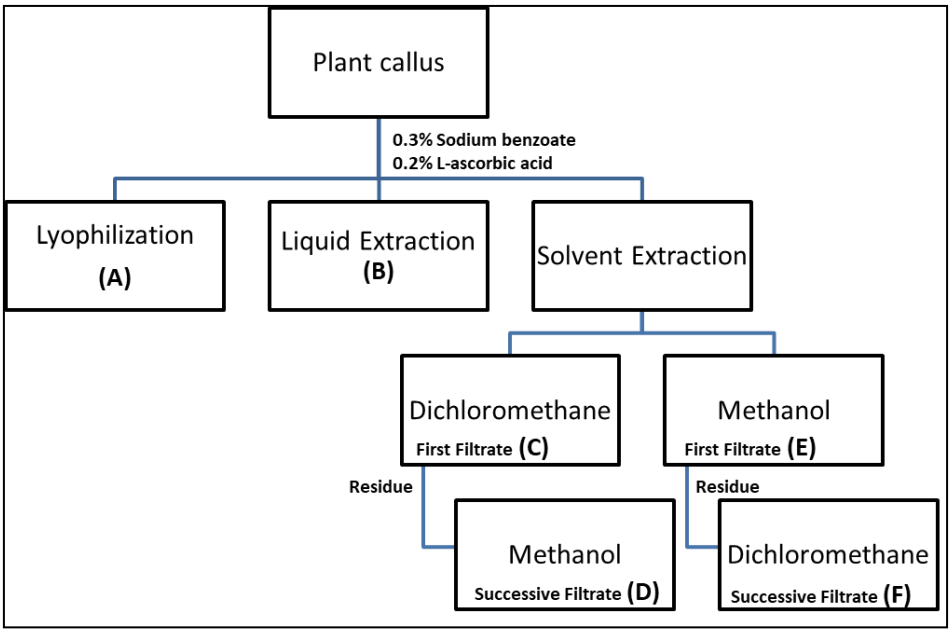

Figure 1: Schematic representation of plant callus extraction

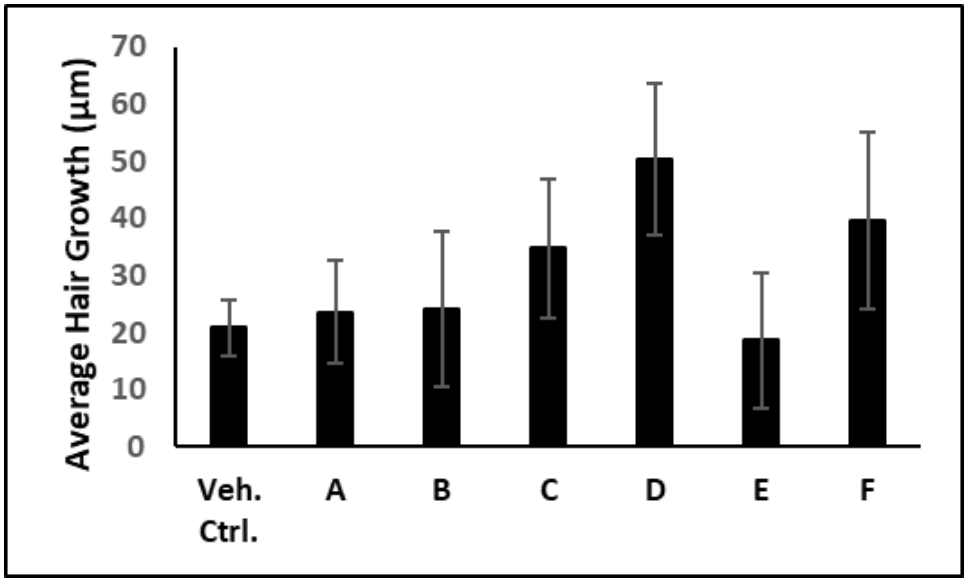

Figure 2: *In vitro* hair growth assay – Goat: A-lyophilized orchid callus extract; B-Aqueous orchid callus extract; C-Dichloromethane orchid callus extract; D-Successive methanol orchid callus extract; E-Methanol orchid callus extract; F-Successive dichloromethane orchid callus extract

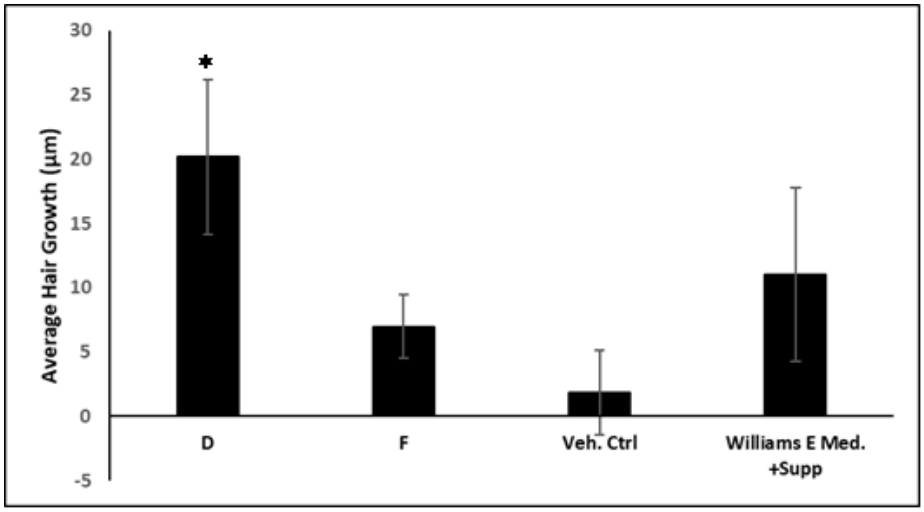

Figure 3: *In vitro* hair growth assay – human, * significant p Value=0.045 D-Successive methanol orchid callus extract; F-Successive dichloromethane orchid callus extract

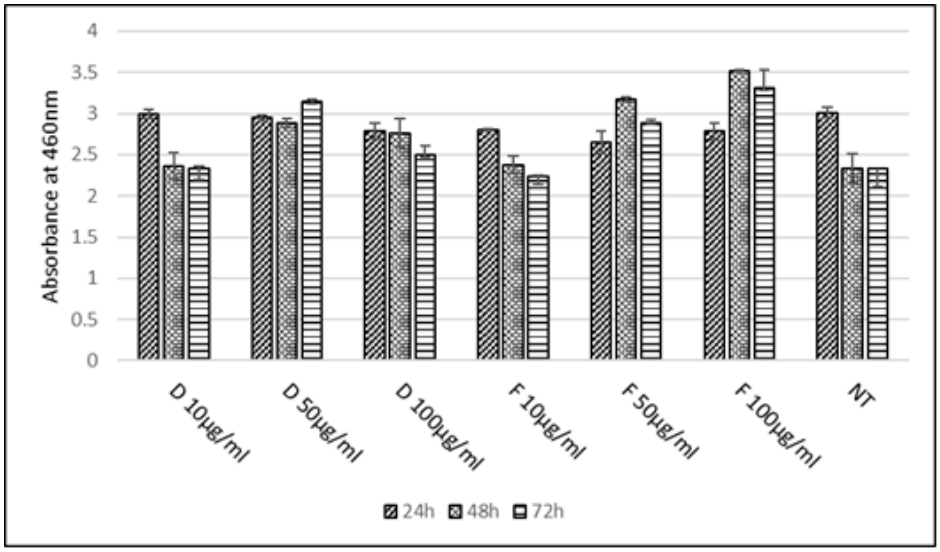

Figure 4: Proliferation assay of human hair follicle stem cells (hHFSC) treated with orchid extract. D-Successive methanol orchid callus extract; F-Successive dichloromethane orchid callus extract

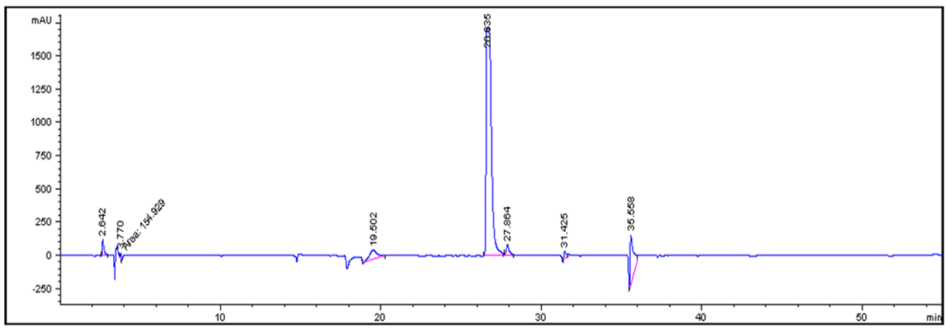

Figure 5: Orchid Plant Callus- Liquid Extract (B)

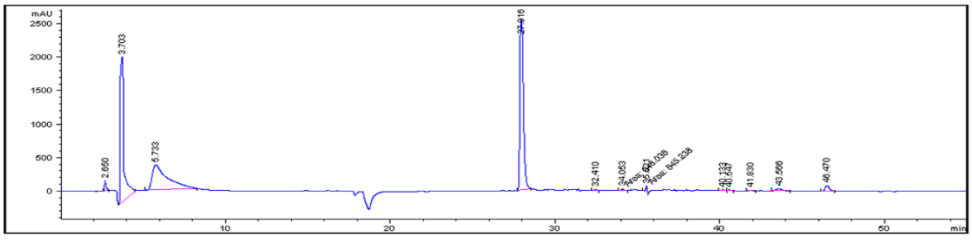

Figure 6: Orchid Plant Callus- DCM Extract (C)

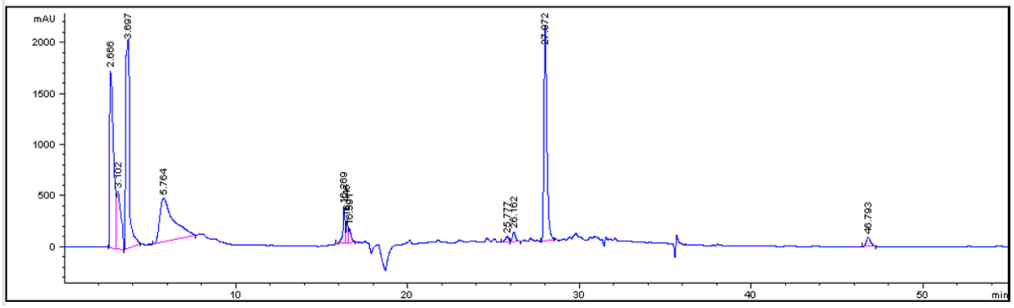

Figure 7: Orchid Plant Callus- Successive Methanol Extract (D)

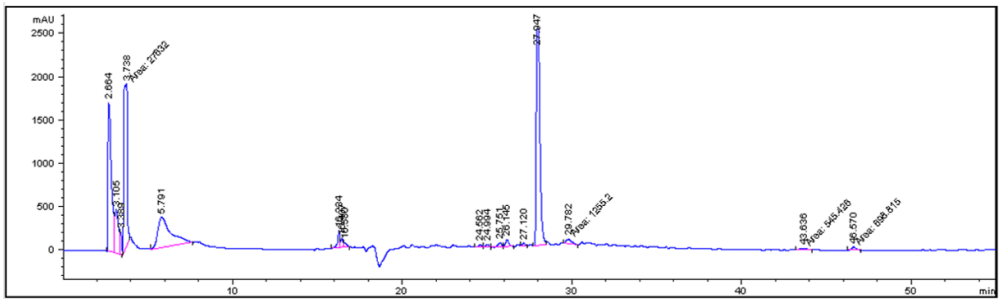

Figure 8: Orchid Plant Callus- Methanol Extract (E)

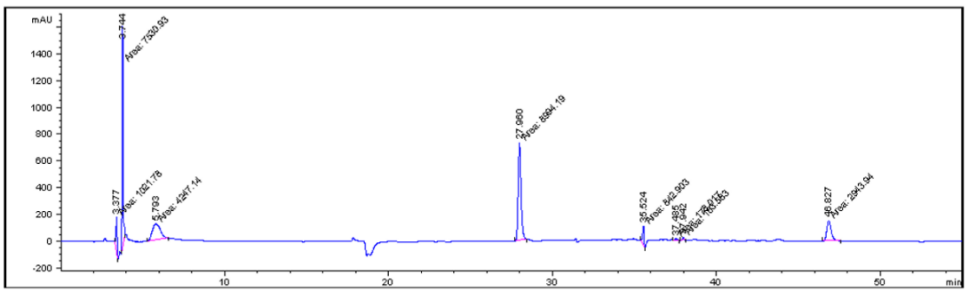

Figure 9: Orchid Plant Callus- Successive DCM Extract (F)

CONCLUSION

HPLC fingerprint analysis of extracts reveal that specific compound/s may be responsible for the observed improvement in hair growth. However, further research is warranted to identify and isolate these active compounds responsible for this effect and to explore their potential in promoting hair growth and elucidate the mechanism behind it. The study has demonstrated the *in vitro* activity of orchid callus extracts in promoting hair growth. However, a clinical trial is necessary to determine its efficacy in humans.

The study presents compelling evidence supporting the positive influence of orchid callus extracts on hair follicle growth and the proliferation capacity of hair follicle stem cells. These finding indicate the potential of orchid callus extract for use in hair care formulations. Furthermore, the utilization of sustainable and natural sources of bioactive compounds, exemplified by orchid callus extract, may offer an environmentally friendly substitute for traditional ingredients and address the increasing consumer preference for natural and sustainable personal care products.

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Conflict of interest

The authors declared no conflict of interest.

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