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Formulation and Development of *Tremella Fuciformis* Whitening Gel

Sammie Loh Pui Shan, Sasikala Chinnappan*, and Ashok Kumar Janakiraman

Faculty of Pharmaceutical Sciences, UCSI University, Kuala Lumpur, Malaysia

*Corresponding author: sasikala@ucsiuniversity.edu.my

Abstract

Cosmetics found in the market are frequently labelled as plant-based or 'chemical-free,' highlighting the absence of any potentially dangerous excipients such as paraben. However, most of them contain synthetic excipients in it, only the herbals were natural. As such, hydroquinone is used as a skin-lightening agent in most whitening products whereby it may cause undesirable side effects. Skin hyperpigmentation is a dermatological condition in which the skin's color darkens due to an increase in melanin production. In this innovation, the natural formulation of gel was developed and characterized for the treatment of hyperpigmentation. Natural excipients and preservatives were used to improve the stability of the innovation. *Tremella Fuciformis* (*TF*) is a species of fungus that is often used in food preparation in Chinese cuisine. *TF* has great skin-brightening properties and can be used to replace hydroquinone. The maceration extraction process was used to produce *TF* aqueous extract. The anti-tyrosinase activity of the aqueous extract was determined using the tyrosinase inhibition test and it showed 86.04% inhibition at 10 mg/ml concentration. Besides, the gel formulation showed maintained its integrity after 3 months of stability testing at various temperatures. Therefore, a novel polyherbal gel was successfully developed. The *TF* extract showed great anti-tyrosinase activity. It can be concluded that T. fuciformis extract is a potential candidate for skin whitening formulations and warrants further studies.

Keywords: *Tremella Fuciformis*, Whitening gel, Hyperpigmentation, Tyrosinase inhibition

Introduction

Skin hyperpigmentation is a dermatological disorder where skin darkens due to excess color melanin production (1,2). Melanin is a pigment that is formed by melanogenesis, and it is accountable for skin pigmentation. Mechanisms involved in skin pigmentation include melanocyte homeostasis, microphthalmia-associated transcription factor (MITF-M)-mediated control of melanogenesis, melanin synthesis by tyrosinase and other melanogenic enzymes, and melanosome transfer (3). Three enzymes namely, tyrosinase, tyrosinase-related protein TRP-1 and TRP-2, directly regulate melanogenesis process. (4) Tyrosinase is a crucial enzyme in the melanogenesis process. Inhibitors of melanogenesis that selectively block tyrosinase catalytic activity use it as their most popular and effective target. It catalyzes the transformation of L-tyrosine into L-DOPA and then dopachrome, which undergoes a series of processes before spontaneously polymerizing into melanin (4,5). Therefore, one of the driving factors of research into the management of hyperpigmentation is the regulation of melanin formation by suppressing the tyrosinase enzyme. Numerous tyrosinase inhibitors, including hydroquinone and arbutin, have been utilized as skin-whitening treatments in the market yet with certain downsides (5). The trend of natural skin care or cosmetics is rapidly evolving in this era of globalization. The fact that natural cosmetics, also known as herbal cosmetics, are composed entirely of herbs is the best reason to use it (6,7). *Tremella fuciformis* is a member of the Tremellaces order and Tremellacea family (8,9). It contains a plethora of bioactive substances, such as fatty acids, proteins,

enzymes, polysaccharides, phenols, flavonoids, dietary fiber, and trace elements. *T. fuciformis* polysaccharide (TFPS) has already been recognized as a key bioactive ingredient that has a variety of physiological and health-promoting properties like immune function augmentation, anticancer, anti-oxidation, and anti-aging, and several others (10). Maceration was employed for *TF* extraction due to its effectiveness in extracting phenolic compounds. The choice of water as the solvent was influenced by its ability to effectively solubilize a wide range of phenolic compounds without the need for more complex solvents (11).

Material and Methods

Chemicals and Reagents

Kojic acid (Sigma-Aldrich, USA), 3-(3,4-Dihydroxyphenyl)-L-alanine 99% (Thermo Fisher Scientific, Waltham, Massachusetts, USA), Folin-Ciocalteu's phenol reagent (Merck, Darmstadt, Germany), Gallic acid (Sigma-Aldrich, USA), Tyrosinase mushroom (Sigma-Aldrich, USA), Phosphate buffer tablets pH 6.8 (Merck, Darmstadt, Germany).

Preparation of Extracts from Dried Tremella fuciformis

The dried *Tremella fuciformis* was bought from Herbal Farmer Sdn. Bhd, Malaysia. The powdered form of *TF* extract was soaked in purified water with a v/w ratio of 20:1. It was kept at room temperature and continuously swirled for four days. The solid-liquid mixture was filtered using suction filtration to obtain the filtrate. The filtrate obtained was transferred into several petri dishes and allowed to dry in the oven which was maintained for seven days. The extracts were then obtained by scraping petri dishes after the drying process. The weight of the extracts was measured and recorded (12).

Preparation of gel

1.5 g of xanthan gum was measured and dispersed in 30 ml of distilled water in a beaker and was kept aside to swell for 30 minutes. After that, mechanical stirring was carried out at 1200 rpm for 30 minutes. Later, 10 ml of vegetable glycerin was mixed with 0.5 ml of Nipaguard SCE preservative and stirred well. Xanthan gum mixture was mixed well with vegetable glycerin-preservative mixture for 15 - 30 minutes. The mixture was homogenised for 5 minutes at 1000 rpm before being cooled to room temperature to form the gel.

Test for phenolic contents

Total phenolic contents (TPC) of *Tremella fuciformis* extracts were employed using Folin-Ciocalteu (FC) reagent by Singleton and Rossi with some modifications. Sample and standard were measured using a UV-Double Beam Spectrophotometer at 765 nm against reagent blank. 100 mg of Gallic acid was measured and dispersed in 10 ml of distilled water and stirred until the particles were dissolved completely and it was then made up to 100 ml stock solution. The gallic acid reference standard stock solution was prepared into 6 different concentrations using the serial dilution technique. The concentration was expressed in micrograms per milliliter with concentrations of (500, 250, 125, 62.5, 31.25, 15. 625). Each of the test tubes was mixed with 0.6 ml of purified water followed by 0.2 ml of FC reagent. Then, 1 ml of sodium carbonate solution was added into the mixture after 5 minutes and purified water was added to make the final volume of 3 ml. The mixture was incubated in the dark for 30 minutes to allow the reaction to take place. The mixture was centrifuged and the absorbances were measured at 765 nm using UV spectrophotometer (13).

Tyrosinase inhibition assay

Tyrosinase inhibition of total of 36 samples was carried done with some minor adjustments employing the dopachrome technique and L-DOPA as the substrate on the procedures. Distilled water was used to dissolve the samples. The tests were carried out on 96-well microplates with 80 µl of phosphate buffered solution (PBS, pH 6.8) followed by 40 µl of samples in each well. Then, 40 µl of mushroom tyrosinase (31 units/ml) and 40 µl of 2.5 mM L-DOPA were

added and incubated for 20 minutes at 37°C to allow the reaction to take place. After incubation, absorbance was determined at 475 nm and 700 nm as a reference. Each sample was accompanied by a blank that contained all the components excluding L-DOPA. Kojic acid was utilised as positive control (13). The results were expressed in percent inhibition (%) calculated as follows:

$$Percent\ Inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Homogeneity and appearance

The formulation was evaluated for homogeneity, visual appearance, and tactile contact once the gel was set in the container. The pearlescence, roughness, and colour were examined to assess the look. The homogeneity and texture of the gel formulation was tested by rubbing a tiny quantity between the thumb and index finger. The consistency of the formulation and the existence of coarse particles were used to assess the texture and homogeneity of the formulation. The immediate skin feels such as greasiness was also assessed.

Grittiness

A light microscope was used to inspect the formulation for the presence of any particles.

Determination of pH

After calibrating the pH meter with a standard buffer solution, 0.5 g of prepared herbal gel was collected and carefully blended with 50 ml of distilled water. The pH of the gel was then measured at room temperature using a pH meter.

Results and Discussion

Based on the results, it was observed that *TF* extract showed high total phenolic content which is 314.38 mg GAE/g *T. fuciformis* extract showed 86.04% percent inhibition of tyrosinase activity (Table 1). The result corresponded to that of the standard tyrosinase inhibitor, kojic acid. Kojic acid inhibits monophenolase activity and has a mixed inhibitory action on mushrooms. tyrosinase diphenolase function (14). This indicates that *TF* extract has good anti-tyrosinase properties which is a potential source for treatment of hyperpigmentation.

Table 1: Percent inhibition calculated for different concentrations of samples for tyrosinase inhibition assay.

Concentration (µg/ml)	Percent inhibition (%)
10000	86.04
5000	54.23
2500	34.32
1250	23.80
625	16.93

Table 2: Evaluation test for gel formulation.

Tests	Gel
Colour	Yellowish-brown colour 
Texture	Smooth
Phase Separation	Absent
Homogeneity	Excellent
Consistency	Excellent
Grittiness	Absent
pH	6.53

The physical appearance, colour, texture, phase separation, homogeneity, consistency and grittiness of the gel formulation were determined. It had a pleasing cosmetic appearance and a smooth texture, and was

homogeneous, with no signs of phase separation (Table 2). In accordance with the quality standards of the United States Pharmacopoeia (USP), any topical application should be devoid of dust and particulate matter. The physiological pH of a healthy adult's epidermis surface ranges between 4 and 6. This formulation's pH was close to that of the skin in order to sustain the skin's natural flora (15). Consequently, this formulation possessed outstanding physiochemical properties. Moreover, during the three-month stability testing at various temperatures including 2°C, 25°C, and 40°C, the formulation maintained exceptional homogeneity, a smooth texture, no grit, and no phase separation. Therefore, *T. fuciformis* extract is compatible with other ingredients and excipients as the final formulation was stable.

Conclusion

From this study, natural formulation of gel was successfully developed and characterized for the treatment of hyperpigmentation. It was observed that *TF* extract demonstrated high phenolic content and high percent inhibition of tyrosinase activity, 86.04%. Besides, natural ingredients and excipients were used to improve stability and acceptability. It can be concluded that *T. fuciformis* extract is a potential candidate for skin whitening formulations and warrants further studies. This formulation is a safer alternative to synthetic whitening formulations due to the usage of fully natural ingredients.

Conflict of Interest

The authors declare no conflict of interest.

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