

Formulation and Development of Poly-Herbal Anti-ageing Cream

Tan Jai Wei, Sasikala Chinnappan*, and Ashok Kumar Janakiraman

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

*Corresponding author: sasikala@ucsiuniversity.edu.my

Abstract

Skin ageing is a complicated biological process that is affected by both intrinsic and extrinsic factors. UV rays produce free radicals that can impair the collagen and elastin networks. Consequently, the cell regeneration process is impeded, resulting in hyperpigmentation and rough, wrinkled skin. As a result, the research advances in natural cosmetics that contribute to restoring a certain level of rejuvenation and delaying or reversing the ageing process are the focus of the study. The purpose of this research is to determine the percentage of radical scavenging activity of aqueous extract of *Tremella fuciformis* (TF) using the DPPH technique, as well as to develop a polyherbal anti-ageing cream containing TF, sea buckthorn, grape seed, rosehip seed, rose water, and natural excipients. The maceration technique was employed in the preparation of the TF extract. Hot water bath equipment was used to prepare oil-in-water emulsions by heating the aqueous phase and oil phase components separately at 75 °C. Subsequently, mixing both phases with the use of a homogenizer set at 1000 rpm for 5 minutes and reduced to 500 rpm until the emulsion cooled down to room temperature was necessary. Following that, the emulsion cream-based was levigated with TF extract to produce a smooth small amount of herbal cream, which was gradually incorporated with a large portion of the cream base until forming a homogenous polyherbal cream. At 2 mg/ml concentration, the TF extract demonstrated 80% radical scavenging activity. The polyherbal cream appeared light yellow with a smooth texture and was homogenous without phase separation. The formulation showed excellent stability across the 3 months of

testing in different temperatures and different packaging materials, including plastic and glass containers. From the above results, it is concluded that the formulation containing TF could be the best formulation to rejuvenate the skin and reduce age-related wrinkles.

Keywords: Anti-ageing, *Tremella Fuciformis*, DPPH test, Polyherbal, Facial cream

Introduction

Aging can be classified into 2 categories which are intrinsic and extrinsic factors. Intrinsic aging also called chronological aging characterised by fine lines, wrinkles, lax skin and flaky skin and it is a natural occurrence due to inherent genetics. Extrinsic aging is mainly attributed to external factors such as photoaging (UV radiation), exhaust, cigarette smoke and so on. It is characterised by dry skin, deep wrinkles, leathery appearance and hyperpigmentation (1). Making attempts to avoid the onset of ageing skin is an ancient phenomenon, and thus herbal compounds have played a crucial role in standard therapy regimens. Nowadays, herbs were integrated into daily use regimens all over the world to preserve skin health and to cure sickness or harm when it happens. Antioxidant properties are the essential portion of the therapeutic benefits offered by phytoconstituents of plant extracts. Herbals are also proven to have a range of naturally occurring antioxidants that preserve and maintain their structural and metabolic integrity, together with their genetic integrity from their seeds (2).

A variety of these phytoconstituents and metabolites are arising as prospective candidate for limiting the impact of ageing on skin by decreasing the biochemical implications of oxidation. The objective of this study aims to formulate and evaluate polyherbal antiaging cream with all-natural base ingredients and to

evaluate the antioxidant activity of *Tremella Fuciformis* by using the DPPH method. A large and growing body of literature have investigated the chemical composition of TF and numerous bioactive components have been indicated present in TF including proteins, phenols, flavonoids, trace element, polysaccharides and dietary fibre (3,4).

Material and Methods

Chemicals and Reagents

The chemicals Folin-Ceocalteu and Sodium acetate was obtained from MERCK KGaA (Darmstadt, Germany), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Gallic acid was obtained from Sigma Aldrich Co. (St Louis, Mo, USA), Methanol was obtained from J.T.Baker (Netherland), Sodium carbonate was obtained from GENE Chem (Westmount Sq, Montreal, Canada), Aluminium Chloride was obtained from Bendosen (Pasir Gudang, Johor, Malaysia). Purified water was obtained by using a Milli-Q system (Millipore). Rutin was bought from Liliana Herbs.

Preparation of Extracts

The preparation of extract from the dried powdered TF was carried out by maceration technique. 75 g of dried TF powders were immersed in 2 litres conical flask containing 1 litre of purified water at the ratio of 3:40 for at least 4 days at room temperature. The conical flask was frequently shaken from time to. After 4 days of maceration, the aqueous extracts were filtered by using the Buchner Funnel filtration set. The collected filtrate in the Buchner funnel was then poured into the petri dishes and a thin layer of liquid was formed. The petri dishes were then evaporated in the oven at a temperature of 40°C overnight and the dried extracts were stored in an airtight container for further experiments test.

Preparation of Cream

The oil-in-water emulsion-based polyherbal cream was prepared. Ingredients of herbal cream will be prepared into 2 parts which are the oil phase and the aqueous phase. The oil phase consisted of 5 g Cetearyl Oliviate, 2.5 g white beeswax, 0.5 g

shear butter, 2 ml of oil and 0.5 ml of Nipaguard; the Aqueous phase consisted of 5.4 g of glycerine and quantum states of rose water. The oil-soluble components and water-soluble components were heated to 75°C separately by hot water bath equipment (5). After heating, the aqueous phase was transferred into the oil phase little by little with continuous stirring manually. Subsequently, the mixer was transferred for homogenization at 1000 rpm for about 5 minutes and reduced to 500 rpm until the emulsion cooled down at room temperature. After this, the prepared emulsion cream-based was mixed with 1 g of TF extract by levigation method to produce a smooth small-scale herbal cream and gradually incorporated more cream base until forming a homogeneous herbal cream (6).

Determination of Total Flavonoid Content (TFC)

TFC of TF extracts was investigated from a calibration curve of Rutin. The procedures of TFC determination were modified according to Chandra S *et al.* (2014) (7). 100 mg of rutin powdered was prepared in 100 ml of methanol-water at the ratio of 1:1. The rutin reference standard stock solution was calibrated in 6 different concentration by serial dilution technique. The concentration unit is expressed in micrograms per millilitre with concentrations (500, 250, 125, 62.5, 31.5, and 15.625). Each of the test tubes was mixed with 3 ml methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1M sodium acetate, and 5 ml of purified water. All of the prepared test tubes were incubated for around 30 minutes at room temperature. The TF extract was prepared similarly to the preparation of rutin standard solution with an unknown concentration. The blank was prepared with ingredients of 3 ml methanol, 0.2 ml sodium acetate, 0.2 ml aluminium chloride and 5 ml purified water (7). Subsequently, the solutions were placed into the cuvette and determined with 415 nm targeted wavelength by using the UV spectrophotometer (8). The TFC was calculated as rutin equivalents RU/g of dried TF extracts based on the plotted calibration curve line of rutin. The equation used is $34x + 0.0023$, $R^2 = 0.9998$

Stability test

Each formulated herbal cream was divided and placed into 3 plastic containers. Each set of herbal cream was placed at 3 different temperatures for a stability test of at least 3 months will be observed. The experimented temperatures are 2-8°C (Fridge), 40°C (Oven) and room temperature.

Determination Antioxidant Activity

200 µM of 2,2-Diphenyl-1-picrylhydrazyl DPPH stock solution was dissolved in 99.5% of ethanol to obtain a constant volume by adding 100 mL of a measuring flask. Meanwhile, 2000 mcg/ml of TF extract was prepared. Both solutions were incubated at 37°C in the darker environment for approximately 1 hour until the absorbance is stabilised. Subsequently, 1 ml of DDPH was added to the diluted TF sample. The absorbance of the test tube with DPPH Blank, TF-DPPH mixtures and gallic acid were measured by using the UV spectrophotometer at 517 nm. Gallic acid played a role as a positive control or standard substance. The percentage of DPPH radical scavenging activity was calculated as follows % Radical Scavenging Activity = $\frac{\text{Sample} - \text{blank}}{\text{Control} - \text{blank}} \times 100$ Gallic acid showed 83.39% radical scavenging activity at 1 mg/ml.

Homogeneity and Physical Appearance

The cream bases were inspected visually regarding its colour and homogeneity. The formulated herbal cream consisting of active ingredients were evaluated visually depending on their colour and homogeneity.

Grittiness

The cream bases were investigated by using a light microscope to observe the presence of any particles. The formulated herbal cream consisting of active ingredients were evaluated by using the light microscope to observe the presence of any particles.

Determination of pH Value

The pH meter will be used to test the pH value of the cream base and formulated herbal herbal cream. Before the test, the pH meter was calibrated by 3 standard buffer solutions with different pHs which are 4 pH, 7 pH, and 10 pH. Subsequently, 0.5 g of samples were gently mixed with 50 ml of distilled water and were determined at room temperature (9,10).

Results and Discussion

Based on the regression equation: $34x + 0.0023$ with a coefficient of 0.9998, exhibiting greatness of linearity, the flavonoid concentration of the TF extracts could be calculated which is 316 mg RU/g (Figure 1). The total flavonoid content of the TF fruiting body was drastically higher than reported

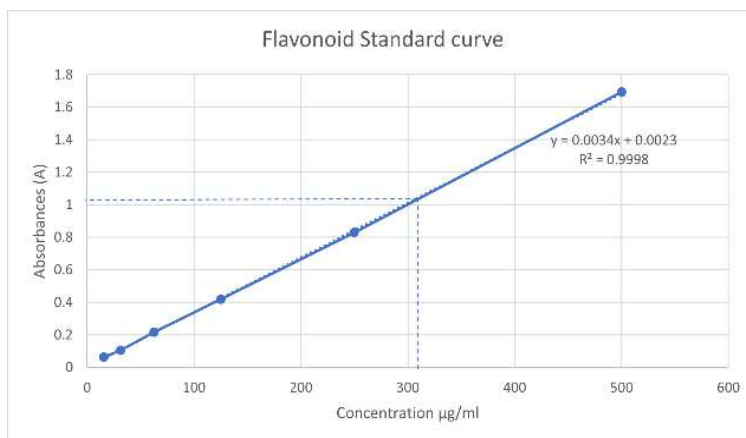


Figure 1: Total flavanoid content of TF
Poly-Herbal Anti-ageing Cream

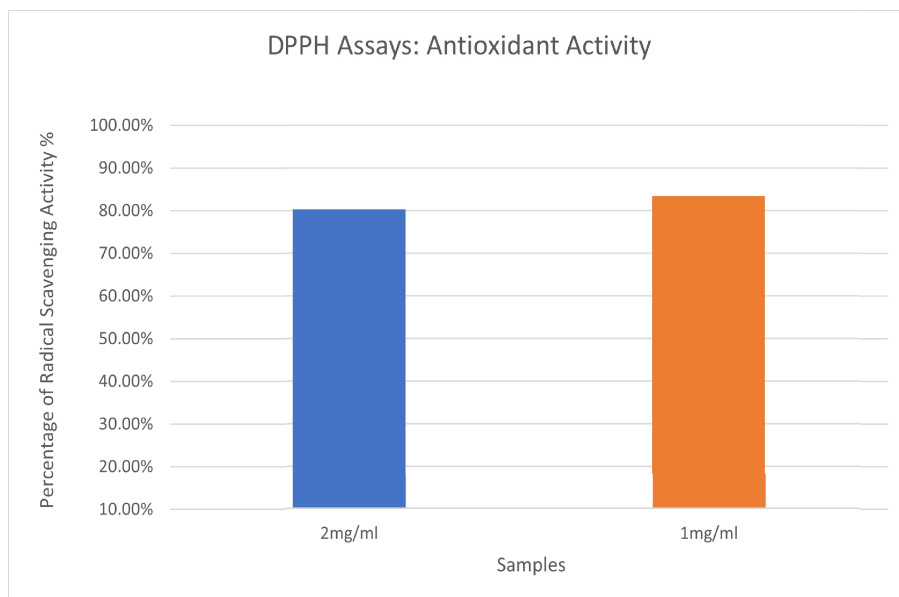


Figure 2: DPPH Assay

(11,12). The present findings confirm 2 mg/ml of TF extracts can produce 80.35% of radical scavenging activity (Figure 2). This could be due to the presence of phytochemical constituents within the TF extract.

Formulated cream possess good absorption properties, are non-gritty, have a great spreadability characteristic, leave no sticky layer, have optimised pH values for skin, are relatively homogenous, have a high acceptance rate of appearance, and leave a smooth protective barrier on the skin after application (Table 1). Another finding is the air bubbles entrapped in the polymer structures is an undesirable condition that could affect the quality of formulation, and physical and chemical stability of an emulsion, introduce oxidation and promote the growth of microorganisms in turn leading to short shelf life. The presence of gas bubbles is difficult to eliminate from a highly viscous solution such that xanthan gum when under buoyancy action alone. In such circumstances, employment of hot plate magnetic stirrer by setting the temperature to 60°C for degassing. Heating is one of the widely used

	F1	F2
		
Colour	Creamy White	Light Yellow Cream
Pourability	Non-Pourable	Non-Pourable
Opacity	+++++	+++++
Homogeneity	+	+
Stickiness	++++	++++
Absorption	++++	++++
Bubble	No	No
pH	6.49	6.40
Grittiness	No	No

thermal degassing methods and the desired outcomes were obtained (13).

A further laboratory test on formulation is the stability test. This is an important finding

to determine the total shelf-life of a product in a container under the circumstance to be marketed. Both formulations showed excellent stability across the 3 months test in different temperatures and different packaging materials including plastic and glass containers as well as stability in the light stability test (14). The physical test parameters involving the colour, odour, appearance, pH, weight and texture showed a constant result from day 1 to day 90. These findings highlight that little is known about the shelf life of the formulations is more than 6 months.

Conclusion

This study demonstrates the successful development of TF polyherbal cream that met the relevant pharmaceutical characteristics form. It also reveals the stable formulated topical cream with antioxidant properties as evidenced by DPPH result which have high economic value. This formulation could be the one of the best formulation to rejuvenate the skin and reduce age-related wrinkles.

References

1. Shin JW, Kwon SH, Choi JY, Na JI, Huh CH, Choi HR. Molecular Mechanisms of Dermal Aging and Antiaging Approaches. *Int J Mol Sci.* 2019;20(9):2126.
2. Ahmed IA, Mikail MA, Zamakshshari N, Abdullah ASH. Natural anti-aging skincare: role and potential. *Biogerontology.* 2020; 21(3):293–310.
3. Wen L, Gao Q, Ma C wah, Ge Y, You L, Liu RH, et al. Effect of polysaccharides from *Tremella fuciformis* on UV-induced photoaging. *J Funct Foods.* 2016; 20:400–10.
4. Yang D, Liu Y, Zhang L. Tremella polysaccharide: The molecular mechanisms of its drug action. *Prog Mol BiolTransl Sci.* 2019; 163:383–421.
5. Parashar Charat, Sharma Pankaj, Kabra Atul. Formulation and Evaluation Of Polyherbal Face Cream. *International Pharmaceutical Sciencia.* 2013; 3(3): 63-68.
6. Sawant SE, Tajane MD. Formulation and evaluation of herbal ointment containing Neem and Turmeric extract. *Journal of Scientific and Innovative Research.* 2016; 5(4):149–51.
7. Chandra S, Khan S, Avula B, Lata H, Yang MH, ElSohly MA, et al. Assessment of Total Phenolic and Flavonoid Content, Antioxidant Properties, and Yield of Aeroponically and Conventionally Grown Leafy Vegetables and Fruit Crops: A Comparative Study. *Evidence-Based Complementary and Alternative Medicine.* 2014;1–9.
8. Amalia Choironi N. Quality standardization of brotowali (*Tinosporacrispa*) stem extract. *Traditional Medicine Journal* 2016;21(1):6-11.
9. Ljaz N, Durrani AI, Rubab S, Bahadur S. Formulation and characterization of Aloe vera gel and tomato powder containing cream. *Acta Ecologica Sinica.* 2022;42(2):34–42
10. Kusmita L, Mutmainah NFN, Sabdono A, Trianto A, Radjasa OK, Pangestuti R. Characteristic Evaluation of Various Formulations of Anti-Aging Cream from Carotenoid Extract of Bacterial Symbiont *Virgibacillus salarius* Strain. *Cosmetics* 2021; 8(4):120
11. Ji Y wei, Rao G wei, Xie G fa. Ultrasound-assisted aqueous two-phase extraction of total flavonoids from *Tremella fuciformis* and antioxidant activity of extracted flavonoids. *Prep Biochem Biotechnol.* 2022;52(9):1060–8.
12. Li H, Lee HS, Kim SH, Moon B, Lee C. Antioxidant and anti-inflammatory activities of methanol extracts of *Tremella fuciformis* and its major phenolic acids. *J Food Sci.* 2014;79(4).
13. Zhao W, Liu Z, Sun Z, Zhang Q, Wei P, Mu X, et al. Superparamagnetic enhancement of thermoelectric performance. *Nature.* 2017;549(7671):247–51.
14. Cosmetics Europe: Guidelines on Stability Testing of Cosmetic Products. CTFA 2004.