

Antioxidant Potential of *Curcuma xanthorrhiza* -based Serum Stick

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ABSTRACT

*Solid skincare products are sustainable and convenient alternatives to traditional liquid or semi-solid formulations. Incorporation of natural active ingredients like *Curcuma xanthorrhiza* (*C. xanthorrhiza*) extract into solid skincare products can offer potential benefits for skin health. This study investigated the Soxhlet extraction of *C. xanthorrhiza* using ethanol solvents. The mean total yield was 8.16%. Then, the extract exhibited a promising IC₅₀ value of 20.86 ppm, indicating significant antioxidant activity. Total phenolic content (TPC) was 45.71 ± 0.36 mg GAE/g, and the total flavonoid content (TFC) was 30.06 ± 0.41 mg QE/g thus suggesting the presence of potentially beneficial bioactive compounds. This study was to formulate a novel serum stick formulation that incorporated *C. xanthorrhiza* extract. The sticks developed have maintained a slightly acidic pH of 5.018 which aligns with healthy skin's natural pH and remains stable. These findings showed that *C. xanthorrhiza* extract could potentially be developed as a solid skin care product. This research lays the groundwork for further development of *C. xanthorrhiza* extract-based topical products with potential antioxidant properties. Future studies could explore the efficacy and safety of the formulated serum stick in clinical trials.*

*Keywords: *Curcuma xanthorrhiza*; Antioxidant; Serum Stick; Skincare*



INTRODUCTION

Skincare is a method or procedure that supports skin integrity, improves the appearance of the skin, and treats skin disorders to maintain the skin's health. Regardless of the pollution, a good skin care regimen will keep the face looking clear and young. The market share of anti-aging products is growing year by year since women are especially worried about skin aging in an era marked by a considerable increase in life expectancy and the resulting growth of the older population. Skin aging has also become an increasingly notable issue of concern as Malaysia transitions into an older nation by 2030, with 15% of the total demography aged 60 and above [1]. In addition to the normal aging process, the skin is constantly exposed to environmental stress and injury, especially from solar ultraviolet (UV) radiation. In most cases, using cosmetics with antioxidant capabilities decreases the skin's aging process. The antioxidants may be coming from synthetic or natural sources. Antioxidants can protect skin health and delay the onset of skin aging. However, the chemicals and synthetic ingredients can contribute to several undesired effects, especially to those with allergic reactions to sensitive skin. The use of natural ingredients in cosmetic products has gained popularity due to increased consumer awareness and demand for eco-friendly and sustainable products. Many different plants, fruits, and vegetables contain natural antioxidants that benefit human skin. *Curcuma xanthorrhiza* (*C. xanthorrhiza*) can be used as it has been known to be effective as a natural product since years ago. It is a potential medicinal plant belonging to the family Zingiberacea. *Curcumin* and *xanthorrhizol* are two of the many active ingredients in *Curcuma xanthorrhiza* (*C. xanthorrhiza*) which are primarily responsible for its positive impacts on the skin [2]. It is a promising element for skincare products because of these chemicals.

The term "skincare" refers to the process of taking care of the skin, which is the largest organ in the human body. Skincare products often contain specific ingredients that are known to have beneficial effects on the skin. The current generation of people is more concerned with their health than ever before. Clean beauty is becoming more and more popular as people look for products without potentially dangerous components. It leads to skincare products made from natural sources. Among all the sources of natural products, plants play a vital role [3]. Keeping one's appearance young can have a positive influence on one's confidence and sense of self.

Younger appearance can be seen in more positive ways in many different settings. The demand for products that promise to maintain or enhance a youthful appearance is reflected in the skincare and cosmetics industries' heavy marketing of such products. The term "skin aging" refers to the slow alterations in the structure and appearance of the skin over time. Skin aging can be designated into categories as intrinsic or extrinsic based on the epidemiological elements influencing the aging process of the skin [4]. Age-related skin changes are varied and impacted by both internal and external variables. Extrinsic aging is impacted by external influences, whereas intrinsic aging is linked to chronological and genetic factors.

Curcuma xanthorrhiza (*C. xanthorrhiza*), also referred to as temulawak or Javanese turmeric, is a plant under the Zingiberaceae family of ginger. Originating from Indonesia, the plant is extensively grown throughout the Malaysian peninsula. This plant (Figure 1) is well-known for its rhizomes, which are underground stems with a variety of uses, including medicinal and culinary like other *Curcuma* genus members. Most of the time, *C. xanthorrhiza roxb* is a medicinal plant that has been traditionally employed for healing a variety of illnesses. This herbal plant is used traditionally as a component in health supplements as it is thought to possess antibacterial, antioxidant, and anti-inflammatory qualities. Prior studies have indicated that the primary benefits of *C. xanthorrhiza* for the skin are attributed to active substances such as *xanthorrhizol* and *curcumin*. The primary feature of an antioxidant is its competence to capture and eliminate free radicals. Xanthorrhizol have its anti-oxidative stress properties to fight the aging process of the skin [5]. It works to counteract free radicals, lessening the oxidative damage that the skin sustains. Xanthorrhizol exhibited photo-protective properties against UV radiation in human skin fibroblasts. The connective tissue cell known as skin fibroblasts oversees extracellular matrix maintenance and protein synthesis. Skin suppleness and support are attributed to proteins like collagen and elastin. According to Yu *et al.* [6], curcuminoids have anti-inflammatory, anti-cancer, and antioxidant properties. It has strong anti-free radical properties. It provides electrons to neutralize free radicals to protect cellular macromolecules from oxidative stress and lessen cellular damage. Curcumin is therefore a particularly promising ingredient in anti-aging products.



Figure 1: *C. xanthorrhiza* powder.

Serums have a very large amount of active ingredients in their formulation when compared to other cosmetic products [7]. Therefore, it is possible to tackle certain issues like wrinkles, hyperpigmentation, or acne effectively. Typically, serums contain minimal or no fillers or additives like emollients due to having the highest concentrations of active chemicals in any cosmetic product. The active components are intended to be strong and efficient, and fillers would merely lessen the concentration of those chemicals. The study aims to develop a stick serum formulation using *C. xanthorrhiza* extract as a natural antioxidant. This will address the limitations of current *C. xanthorrhiza* products, such as low bioavailability and instability. The stick serum format offers convenience and accessibility for consumers, making it a promising alternative to traditional formulations.

EXPERIMENTAL METHODOLOGY

Reagents and raw material

Fresh samples of *C. xanthorrhiza* were collected from a local supplier. The mature rhizomes were harvested. The chemicals used are ethanol, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, sodium carbonate, methanol, sodium nitrate, sodium hydroxide, quercetin, aluminium chloride, virgin coconut oil, cera alba, cetyl alcohol and lavender essential oil.

Sample preparation and extraction

The rhizomes were manually cleaned using water. Then, the rhizomes were prepared for use in the analysis by air drying. Soxhlet extraction was used to obtain the extracts from the powders. A total of 20 g of powdered rhizomes was extracted with 200 mL of ethanol for 6-8 h. The extract was concentrated in rotary evaporator and then kept in a hot air oven at 40-50 °C till all the solvent evaporated. The dried extract was kept in the refrigerator at 4 °C for their future use.

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

The antioxidant analysis was done according to the study by Akter *et al.* [8]. Ascorbic acid is used as the positive control. 3 mL of DPPH solution was added into 1 mL of samples at different concentrations (100, 150, 200, 250 and 300 ppm). This allows for measuring the antioxidant activity across a different range of concentrations and potentially identifying the effective dosage. The decrease in absorbance at 517 nm was recorded after 30 min of incubation at 37 °C in the dark using the UV-VIS spectrophotometer. The DPPH assay is commonly used to determine the IC₅₀ values of antioxidants. IC₅₀ represents the concentration of the antioxidant required to scavenge 50% of the DPPH radicals in each reaction mixture. The percentage of inhibition of DPPH free radicals was evaluated by using Equation (1).

$$\text{Percentage inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (1)$$

Determination of Total Phenolic Content (TPC)

The TPC test was determined using the Folin-Ciocalteu method that was shown in a study with some modifications [9]. Firstly, 0.2 mL of sample and 15.8 mL of distilled water were added into the flask. Then, 1 mL of diluted Folin-Ciocalteu (10%) and 3 mL of 7.5% sodium carbonate (7.5%) were added to the mixture, and it was shaken for 5 min. The mixture was incubated for 30 min at room temperature in a dark environment. The UV-VIS spectrophotometer was used to detect absorbance at 760 nm. TPC value was calculated according to the calibration curve of gallic acid standard

solutions in ethanol (10 to 100 ppm) with the absorbance measurement. The results were in milligrams of gallic acid per gram of extract (mg GAE/g) and three duplicates of the experiment were conducted.

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the extracts was determined by the aluminium chloride colorimetric method [10]. A mixture of distilled water (2.0 mL), 5% sodium nitrate solution (0.3 mL) and extracts (0.40 mL) were used. The mixture was mixed with 0.3 mL of 10% aluminium chloride solution for 6 minutes after standing for 5 minutes. After that, the mixture was combined with 2.0 mL of sodium hydroxide (1 M) and it was left to stand for 15 minutes at room temperature. The absorbance of the solutions was determined at 422 nm using a UV-VIS spectrophotometer. Then, the TFC value of the extract was calculated according to the quercetin standard curve (20 to 600 ppm) and the absorbance measurement. The findings should be expressed in milligrams of quercetin equivalents for each gram of dry weight sample (mg QE/g) and three copies of the experiment were conducted.

Formulation of the serum stick

Two different formulations were made with alterations [11]. Two serum sticks were prepared and labeled as F1 and F2. F1 was a control formulation without the extracts while F2 was the active formulation containing 1% of *C. xanthorrhiza* extracts for comparison. Firstly, cera alba was melted in a water bath at 65 °C. Cetyl alcohol and adeps lanae were added to the melted cera alba and stirred until well blended. Then, slowly add virgin coconut oil to the mixture. It was continuously stirred until the mixture became fully homogeneous. Lastly, lavender essential oil was added. *C. xanthorrhiza* extracts were added into F2 and continued to stir to ensure homogenous mixing before adding the essential oil. Then, the mixture was poured into appropriate molds and allowed the sticks to cool and solidify completely before use. Table 1 shows the formulations for the serum stick.

Table 1: Formulation of serum stick.

Materials	Function	Amount	
		F1 (mL)	F2 (mL)
<i>Curcuma Xanthorrhiza</i> extracts	Active substance	-	1
Cera Alba (Beeswax)	Hardener	15	15
Adeps lanae (Lanolin)	Fastener / Hydration	5	5
Cetyl Alcohol	Stabilizer	5	5
Virgin coconut oil	Emollient	25	24
Lavender essential oil	Perfume	5 drops	5 drops
Total		50	50

Physical tests on serum stick

An organoleptic test, also known as a sensory evaluation, is a method used to assess the properties of a product using the five senses which are sight, smell, taste, touch, and hearing. Organoleptic observations include observations of changes in odour, shape, colour, homogeneity and texture. However, the focus would be on the product's visual appearance, scent and feel on the skin. The organoleptic test was done by observing the change in colour, texture and smell of serum stick of each formula during storage at room temperature on days 1, 5, 10 and 15

The patch test was carried out by hand. This test was used to evaluate the potential of a formulation to cause skin irritation when being used. It is carried out by applying the product to the skin for 10 min. A small amount of the serum stick was applied to the chosen area, which was hand. During the test period, pay close attention to the area for any signs of irritation such as redness, itching, burning, or stinging.

The pH tests were carried out by dipping a pH meter into the mixture of melted serum sticks and distilled water. The pH meter was calibrated by using a buffer solution. It was determined to keep the pH of the serum stick as neutral as possible and to investigate the possibility of any side effects. Using a serum stick with a significantly different pH could disrupt the skin barrier and lead to irritation or other adverse effects.

RESULTS AND DISCUSSION

Percentage Yield

The efficiency of the extraction is measured by the percentage yield, which is the weight of the extract to the starting weight of the powder sample. The percentage yield of the extraction for extraction 1 and extraction 2 is 8.84% and 7.47% respectively. According to Sari *et al.* [12], the ethanol extract of the *C. xanthorrhiza* using Soxhlet extraction produced a yield of 5.96%. The yield of this extract is higher due to the concentration of solvents used in the extraction. There is also a difference between the percentage of the two extractions due to the time used for the rotary evaporator. Table 2 shows the percentage yield of the extraction.

Table 2: The weight of crude extract and dry extract of *Curcuma xanthorrhiza* sample.

Extraction	Crude extract (g)	Dry sample (g)	Percentage yield (%)	Mean±SD
1	1.768	20	8.84	8.16±0.97
2	1.495	20	7.47	

2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

DPPH assay was used to assess the *C. xanthorrhiza* extract's capacity to scavenge free radicals. As a stable radical, DPPH can combine with other radicals to generate a stable molecule or with hydrogen atoms from an antioxidant to make reduced DPPH. Shifting or fading of the dark purple to a somewhat yellowish colour indicates the reduction process. The visible light from the spectrophotometer has less absorption due to colour fading. The level of antioxidant activity increases with decreasing absorbance value. The observed absorbance value has dropped because of fewer antioxidants interacting with radicals.

The calibration curve was shown in Figure 2, with ascorbic acid acting as the positive control. The figure illustrates the linear relationship between the absorbance and the concentration of ascorbic acid. We may conclude that a higher percentage of DPPH inhibition was caused by an increase in the concentration of the ascorbic acid.

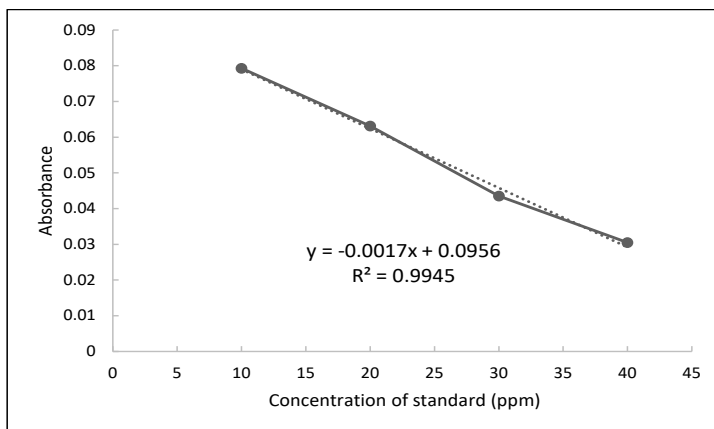


Figure 2: The calibration curve of ascorbic acid standard (positive control).

The percentage of DPPH inhibition of various concentrations of the extracts was also examined and the results are displayed in Figure 3 at a wavelength of 517 nm ($r^2 = 0.829$). The ethanolic extract of *C. xanthorrhiza* produced an IC₅₀ value of 20.86 ppm in the DPPH experiment as displayed in Table 3. According to Qader *et al.* [13], the ethanolic extract of *C. xanthorrhiza* produced an IC₅₀ value of 64.0 ppm. Another study stated that the extract had moderate antioxidant activity with an IC₅₀ value of 26.38 ppm [14]. Based on the result, the extract demonstrated strong free radical scavenging ability. Curcuminoid polyphenol compounds that comprising of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in *Curcuma* species exhibit high antioxidants that contribute to the assay [15]. The potential reasons for any observed differences due to the variations in extraction conditions such as concentration of ethanol used and the plant source itself. The prolonged storage time of the extracts showed an increase in DPPH absorbance value thus reducing the antioxidant activity.

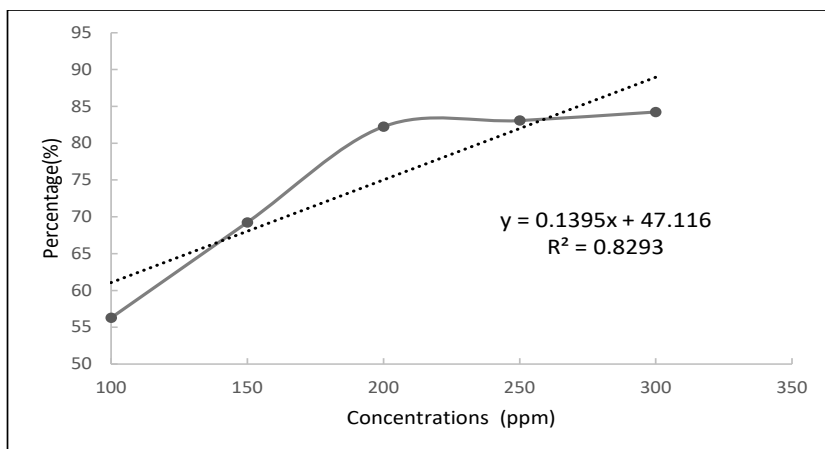


Figure 3: Percentage inhibition of *Curcuma xanthorrhiza* extract.

Table 3: Antioxidant value of *Curcuma xanthorrhiza* extract.

Sample	IC50 (ppm)	TPC (mg GAE/)	TFC (mg QE/g)
<i>C. xanthorrhiza</i>	20.86	45.71 ± 0.36	30.06 ± 0.41

Total Phenolic Content (TPC)

This test quantifies the total amount of phenolic compounds in a sample. A higher total phenolic content suggests a greater potential for antioxidant activity. The total phenolic content of the extract was measured using the Folin-Ciocalteu method and expressed as gallic acid equivalents per gram of dry weight (mg GAE/g). Gallic acid was used as the standard and the calibration curve was illustrated as shown in Figure 4. Using the equation obtained from the standard curve, the concentration of phenolic compounds in the sample was calculated. The absorbance of the sample extract is compared to the calibration curve to determine its equivalent gallic acid concentration. The absorbance of the resulting blue solution is measured at a 759 nm wavelength.

The TPC of the extract was obtained was 45.71 ± 0.36 mg GAE/g (Table 3). According to research by Asyhar *et al.* [16], it was found that the total phenolic content of the ethanolic extract of *C. xanthorrhiza* between

the ten accessions ranged from 1.74 ± 0.085 to 18.72 ± 1.47 mg GAE/g. The phenolic antioxidant content in *C. xanthorrhiza* is influenced by altitude, rainfall, and temperature conditions of the location where the tree was planted [17]. The concept with moderate rainfall and warmer temperatures might promote higher phenolic production.

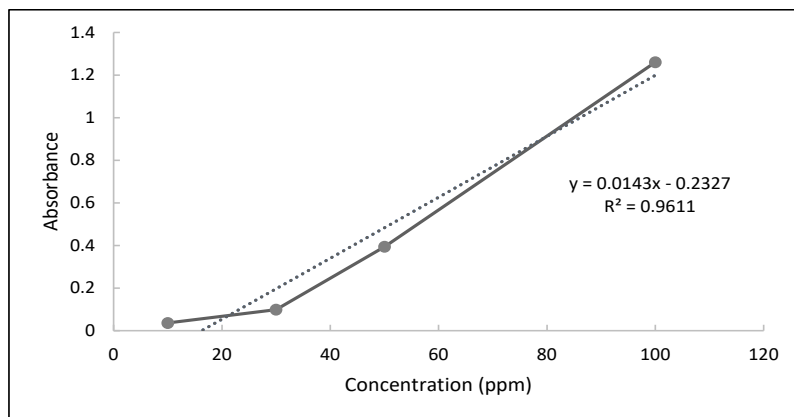


Figure 4: The calibration curve of gallic acid standard.

Total Flavonoid Content (TFC)

The test measures the total amount of flavonoids which are another class of antioxidant compounds. A higher total flavonoid content indicates the presence of antioxidants with specific structures. The total flavonoid content of the extract was measured using the Aluminium Chloride method and expressed as quercetin equivalents per gram of dry weight (mg QE/g). The absorbance of the resulting yellowish solution was measured at 508.9 nm. A standard curve was prepared using quercetin and the absorbance of the standard solutions at various concentrations was measured in Figure 5. Using the equation obtained from the standard curve, the concentration of flavonoid compounds in the sample is calculated.

The TFC value of the extract was 30.06 ± 0.41 mg QE/g as shown in Table 3. A study ranged the content in the ethanol extract of the rhizome from 3.47 ± 0.40 to 39.54 ± 4.08 mg QE/g [16]. Another study found that the total flavonoid contents of *C. xanthorrhiza* ranged from 25.07 to 143.03 mg QE/g according to the solvents [18]. The difference might be attributed to

the types of flavonoids and polarity of solvents. The less polar flavonoids could be better extracted by nonpolar solvents, whereas the more polar ones are better extracted by alcohol solvents [19].

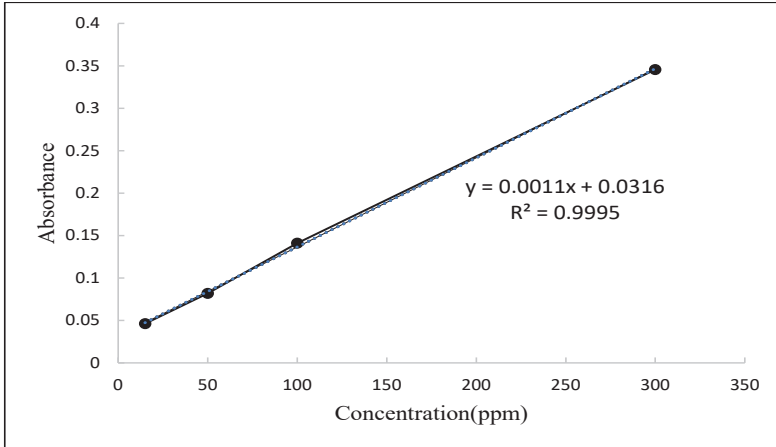


Figure 5: The calibration curve of quercetin standard.

Formulation serum stick

Both sticks share a base of beeswax, lanolin, cetyl alcohol and virgin coconut oil for structure and spreadability. Lavender essential oil adds a calming scent in both. The formulations were prepared by melting the base ingredients, then incorporating the *C. xanthorrhiza* extract (active formulation only) and lavender essential oil at a cooler temperature.

Physical Appearances

The key goal in designing the serum sticks was aesthetically pleasing and easy to use. The serum sticks were contained in a cylindrical tube for ease of grip and handling during application. The sticks had a smooth, unpatterned surface that gave them a tidy, commercial appearance. As a reflection of the natural curcumin level in *C. xanthorrhiza*, the colour was consistently bright yellow. The opaque nature of the serum sticks allowed users to see a little of the extract inside. The solid firmness of the serum sticks allows for good control during application without being too brittle. The sticks have a firm consistency and a robust texture that make them

easy to handle and apply smoothly to the skin. The firmness for controlled application and the slightly yielding texture for easy glide-on application are perfectly balanced in the serum sticks. Figure 6 shows serum sticks containing *C. xanthorrhiza* extracts while Figure 7 shows the serum sticks without containing the extracts that acts as a control.



Figure 6: Serum sticks containing *Curcuma xanthorrhiza* extracts.



Figure 7: Serum sticks not containing *Curcuma xanthorrhiza* extracts.

Organoleptic test

The organoleptic properties of the *C. xanthorrhiza* serum sticks were designed to enhance the user experience. The light-yellow colour provides a natural and aesthetically pleasing appearance. The faint earthy aroma of the *C.xanthorrhiza*, while noticeable, was not overpowering due to the light coconutty of the virgin coconut oil which incorporated a lot in the formulation. The smooth and waxy texture allows for effortless application without applying extra pressure. It is important to acknowledge that organoleptic testing is subjective. While the testers generally found the colour, odour, and texture to be acceptable, individual preferences may vary. Further user testing with a larger population could provide more specific insights into user perception of these organoleptic characteristics. The organoleptic evaluation of serum sticks on different days was displayed in Table 4.

Table 4: Organoleptic test on serum sticks developed.

Product	Characteristic	Day 1	Day 5	Day 10	Day 15
F1	Colour	Clear	Clear	Clear	Clear
	Odour	Faint coconut odour	Faint coconut odour	Faint coconut odour	Faint coconut odour
	Texture	Smooth	Smooth	Smooth	Smooth
F2	Colour	Light yellow	Light yellow	Light yellow	Light yellow
	Odour	Faint coconut odour	Faint coconut odour	Faint coconut odour	Faint coconut odour
	Texture	Smooth	Smooth	Smooth	Smooth

Patch test

The serum stick was applied to a small area of skin as the patch test was carried out. Any indications of irritation, such as redness, itching, or burning, were assessed at the patch location. The stick gives the skin a faint hint of yellow after application. The patch test revealed no irritation, which is encouraging for the serum stick's user safety. In individuals, it implies a less likelihood to develop any skin reactions. It is crucial to recognize

the limitations of patch skin as some individuals may experience irritation because of personal sensitivity. The serum sticks produced did not cause any irritation, but some people could inherently be sensitive to certain substances.

pH test

The measured pH of the F1 which is the control was 6.829 while the measured pH for the *C. xanthorrhiza* serum stick (F2) was 5.018. It falls within this desirable range, suggesting a lower risk of irritation for usage. A slightly acidic pH is considered ideal for topical skin care products [20]. This pH range closely aligns with the natural pH of healthy human skin which normally is around pH 4.7-5.75. A formulation with a pH closer to the skin's natural pH feel more comfortable upon application and be less likely to cause irritation as it helps minimize any potential disruption of the natural barrier function of the skin.

CONCLUSION

This study successfully demonstrated the potential of a natural antioxidant serum stick derived from *C. xanthorrhiza* as a promising skincare product. The formulation of two serum sticks exhibited significant antioxidant activity in vitro. The ethanolic extract of *C. xanthorrhiza* produced a strong IC₅₀ value of 20.86 ppm. The TPC and TFC tests indicate 45.71 ± 0.36 mg GAE/g and 30.06 ± 0.41 mg QE/g. The physical properties of the serum stick including firm texture, make it a convenient and user-friendly skincare solution.

While this study provides a strong foundation for the development of natural antioxidant serum sticks, further research is necessary to optimize the formulation for long-term stability and to explore additional potential benefits. Future studies could focus on formulation using various extract concentrations as extracts can affect the look of the final products and exploring the product longevity. Either HPLC (High-Performance Liquid Chromatography) or GC (Gas Chromatography) can be used to obtain deeper insights into the sample's antioxidant capacity considering the encouraging results from the DPPH, TPC and TFC protocols. By addressing these areas,

we can develop a highly effective and sustainable skincare product that harnesses the power of nature to promote youthful skin.

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