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Research Article

The Combination of Turmeric (*Curcuma domestica*) Rhizome Extract and Collagen in A Serum Formulation as an Antioxidant

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Abstract

Turmeric (*Curcuma domestica*) has nutritious compounds called curcuminoids, which can be used as antioxidants. As an antioxidant, *C. domestica* extract can be used to ward off free radicals that damage collagen and elastin, a protein that keeps skin moist. This study aimed to determine the antioxidant activity of serum combined with collagen's addition using the DPPH method. The DPPH was made at a concentration of 80 µg/mL, and the absorption was read at a wavelength of 520 nm using a microplate rider. The study was conducted by making six formulations: F0, F1, F2, F3, F4, and F5 obtained the results of serum made from *C. domestica* extract that could inhibit free radicals and meet the physical evaluation test requirements of serum. Furthermore, the formula was made using only one active ingredient and only collagen to determine the extract or collagen's antioxidant activity. The results obtained indicate that collagen had a supporting role in adding antioxidant activity apart from its extract. The highest % inhibition value at F5 with 90.526% could ward off free radicals.

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INTRODUCTION

A free radical is a relatively unstable molecule with an atom in its outer orbit having one or more unpaired electrons. The molecule that loses the pair becomes unstable and radical. Therefore, this molecule always tries to find its electron pair by seizing electrons from other molecules[1,2]. Free radicals can damage collagen and elastin, which are a protein that keeps skin moisturized, smooth, flexible, and elastic. Excessive free radical levels trigger various degenerative diseases and conditions in the skin, such as premature aging, wrinkles, erythema, skin cancer, and others[3,4].

Aging is a process that is a certainty, but premature aging is something that is not expected. The skin's premature aging is characterized by thinning skin, dry skin, wrinkles, and uneven coloration[5]. One of the causes of premature aging is free radical[6]. One of the ways used to prevent premature aging is to use cosmetics that contain antioxidants. These antioxidants can inhibit the development of oxidation reactions by binding to free radicals and highly reactive molecules to inhibit cell damage[7].

One of the cosmetic dosage forms that have developed lately to prevent premature aging is serum. The serum has the advantage of having a high concentration of active ingredients so that the skin more quickly absorbs the effect, can provide a more relaxing effect, and is easier to spread on the surface of the skin because the viscosity is not too high[8].

Research on antioxidant activity from medicinal plants in serum form has been carried out, including by Mardhiani

by testing the formulation and stability of the serum from green coffee (*Coffea canephora* var. Robusta) extract as an antioxidant where the results showed an IC_{50} value of 68.89 µg/mL, which is classified as a strong antioxidant[9]. Another plant whose antioxidant activity has been studied is turmeric (*Curcuma domestica*). *Curcuma domestica* is a type of spice with nutritious compounds called curcuminoids consisting of curcumin, desmethoxycurcumin, and bisdemethoxycurcumin, which have various important pharmacological activity such as antioxidants[10].

Curcuma domestica rhizome extract in cosmetics has been widely used, and there is some clinical evidence showing that preparations containing *C. domestica* are either oral or topical provide benefits to treating various skin diseases and overall skin health[11]. In other studies, it was found that the hexane fraction and ethyl acetate fraction from the ethanol extract of *C. domestica* rhizome were included in the non-toxic category[12]. Based on studies that have been carried out regarding *C. domestica* and curcumin's safety evaluation, it is known that it does not cause toxic effects at doses. Therefore, *C. domestica* and curcumin can be developed in modern medicine for the therapy of various diseases[13].

Naturally, at least 1% of the collagen in the human body is lost every year, and at the age of 40, humans do not produce collagen anymore, so the loss of collagen reaches 35-40%. Therefore, collagen from outside the body is needed, which has an anti-aging activity to prevent aging from occurring faster[14]. Collagen has a vital role in the food, biomedical, pharmaceutical, and cosmetic industries; therefore, collagen is needed for the skin.

Research on collagen has been carried out by Budiarti[16] by testing collagen from chicken bone waste (*Gallus gallus domesticus*) against anti-aging activity *in vitro*, where the results obtained by stirring for six hours have a smaller particle size (1.34 µm) compared to eight hours (1.80 µm) stirring. Collagen with a size of 1.34 µm showed the best activity, namely antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) of 24.7% and tyrosinase inhibitor of 26.77%. Based on the antioxidant, anti-glycation, and antityrosinase activities of collagen by immersing 0.10 M NaOH and stirring for six hours, it has anti-aging properties.

Antioxidant testing can be done in several ways, one of which is using the DPPH method. The DPPH method is one of the most popular methods because it is practical and sensitive[17]. Antioxidant testing using the DPPH method can be seen from the IC_{50} value. The research aims to see the effectiveness of the combination between *C. domestica* rhizome extract and collagen from fish skin in serum by determining the antioxidant using the DPPH method.

MATERIALS AND METHODS

Materials

The materials used were *C. domestica* rhizome obtained in Pekanbaru and determined at the Botanical Laboratory, Universitas Riau, collagen, ethanol 96% (Merck), methanol (Merck), demineralized water (Brataco), disodium EDTA (Merck), NaCl (Merck), DPPH (Merck), natrosol hydroxyethyl cellulose, glycerin, DMDM hydantoin, and ethoxydiglycol. The instrument used were analytical scales (Boeco), ultrasonic bath (Branson), rotary evaporator (Buchi R-14), micropipette, viscometer (Scorex), microplate (Bertholt Tristar), and microplate reader (Bertholt Tristar).

Methods

Extraction

Curcuma domestica rhizome was blanched by boiling at 100°C for five minutes with a medium of 0.05% citric acid solution. Each treatment sample was subjected to an extraction process of 200 g of macerated samples for 72 hours using 96% ethanol solvent and then sonicated

using an ultrasonic bath. The solvent of macerate was collected, and it was evaporated using a rotary evaporator until a thick extract was obtained.

Serum formulation

The serum made was based on the results of research by Mardhiani[9]. Variations were made to the concentrations of *C. domestica* and collagen extracts, each with varying levels of 0, 0.5, 0.8, and 1.1; as well as 0 and 2, respectively. The serum formulation in this study was presented in **Table I**.

Table I. Serum formulation

Ingredients	Concentration (%)					
	F0	F1	F2	F3	F4	F5
Curcuma domestica extract	-	-	0.5	0.5	0.8	1.1
Collagen	-	2	-	2	2	2
Natrosol	0.75	0.75	0.75	0.75	0.75	0.75
Glycerin	10	10	10	10	10	10
DMDM Hydantoin	0.05	0.05	0.05	0.05	0.05	0.05
Ethocydiglycol	2	2	2	2	2	2
Demineralized water	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

F0 = negative control; F1 = collagen 2%; F2 = ethanol extract 0.5%; F3 = *C. domestica* ethanol extract 0.5% + collagen 2%; F4 = *C. domestica* ethanol extract 0.8% + collagen 2%; F5 = *C. domestica* ethanol extract 1.1% + collagen 2%

Physical evaluation of serum

The physical evaluation of serum could be carried out with several tests, including:

1. Organoleptic test: The organoleptic test was intended to see the physical appearance of the preparation, which includes shapes, colors, and smells.
2. Homogeneity test: Homogeneity checks were carried out using an object-glass, with a certain amount of the serum applied to a piece of glass or other suitable transparent material. The preparation must exhibit a homogeneous arrangement and did not show any coarse grains.
3. pH test: pH determination was carried out using a pH meter.
4. Irritation test: The test was carried out using a closed patch test on human skin, with 1 g of serum is taken, then applied to the inner arm with a diameter of 2 cm, covered with a bandage, left plastered for 24 hours, and observed symptoms such as redness and itching of the skin. This irritation test was carried out on three panelists for one formula[18].
5. Viscosity test: The serum's viscosity was measured by placing the sample in a viscometer until the spindle is submerged[19].

Antioxidant activity test using DPPH

DPPH solution was made with a concentration of 80 µg. Then, a main standard solution of the sample was made with a 1000 µg/mL concentration. Determination of the serum's antioxidant activity was carried out using a microplate reader with the DPPH method at a wavelength of 520 nm. The mixture was incubated in a dark place for 30 minutes and then measured its absorbance at a wavelength of 520 nm using 80 µg/mL DPPH 80 µg/mL, while for the blank used 50 mL methanol absolute[20].

Data analysis

The absorbance measurements using a microplate reader were used to calculate the percentage of DPPH free radical reduction. The percentage of DPPH free radical reduction was calculated using the following equation:

$$\%inhibition = \frac{ABS\ control - \ ABS\ sample}{ABS\ control}\ x\ 100$$

ABS control : Absorbance of DPPH + methanol) - Absorbance of methanol
ABS sample : (Absorbance of sample) - Absorbance of methanol

RESULTS AND DISCUSSION

The sample used was fresh *C. domestica* rhizome. The middle part of the clean sample was taken and blanched at 100°C for five minutes. This blanching process was able to increase antioxidant activity by changing fewer active compounds to be active. In the blanching process, it was suspected that there would be degradation of

complex phenol compounds into simple phenols, and phenol compounds did not undergo enzymatic oxidation, so their numbers did not decrease[21]. Besides, the blanching process was able to inactivate enzymes in the material and optimize the extraction process. The active substances said to provide antioxidant activity in *C. domestica* were curcuminoid compounds[22]. The addition of 0.05% citric acid solution to the boiling process served to denature the cells so that the higher the concentration used, the more cell membranes were degraded, which results in the pigment components coming out quickly and producing more yield[23]. The yield of the extract obtained was 50.87%. In the physical evaluation of the serum, the results were displayed in **Table II**.

Table II. Organoleptic examination result, homogeneity, pH and viscosity of the serum

Formulation	Color	Smell	Form	Homogeneity	Viscosity (cP)	pH
F0	Transparent	Weak smell	Gel	Homogeneous	30860	4.5
F1	Transparent	Weak smell	Gel	Homogeneous	34250	5.9
F2	Light Yellow	Distinctive smell	Gel	Homogeneous	35430	5.4
F3	Dark Yellow	Distinctive smell	Gel	Homogeneous	32020	6.1
F4	Dark Yellow	Distinctive smell	Gel	Homogeneous	32380	6.0
F5	Dark Yellow	Distinctive smell	Gel	Homogeneous	32440	5.9

The finished serum formulation was followed by a physical evaluation test of the preparation. The color and smell test obtained results following **Table II**. As for the form, all were in gel form. Furthermore, the pH test for serum in the six formulas was still in the skin pH range of 4.5-6.5[24]. If serum has a pH too acidic, it will cause skin irritation. On the other hand, if it is too alkaline, it can cause scaly skin[25]. The homogeneity test indicated that all formulas are homogeneous, marked by the absence of coarse grains at the time of testing[26]. The serum's viscosity test showed that the results still met the serum's viscosity requirements by SNI 16-4399-1996[27]; the standard viscosity value for serum was 6000-50000 cP or 6-50 Pa.s. Viscosity testing aims to determine the viscosity value of a substance. The higher the viscosity value, the higher the substance's viscosity level[28]. Also, viscosity is related to the ability of a liquid to flow. Viscosity is inversely proportional to dispersibility, in which the higher the viscosity, the lower the dispersibility.

Viscosity also determines the length of the skin's adhesion supply so that the supply can adhere well[29]. In this study, the lowest viscosity obtained at F0 (base) was 30860 cP; collagen's addition increased the preparation's viscosity, which was 34250 cP. The formula containing a combination of collagen and ethanol extract of *C. domestica* rhizome had decreased viscosity value, increasing the dispersibility of the preparation. In the irritation test on ten panelists, there were no allergic reactions such as redness, itching, heat, and swelling; it could be seen in **Table III**.

Table III. Irritation test results

Irritation	F 0	F1	F2	F3	F4	F5
Itching	-	-	-	-	-	-
Heat	-	-	-	-	-	-
Swelling	-	-	-	-	-	-
Redness	-	-	-	-	-	-

(-) : No irritation was observed

Antioxidant testing of *C. domestica* rhizome serum was carried out using the DPPH radical absorption method. DPPH is a purple radical compound that has one unpaired atom. The antioxidant activity is indicated by the DPPH absorption measurement that reacts to antioxidant compounds at a maximum wavelength range of 515-520 nm[17]. The DPPH was a free purple radical molecule that turns into a stable yellow compound caused by the reaction to antioxidants, in which antioxidants give one electron to DPPH so that there was a reduction in DPPH free radicals. The sample's antioxidant activity caused a color change in the DPPH solution, which was initially dark purple to pale yellow and colorless[30]. The test results of the % inhibition

value obtained in the ethanol extract serum formulation of *C. domestica* rhizome with the addition of fish skin collagen were shown in **Table IV**.

Table IV. The % inhibition value of the serum

Formula	P1	P2	P3	% Inhibition ± SD
F0	93.494	87.732	90.335	90.526 ± 1.87
F1	70.260	70.074	76.952	72.446 ± 0.11
F2	59.294	59.108	68.960	60.805 ± 0.94
F3	60.967	59.108	60.223	60.124 ± 5.63
F4	45.540	45.725	45.539	45.635 ± 3.92
F5	34.572	38.290	36.803	36.594 ± 2.89

The % inhibition value shows that the higher the extract concentration, the higher the antioxidant activity. At F0 and F1, the % inhibition value increased. This finding shows that the collagen used had a supporting role in increasing antioxidant activity, although only partially. The addition of collagen aims to help prevent premature aging because collagen had anti-aging activity. Collagen with a smaller size would have a higher antioxidant activity[16].

Furthermore, the F2 test was carried out using only turmeric rhizome extract, where *C. domestica* had various functions, one of which was as an antioxidant. Following Pratiwi and Wardaniati[31], one of the natural antioxidants was found in the *C. domestica* plant because *C. domestica* rhizomes contain active compounds with medicinal properties. It was called curcuminoids, which belong to the group of phenolic compounds.

The extract and collagen were combined into F3, F4, and F5 to increase the % inhibition value obtained. At F5 with an extract concentration of 1.1%, it shows % inhibition of 90.526%. A higher % inhibition value results in lower absorbance and high antioxidant activity. It could be said that the extract used was able to inhibit free radicals plus the addition of collagen so that free radical inhibition increased. The % inhibition result increased due to differences in extract concentration and collagen's addition in the formula. This study also used a negative control to determine whether there was an effect of each ingredient used in the manufacture of serum and was also carried out on serum-containing only one active ingredient, which aims to determine which active ingredient in serum had antioxidant activity.

CONCLUSION

From this research, it was concluded that the serum formula used the active ingredient of *C. domestica* rhizome extract with the addition of collagen had a % inhibition value that inhibited free radicals. The collagen used affected increasing antioxidant activity apart from the extract. At F5, the concentration of 1.1% extract indicated the highest % inhibition value in inhibiting free radicals, which was 90.526%.

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