International Journal of Scholarly Research in
Engineering and Technology
Journal homepage: https://srrjournals.com/ijsret/
ISSN: 2961-3280 (Online)

(RESEARCH ARTICLE)

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The antiaging cream characteristics from extract of turmeric (*Curcuma domestica* Val.) rhizome and tamarind (*Tamarindus indica* L.) leaves

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International Journal of Scholarly Research in Engineering and Technology, 2023, 02(01), 020–030

Publication history: Received on 27 November 2022; revised on 14 January 2023; accepted on 17 January 2023

Article DOI: https://doi.org/10.56781/ijsret.2023.2.1.0031

Abstract

Chemical-based cosmetics tend to have adverse side effects on human skin so that public awareness arises to use cosmetics made from natural ingredients that do not have side effects. Extract cream of turmeric and tamarind leaf is one of the cosmetics made from natural ingredients that has the potential to replace chemical-based cosmetics. This study aims to determine the hydrophilic lipophilic balance (HLB) value and perfume type to get the cream with the best characteristics, an extract mixture concentration of turmeric rhizome and tamarind leaves used in the manufacture of anti-aging cream, and its application. The experiment used randomized block design with HLB treatment: 9,10,11 and the perfume type : 1,5% jasmine, 2% champagne, 2% rose, while the mixed treatment of turmeric extracts and tamarind leaves extracts were used with the concentration of 0, 1, 2, 3, 4, 5%. The application of the cream to experimental animals, using 36 wistar rats (*Rattus norvegicus*), treated without cream, smeared with placebo cream, smeared with cream of a mixture of tamarind leaves and turmeric extracts, for 4 weeks all groups of rats received exposure chronic UV-B rays 840 mJ/cm. The results showed that the type of perfume had a significant effect on the dispersion, but HLB and the type of perfume had a significant effect on overall acceptance. HLB 10 cream in all types of perfumes showed the best cream characteristics. The lowest anti-collagenase IC50 value was obtained in the cream with a 5% concentration of turmeric extract and tamarind leaves, IC50 value: 0.36 g/ml. Application of cream mixture of turmeric rhizome extract and tamarind leaves with a concentration of 5% gave a significant effect on the decrease in MMP-1 expression up to 12.147+2.380 µg/ml and increase the amount of collagen by 23. 67% in the skin of wistar rats.

Keywords: Anti-aging; HLB; Perfume; Extract concentration; Turmeric; Tamarind

1 Introduction

Public awareness about harmful chemicals in cosmetics has led to an increase in the demand for natural cosmetic ingredients and the use of safe cosmetic ingredients. Turmeric (*Curcuma domestica* Val.) and tamarind leaves (*Tamarindus indica* L.), are safe natural cosmetic raw materials, and have potency as anti-aging ingredients [1]. Cream emulsion is a semi-solid form of cosmetic ingredients that are commonly used because they are easy to apply [2].

The use of extracts of turmeric and tamarind leaves with tween 80 and span 80 as emulsifiers produce a safe cream. Span 80 as a non-ionic emulsifier is also used as a stabilizer in food products [3], tween 80 is used as a stabilizer in ice cream, dairy products, lotions and creams [4]. The use of tween 80 and span 80 in the manufacture of cream generally has an HLB (hydrophilic-lipophilic balance) value of 9-11 so that the cream is easy to rinse and dissolves in water [5]. The use of virgin coconut oil (VCO) in cream of turmeric extract and tamarind leaves as a result of research by Mulyani[1] has a weakness in aroma. The dominant cream is VCO flavored so that the organoleptic value is low. Based on this, the addition of perfume is needed to increase the organoleptic acceptance.

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A mixture of turmeric extract and tamarind leaves has been developed into cosmetic ingredients, one of which is to prevent skin damage and inhibit the aging process or as an antiaging ingredient [1]. Skin damage often occurs in people who live in areas that are often exposed to direct sunlight, such as in Indonesia[6]. Prevention of extrinsic skin aging is done by inhibiting the increase in matrix metalloproteinase enzymes and providing antioxidants [6]. Giving creams that contain antioxidants will increase the skin's defense against the accumulation of free radicals so that the process of forming wrinkles is inhibited. Antiaging cosmetics that have anti-collagenase and antioxidant abilities, have a mechanism of action by preventing damage caused by UV radiation or repairing damage that has already occurred. Anti-collagenase properties are the ability to inhibit the activity of collagenase enzymes including matrix-metalloid proteinase [7]. Antioxidants are often added because they can reduce oxidative damage caused by an increase in reactive oxygen species (ROS) due to UV radiation [8]. Ingredients that have anti-collagenase and antioxidant activity, will be able to prevent skin damage and repair the damage.

Phenolic compounds are ingredients with a content of 0.05-0.26 mg GAE/ml, have great potency as antioxidants and have prospects for the development as anti-aging cosmetic treatments [9, 10]. The phenolic content of a mixture of tamarind leaves and turmeric extract reaches 0.12 mg GAE/ml [11], so the mixture has the potency as an anti-aging cosmetic treatment. Research conducted by Mulyani et al. [1], showed that the ability of turmeric extract cream and tamarind leaves as an anti-collagenase was still low, IC_{50} value: 1,545.03 g/ml. Therefore, it is necessary to optimize all influencing factors and look for other unknown factors so that the activity of cream as an anti-collagenase is increased and optimal. The purpose of this study was to determine the HLB value and type of perfume to obtain the cream with the best characteristics, determine the concentration of the mixture of turmeric extract and tamarind leaves used in the manufacture of anti-aging cream and its application to experimental animals. The expectation of this research is that turmeric extract cream and tamarind leaves can be a solution to the increasing demand for safe natural cosmetic ingredients and the commercial development of anti-aging cream cosmetics.

2 Material and methods

The materials used include turmeric and leaves of local varieties, turmeric from the Village of Petang Badung Bali, and tamarind leaves from the Village of Jimbaran Badung, Bali. folin ciocalteu and gallic acid, mineral oil, FALGPA and collagenase enzymes from *Clostridium histolyticum* (CHC-EC.3.4.23.3), MMP-1 kit, MMP-1 antibody (Sigma). Tween 80, Span 80, stearic acid, cetyl alcohol, glycol, glycerol and sorbitol, Ethanol, formaldehyde, NaH_2PO_4, Na_2HPO_4, paraffin, xylol (Emsure), virgin coconut oil (Legenda), methanol (Brataco) and jasmine perfume. Sirius red, Avidin-HRV and DAB dyes. Wistar rats with aged 10-12 weeks weighing 150-160 g, animal feed.

The research was carried out in stages, namely 1) determination of HLB and type of perfume from turmeric and tamarind leaves extract cream, 2) determination of the concentration of a mixture of turmeric and tamarind leaves extract, 3) application of cream mixture of turmeric and tamarind leaves extract cream in experimental animals.

2.1 Determination of HLB and type of perfume from turmeric and tamarind leaves extract cream

This study used a randomized block design with HLB treatment (9, 10, 11) and type of perfume (1.5% jasmine, 2% champagne, 2% rose), and the treatments were divided into 3 blocks.

Simplicia turmeric and tamarind leaves 10 ± 1% moisture content made into powder with the size of 80 mesh. Furthermore, each simplicia powder was extracted with 96% ethanol solvent by maceration with a ratio of material: solvent = 1:6. Maceration was carried out twice each for 24 hours, the filtrate was evaporated using an evaporator, to obtain turmeric extract and tamarind leaf extract, then mixed with the ratio of turmeric extract: tamarind leaf extract = (10:2).

The manufacture of cream emulsions according to Mulyani's research [1] with HLB treatment and type of perfume, the cream formula is as shown in Table 1. The oil phase consists of stearic acid, virgin coconut oil (VCO), mineral oil, cetyl alcohol, moisture conditioner, tween 80 and span 80. The aqueous phase is distilled water, all ingredients are weighed and put into a beaker according to the phase. The oil phase and the water phase are heated at 65°C on water bath. Heating is carried out until all the ingredients are melted and then removed from the water bath and put in the tamarind leaf turmeric extract (10:2). Furthermore, the water phase is added to the oil phase while mixing until a homogeneous cream is obtained [12].

2.2 Determination of the concentration of a mixture of turmeric and tamarind leaves extract

This study used a completely randomized design with a mixture of turmeric extract and tamarind extract with a concentration of 0, 1, 2, 3, 4, and 5%, which was repeated 4 times.

The cream emulsion was made based on the best results of the first phase of research, namely jasmine perfume 1.5%, tween 80 (2.20%) and span 80 (2.80%), treatment of a mixture of turmeric and tamarind leaves extract with concentrations of 0, 1, 2, 3, 4, and 5%, other cream ingredients as shown in Table 1.

Table 1 Cream formula of a mixture of turmeric extract and tamarind leaves treated with HLB and types of perfume [1, 13]

Material (g)	**Amount in 100 g**		
	E1 (HLB 10)	**E2 (HLB 11)**	**E3 (HLB 12)**
Stearic acid	10.9	10.9	10.9
VCO	3.64	3.64	3.64
Mineral oil	2.27	2.27	2.27
Cetyl alcohol	0.91	0.91	0.91
Span 80	2.80	2.34	1.87
Tween 80	2.20	2.66	3.13
Moisturizer conditioner	10	10	10
The type of perfume	according to treatment	according to treatment	according to treatment
Turmeric and tamarind leaves extracts	0.2	0.2	0.2
Water addition up to	100	100	100

The presence of total phenolic has a direct impact on the inhibition of the cream on the activity of the collagenase enzyme [14]. The measurement of inhibition is based on the Moore and Stein method [15] which is carried out as follows: preparation of a pH buffer solution of 7.5 and preparation of a furylacryloyl-leusin-glycyl-propil-alanin /FALGPA solution: 1.0 mM solution using FALGPA in a buffer, for good dissolution, stirring is required for approximately 30 minutes using a stirrer. Preparation of an enzyme solution, containing 2 units/mL of collagenase in cold ultrapure water (2–8 °C).

Test procedure: in a 3.00 mL test tube, enter the following reagents: (a) test: 2.9 ml FALGPA and (b) blank: 2.9 mL FALGPA + 0.1 ml ultrapure water. The mixture with inversion and equilibrate temperature of 25 °C, monitored at absorbance (λ) = 345 (A345) to constant, used a spectrophotometer with a thermostat. Then added to the test tube (a):0.1 ml of enzyme solution. Immediately mixed with inversion and noted the reduction in absorbance wavelength (λ) = 345 for 5 minutes. Determined the maximum speed for both, (a) test and (b) blank using at least one minute interval and a minimum of 4 (four) points of data.

$$\text{Inhibition (\%)} = ((\text{A control} - \text{A sample})/\text{A control} \times 100$$

Explanation:

A control = absorbance buffer, collagenase + solvent
A sample = absorbance buffer, collagenase + extract

2.3 Application of cream mixture of turmeric and tamarind leaves extract cream in experimental animals

This study used a randomized post-test-only control group design, with a sample of 36 white rats (*Rattus norvegicus*). The rats were randomly divided into 3 groups so that each of them contained 12 rats as experimental animals with the following treatments: without cream, placebo cream smeared, and tamarind leaf turmeric extracts mixed cream smeared.

A sample of 36 male and healthy white rats (*Rattus norvegicus*), aged 18 months at that age they have similarities with humans aged about 45 years and have experienced an intrinsic aging process, weighing 450-550 g [16]. White rats adapted for 2 weeks were fed and watered *ad libitum*, each cage was occupied by 2 rats. Rats were randomly divided into 3 groups (P0, P1 and P2) so that each group had 12 rats. All groups of rats had their backs shaved, then applied the cream as a base material, cream was smeared on P1 and P2, each as much as 0.05mg/cm^2 of rat skin surface area, while

P0 was not given cream. Chronic UV-B exposure was given to all groups, exposure was carried out 3 times, total UV-B rays received by all groups of rats was 840 mJ/cm for 4 weeks. After four weeks of treatment, the rats were euthanized with ketamine-xylazine; The dorsal dermis skin tissue of the rats was taken with a 2 x 2 cm knife for immune histochemical examination.

The variables observed included the amount of collagen (Pico-Sirius-Red staining) and levels of MMP-1 (immune histochemical observations). MMP-1 expression was examined immune histochemical and the amount of skin dermal collagen was examined using Pico-Sirius-Red staining.

$$\text{Rate MMP} - 1\ (\%)\ = \frac{\text{Fibroblast that expressive MMP} - 1}{\text{total fibroblast in the field of view}} \text{x } 100\%$$

2.4 Data analysis

The data of the research in stages I and II were carried out by Analysis of Variance and continued with DMRT, organoleptic test using Friedman test. Phase III the research data was carried out in normality of the data with the Shapiro-Wilk & Kolmogorov-Smirnov test and tested for homogeneity with Levene's test. Furthermore, analyzed of variance was done to the data and continued with DMRT.

3 Results and discussion

3.1 Adhesiveness

Table 2 shows that the adhesion time of the cream on the 6th week in all HLB treatments is not significantly different. This is due to the addition of the same amount of tween 80 and span 80 non-iconic surfactants; although the ratio is different, but it still produces the same stability. This causes the same adhesion time. The adhesion time until the 6th week still meets the SNI (Indonesian National Standard) requirements which is more than 4 seconds. The stability of adhesion time is also due to the presence of cetyl alcohol which functions as an emulsifier. The emulsifier works to form a layer around the dispersed droplets thus preventing the separation of the dispersed liquid [17].

Table 2 Average value of Adhesiveness (seconds) of turmeric and tamarind leaf extract cream on the 6th week

Perfume type	HLB			Average
	10 (E1)	11 (E2)	12 (E3)	
Jasmine (P1)	14.79	9.48	22.84	15.70a
Champagne (P2)	23.45	16.31	6.51	1.42 a
Rose (P3)	2.70	31.61	26.28	27.20 a
Average	20.64 a	19.13 a	18.54 a	

Description: the same letters behind the average value in the same column and row indicate no significant difference ($p < 0.05$)

3.2 Dispersive ability

Table 3 show that the HLB treatment is not affected because the HLB value only distinguishes the span and tween surfactant ratios, while the number of additions is the same. Emulsion stability is more influenced by surfactant concentration. Because the concentration is the same, the HLB value does not affect the dispersive power of the cream.

The perfume treatment has an effect on the dispersive power of the cream. It might be due to the difference in the base of the perfume solvent. In general, perfume compositions consist of: ethanol as a solvent, aquadest as a perfume solvent base and active ingredients or perfume core and polyethylene glycol (PEG-40) as a fixative/binding agent and moisturizer. The three types of perfume used have different amounts of aquadest addition as a solvent base. Based on the value of dispersive power, it shows that the amount of rose perfume added aquadest is smaller than those in jasmine and champagne perfume. The difference in the amount of water in each perfume will affect the dispersive power of the cream.

Table 3 Average value of dispersive ability (cm) of turmeric and tamarind leaf extract cream on the 6th week

Perfume type	HLB			Average
	10 (E1)	11 (E2)	12 (E3)	
Jasmine (P1)	5.52	5.35	5.58	5.48 a
Champagne (P2)	5.13	5.66	5.57	5.45 a
Rose (P3)	5.06	5.04	5.13	5.08b
Average	5.23 a	5.35 a	5.43 a	

Description: the same letters behind the average value in the same column and row indicate no significant difference ($p < 0.05$)

3.3 Acidity (pH)

The analysis of variant shows that the HLB treatment, perfume type and their interactions do not significantly influence ($p > 0.05$) the pH of the cream (Table 4). The pH range of the cream is 6.08-6.38. The selection of tween and span non-iconic surfactant emulsifiers has these advantages: safe ingredients, does not irritate the skin and produce a smooth and stable texture [18], and does not affect pH. The difference in the type of perfume does not affect the pH, this is due to the perfume concentration of no more than 2%. The pH of the perfume tends to be neutral and close to the skin's pH so that the treatment of added perfume does not affect the pH of the cream. A good cream has the same pH value as the normal pH of the skin ranging from pH 4.5 to 6.5 [19]. All research creams meet SNI requirements.

Table 4 Average value of turmeric and tamarind leaf extract cream pH on the 6th week

Perfume type	HLB			Average
	10 (E1)	11 (E2)	12 (E3)	
Jasmine (P1)	6.25	6.22	6.10	6.19 a
Champagne (P2)	6.25	6.20	6.13	6.19 a
Rose (P3)	6.38	6.17	6.13	6.23 a
Average	6.29a	6.19a	6.12a	

Description: the same letters behind the average value in the same column and row indicate no significant difference ($p < 0.05$)

3.4 Separation ratio

The results show the HLB treatment, the perfume type and the interaction has no significant effect ($p > 0.05$) on the cream separation ratio (Table 5). The number of additions of the same tween 80 and span 80 non-iconic surfactants, with different ratios, does not affect the density of the two phases in the emulsion so that it does not affect the separation ratio. This also applies to the type of perfume treatment. The amount of perfume addition which is only a maximum of 2% has no effect on the density of the two phases so that the cream separation ratio is not different.

Table 5 Average value of separation ratio of turmeric and tamarind leaf extract cream on the 6th week

Perfume type	HLB			Average
	10 (E1)	11 (E2)	12 (E3)	
Jasmine (P1)	1.00	0.78	1.00	0.93 [a]
Champagne (P2)	1.00	0.77	0.70	0.82 [a]
Rose (P3)	0.88	0.81	0.77	0.82 [a]
Average	0.96[a]	0.79[a]	0.82[a]	

Description: the same letters behind the average value in the same column and row indicate no significant difference ($p < 0.05$)

3.5 Viscosity

The results show that HLB treatment, the perfume type and their interactions do not significantly affect (p> 0.05) the viscosity of the cream (Table 6). The viscosity range of the cream is 17500-38000 cp. The viscosity of an emulsion is affected by the size of the droplet. The small droplet size will increase the surface area and increase the emulsion resistance to flow which then increases the viscosity [20]. The size of the droplet is influenced by the time and method of homogenization, this will affect the stability of the emulsion [21]. In this study, the homogenization time and method do not differ those results in the same droplet size, so that the treatment has no effect on the viscosity of the cream. The viscosity of all creams produced meets SNI requirements.

Table 6 Average value of viscosity (x 10,000 cp) of turmeric and tamarind leaves extract cream on the 6th week

Perfume type	HLB			Average
	10 (E1)	11 (E2)	12 (E3)	
Jasmine (P1)	2.50	3.80	2.50	2.93 [a]
Champagne (P2)	2.85	2.50	2.00	2.45 [a]
Rose (P3)	2.75	2.43	3.25	2.81 [a]
Average	2.70[a]	2.91[a]	2.58[a]	

Description: the same letters behind the average value in the same column and row indicate no significant difference (p < 0.05)

3.6 Organoleptic test

Friedman test results show that the HLB treatment and perfume type have significant effect (p<0.05) on the overall acceptance of the cream (Table 7). The acceptance values range from 4.37-6.10. The characteristics of the cream in all treatments until the 6th week of storage still meet SNI standards. This shows that tween 80 and span 80 emulsifiers with a concentration of 5% are able to maintain the stability of turmeric and tamarind leaf extract cream emulsion. Based on the organoleptic test, the highest results were obtained from the cream with HLB10 at all storage until the 6th week, with a value of 5.1 to 6.1 (quite like to like).

Table 7 Friedman test result of the cream in HLB and perfume type towards the overall acceptance of the cream

Product	Value
HLB 10 Jasmine	6.10a
HLB 10 Champagne	5.57a
HLB 10 Rose	5.10a
HLB 11 Jasmine	4.51b
HLB 11 Champagne	4.64b
HLB 11 Rose	4.47 b
HLB 12 Jasmine	4.61 b
HLB 12 Champagne	4.57 b
HLB 12 Rose	4.37 b

Description: the same letters behind the average value indicate no significant difference (p < 0.05)

3.7 The total phenolic cream mixture of turmeric extract and tamarind leaves

The total phenolic value of the cream treatment concentration of turmeric and tamarind leaves extract ranged from 1.26 to 18.2 mg GAE/g (Table 8). The total phenolic value of the cream increases with the increase in the concentration of turmeric and tamarind leaves extract, this is because the main content of turmeric is flavonoids which are phenolic compounds [22]. The phenolic compounds in turmeric include curcumin which acts as an antioxidant [23, 24]. Mukhopadhyay et al. [25] clinically stated curcumin as a potent free radical scavenger. Curcumin interacts with a number of biomolecules through non-covalent and covalent bonds [26]. The addition of tamarind leaves is intended to increase antioxidant activity [27], and it is proven that both show synergism[11]. Huang et al. [28] stated that antioxidant

activity is directly proportional to total phenolic, the higher the phenol content in the ingredient, the higher its activity as an antioxidant.

Table 8 The average of total phenol cream in the treatment of the concentration of extract turmeric and tamarind leaves

Treatment concentration of turmeric extract mixture and tamarind leaves (10:2)	Average total phenol mg GAE/g
K1 (0%)	1.26c
K2 (1%)	1.30c
K3 (2%)	1.33c
K4 (3%)	1.47bc
K5 (4%)	1.53ab
K6 (5%)	1.82a

Description: the same letters behind the average value indicate no significant difference ($p < 0.05$)

3.8 Inhibition of cream against collagenase activity

The effect of the concentration of turmeric and tamarind leaves extract on anti-collagenase activity (IC_{50}) and its coefficient of determination (R^2) is presented in Table 9. The cream treatment of turmeric and tamarind leaves extract that produced the lowest IC_{50} value was 0.36 µg /ml at 5% extract concentration. The smaller IC_{50} value indicates that the cream has the ability to inhibit the activity of the collagenase enzyme which is getting bigger or higher. The decrease in IC_{50} value in the cream with increased concentrations of turmeric and tamarind leaves extract was due to the higher phenolic compounds contained in the cream, so that the inhibition of collagenase activity was also greater. Plant extracts with total phenolic content (0.05-0.26) mg GAE/mL, were known to have inhibitory activity against collagenase [10]. According to the statement of Smet et al [29] the overall height phenolic content in cream is also due to the synergism of antioxidants and the combination of two types of antioxidants is proven to increase its effectiveness. A mixture of turmeric and tamarind leaves extracts increased the effectiveness of phenolic compounds, a mixture of the two extracts in a ratio (10:2), showed the highest antioxidant synergism, amounting to 115.969% [30]. The total phenolic value of the cream was 1.26–17.2 mg GAE/g, indicating that all creams had an inhibitory effect on collagenase because the value was higher than the range stated by Thring et al [10].

Table 9 Average IC_{50} of the anti-collagenase cream treatment concentration of a mixture of turmeric extract and tamarind leaf

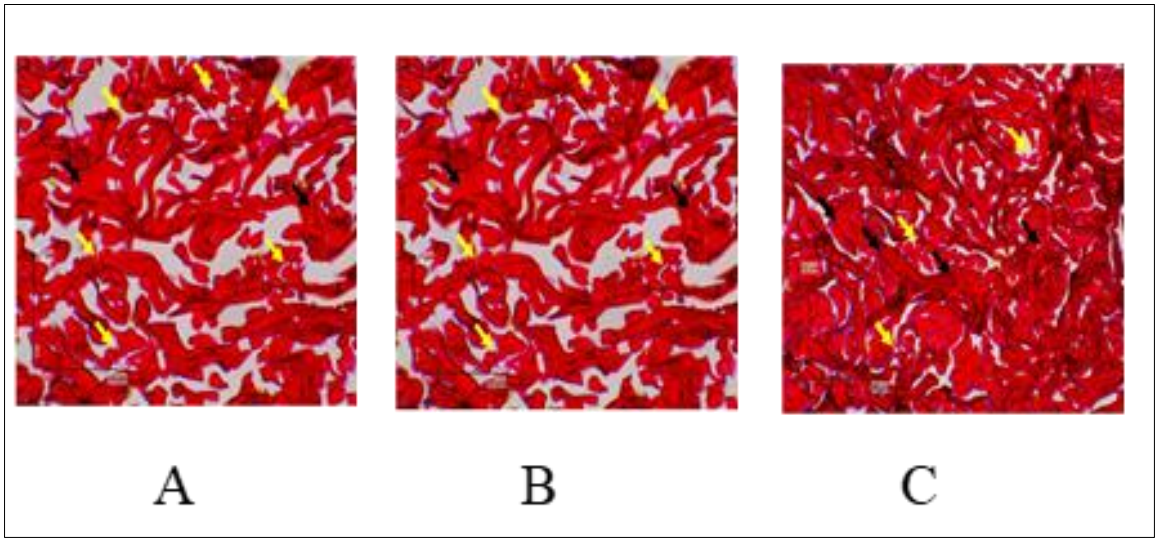
Treatment concentration of turmeric extract mixture and tamarind leaves (10:2)	Average IC50 (µg/ml)
K1 (0%)	2.20a
K2 (1%)	2.12a
K3 (2%)	2.03a
K4 (3%)	1.88a
K5 (4%)	0.89b
K6 (5%)	0.36c

Description: the same letters behind the average value indicate no significant difference ($p < 0.05$)

3.9 The ability of the cream to increase the amount of skin collagen

In the control treatment (P0), the amount of collagen in the dermis tissue decreased because UV radiation energy damaged cell membranes and proteins, resulting in the production of ROS (reactive oxygen species). The results of this study prove that the antioxidants in turmeric and tamarind leaves are able to act as free radical scavengers, as evidenced in the skin of rats given turmeric and tamarind leaves extract cream (P2) produced higher collagen values and different from the other treatment groups. The higher amount of collagen in the dermis in the P2 treatment was due to the energy of UV radiation which damages cell membranes and proteins suppressed by the antioxidants in the cream. The IC_{50} value of turmeric and tamarind leaves extract is very strong because of $IC_{50} < 50$ g/ml [31].

The result of the observation of the amount of collagen the rat dermis tissue with Picro Sirius painting is presented in Figure 2. In the control treatment (Figure 1A) it was seen that the collagen on the rat dermis was damaged in the structure and structure of the collagen with red collagen fibers that was thin. Intact collagen fibers are indicated by black arrows. Incomplete collagen fibers are indicated by yellow arrows. Damage to the composition and structure of collagen is caused by UV-B exposure which reduces the amount of collagen in the dermis tissue because UV radiation energy damages cell membranes and proteins, thereby producing ROS. Approximately 50% of UV induces damage stemming from the free radical formation[2].



(Note: black arrows indicate intact collagen fibres. Yellow arrows indicate collagen that is not intact)

Figure 1 Expression of mouse dermis tissue collagen at 400x magnification with Picro-Sirius Red painting: (A) UV-irradiated rats' skin without cream; (B) UV irradiated rat skin given a placebo cream, (C) UV irradiated rats' skin given a cream of turmeric extract and tamarind leaves extract

Placebo treatment group Figure 1B, the results show collagen with red fibers that are wider and thicker. Compared to the control group (Figure 1A), the placebo treatment group (Figure 1B) showed a higher expression of rat dermis tissue collagen, this indicates that the placebo cream was able to inhibit UV-B light exposure. Placebo cream was able to inhibit UV-B light exposure because it contains ingredients such as VCO, mineral oil, and moisturizer conditioner. VCO contains 92% saturated fatty acids consisting of lauric acid. Oleic acid, lauric, and oleic acid content which has properties to smooth the skin [32]. Mineral oil has a role as an emollient, moisture conditioner is useful for maintaining water content in the skin because water is important for maintaining skin plasticity and integrity [12].

In the treatment of turmeric and tamarind leaves extract cream (Figure 1C), the amount of collagen with red collagen fibers appeared wider, thicker and more intact collagen fibers also appeared. This condition was caused in addition to the placebo cream as well as the presence of turmeric and tamarind leaves extract in the cream. UV radiation will induce an increase in collagenase-1 (MMP-1) [33]. The main enzyme responsible for breaking down collagen-1 is the collagenase group[7]. The results of this study proved that the turmeric and tamarind leaves extract at a concentration of 5% was able to inhibit the activity of the collagenase enzyme. So that the treatment of rats given cream of turmeric and tamarind leaves extract have less collagen damage. Intact collagen fibers also prove that phenolic compounds are able to inhibit the collagenase enzyme that causes collagen damage.

3.10 The ability of the cream to decrease MMP-1 expression on rat

Table 10 shows that the cream treatment of extracts of turmeric rhizome and tamarind leaves had a significant effect ($P<0.05$) on the levels of matrix metalloproteinase-1 (MMP-1) in the skin of wistar rats. The results showed that exposure to UV-B ray increased MMP-1 levels up to 32.530 ± 2.889 µg/ml in the skin tissue of control rats (P0). On the skin of rats given cream of turmeric rhizome and tamarind leaf extract (P2), MMP-1 levels decreased to 12.147 ± 2.380 µg/ml. This shows that the cream is able to inhibit UV exposure or the cream has reducing activity against free radicals. This inhibition is due to the cream containing phenolic compounds and vitamin C.

Mulyani et al. [1], stated that turmeric and tamarind leaves extract cream had anti-collagenase and antioxidant activity, with the phenolic content of turmeric and tamarind leaves extract reaching 0.12 mg GAE/ml [11]. Turmeric and

tamarind leaves are one of the plants as sources of antioxidants [30], the antioxidant properties of tamarind leaves come from the content of vitamin C[1] and phenolic compounds [34]. Phenolic compounds in turmeric are included in primary antioxidants or chain-breaking antioxidants, ascorbic acid in tamarind leaves functions as a secondary antioxidant, namely as a chelating agent [35]. Both types of antioxidants stop the chain reaction that destroys the formation of MMP-1, so that the levels of MMP-1 in the cream-treated rats are lower. The combination of two types of antioxidants has been shown to increase their effectiveness or have synergism [36].

The cream of turmeric and tamarind leaves extract with a concentration of 5% has the ability to increase the amount of skin collagen by 23, 67%. This value is much higher than the inhibitory value of green tea extract of 19.76% [8], purple corn of 6.16% [3], corn silk of 15.13% [36] and bitter melon extract of 23.17% [4]. In lowering the expression of MMP-1, the value of 20.383% was obtained, this proves that the cream of turmeric and tamarind leaves extract with a concentration of 5% has a better ability than green tea extract of 27.45% [8], and bitter melon extract 40.59% [4]. Based on these data, it shows that turmeric and tamarind leaves extracts have great potency as active ingredients in anti-aging creams.

Table 10 Average levels of MMP-1 in rat skin dermis tissue in each treatment

Treatment group	Average MMP-1 (µg/ml)
P0 (UV-irradiated without cream)	32.530+2.889a
P1 (UV irradiated-given placebo cream)	24.728+1.929ab
P 2 (UV irradiated-given cream of turmeric extract and tamarind leaves)	12.147+2.380b

Description: the same letters behind the average value indicate no significant difference ($p < 0.05$)

4 Conclusion

HLB, perfume type, and the interaction of the two do not significantly affect adhesion time, pH, separation ratio, viscosity, and total phenol. The type of perfume affects the dispersive power, but the HLB and perfume type has a significant effect on overall acceptance. HLB 10 cream on all types of perfumes shows the best characteristic cream. The lowest anti-collagenase IC_{50} value was obtained from the cream with a 5% concentration of turmeric and tamarind leaves extract, IC_{50} value: 0.36 g/ml. Application of cream mixture of turmeric rhizome extract and tamarind leaves with a concentration of 5% gave a significant effect on the decrease in MMP-1 expression up to 12.147+2.380 µg/ml and increase the amount of collagen by 23. 67% in the skin of wistar rats.

Compliance with ethical standards

Acknowledgments

Thank you to the directorate general of higher education, the ministry of culture for providing research grants and publications. In addition, the Chancellor, Head of LPPM and Dean of the Faculty of Agricultural Technology, Udayana University who have facilitated the implementation of this research and publication.

Disclosure of conflict of interest

The researchers as well as the authors of this manuscript have no conflict of interest either between the authors or with other parties

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