VOL 35 (3) 2024: 521-530 | RESEARCH ARTICLE

Formulation of a Lotion with Ethanol Extract of *Curcuma manga* Val. as a UV B Sunscreen and Activity Test by UV-Vis Spectrophotometry

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Article Information	ABSTRACT
Submitted: 24-08-2023 Revised: 12-01-2024 Accepted: 15-01-2024	Temu mango (<i>Curcuma manga</i> Val) is a rhizome plant native to Indonesia that contains curcuminoid and flavonoid antioxidant compounds. These compounds are believed to act as UV-light protectors, especially UV B.
*Abdul Karim Zulkarnain	In this study, we performed a qualitative test of the compound content in temu mango followed by formulation and activity testing of water in oil (w/o)
Email: <u>akarimzk@ugm.ac.id</u>	sunscreen lotion of temu mango in vitro. The optimization of the w/o lotion formulation was performed using Design Expert [®] (DE) software version 9.0.4.1 with the Simplex Lattice Design method. The DE software obtained 13 runs of the w/o lotion formula and continued testing the physical property to determine the optimum formula. The optimum formula of lotion w/o predicted by DE software was tested for physical stability for over 4 weeks; and the SPF test, percent pigmentation, and percent erythema were determined by UV-Vis spectrophotometry. The present results suggested that temu mango extract provided an SPF value at a concentration of 0.3 mg/mL with an SPF value of 16.62. The optimum formula of the lotion w/o were 7% glycerin, 7% cera alba, 5% cetyl alcohol with a value of stickiness of 0.70 ± 0.03 s, spreadability of 13.20 ± 0.83 cm ² , and viscosity of 95.19 ± 3.06 dPa.s. Referring to the storage results, the optimum formula of w/o lotion from temu mango extract was stable during a month of storage. The w/o lotion in the optimum formula produced an SPF value of 15.06 ± 0.39, % erythema of 10.95%, and percent pigmentation of 10.41%.
	Reyworus: <i>curcuma manga</i> , antioxidant, sunscreen, curcuminoids, flavonoids

INTRODUCTION

Excessive sun exposure is a major exogenous mediator of skin damage that can accelerate aging and increase the risk of skin cancer (Bhattacharya & Sherje, 2020; Stevanato *et al.*, 2014). UV rays are beneficial for the formation of vitamin D3 (cholecalciferol), which is required to metabolize bone formation and the immune system. However, continuous exposure to UV light can have adverse health effects, such as skin cancer, sunburn, melanoma, premature skin aging, pigmentation, erythema, and immune system damage (Almeida *et al.*, 2019; Bustamante *et al.*, 2020; Gollavilli *et al.*, 2020).

Human skin has a mechanism to protect against the dangers of UV rays, that is by forming pigment grains (melanin) that reflect UV rays. Exposure of the skin to sunlight can induce melanin reactions, the rapid addition of melanin to the skin surface and the formation of additional new melanin. However, continuous exposure to UV rays can cause hyperpigmentation, leading to the information of dark spots on the skin and other skin damages, such as premature aging and skin cancer (Imokawa, 2009; Longo *et al.*, 2013; Shanbhag *et al.*, 2019). Therefore, sunscreen is required to protect the skin from the adverse effects of UV radiation (Zastrow *et al.*, 2017).

Compounds with sun protection activity are very useful in reducing the adverse effects of UV radiation on the skin. However, several UVabsorbing active substances can cause photoallergies, skin irritation, rash, active sunburn, and active autoimmune diseases (Matta et al., 2019, 2020; Nash, 2006; Parwaiz et al., 2019). Therefore, a formulation containing plant extracts is currently being developed. Cosmetics prepared from plants that are commonly used to avoid aging contain antioxidant compounds. The polyphenol content of antioxidant compounds can minimize free radical activity and protect the skin from UV radiation. Compounds containing aromatic rings can absorb UV rays, especially UV A and UV B, at wavelengths of 200- 400 nm (Ferreira *et al.*, 2023; Goyal *et al.*, 2022; Kageyama & Waditee-Sirisattha, 2019).

A few sunscreens still use active substances derived from active compounds of natural ingredients. Therefore, the researcher intends to prepare a sunscreen using active compounds of natural ingredients from temu mango (Curcuma manga Val). Previous studies have shown that temu mango contains antioxidant compounds, including chalcones, flavanones, flavones, and curcumin which have chromophore groups and aromatic rings (Muchtaromah et al., 2021; Pujimulyani et al., 2020). The active compounds found in temu mango, such as rhizome, curcumin, and flavonoids, can be used in sunscreen formulations (Liu et al., 2018; Pujimulyani *et al.*, 2020). Thus, they can be considered as the basic components when testing for the optimization of the temu mango ethanol extract formula as a sunscreen in w/o lotion dosage form.

The lotion w/o dosage form was selected because it is more commonly used for topical sunscreen preparations (Da Silva & Ricci-Júnior, 2020; Qian et al., 2016). Lotions can be suspensions, emulsions, or solutions without or with drugs intended for topical application, whose level of liquidity enables even and rapid application on a large surface of the skin to allow it to dry quickly, apply and spread easily and coat a thin layer of the components on the skin surface. The w/o type lotion offers several advantages, such as waterproof adhesion, which make it suitable for outdoor activities such as exercising, suitable for dry skin, and long staving properties, which make the composition and effectiveness better in comparison to those of the o/w type (Barnes et al., 2021; Radhakrishnan et al., 2018).

For lotion preparations to meet the criteria for good skin protection, it is necessary to optimize the lotion w/o sunscreen formula by using cera alba, cetyl alcohol, and a glycerin base. Cera alba improves the lotion consistency, cetyl alcohol serves as an emulator and emollient thereby improves the lotion stability, and glycerin serves as a humectant and emollient as well as contributes to the stability of the lotion. Optimization of the content of these three ingredients is expected to provide good physical stability and comfort.

MATERIALS AND METHODS Instrumentation

Oven, Buchner funnel (Pyrex®), rotavapor R-100 (Buchi), water bath (Wnb14, Memmert[®]), moisture balance (Ohauss Mb23), thin layer chromatography apparatus, UV-Vis spectrophotometer (Genesys 10S, USA), analytical balance (Ohauss PA 214), Ultra-turrax T25 (Janke & Kunkel, IKA[®]), universal pH, centrifuge (EBA[®]), spreadability test kit (Pharmaceutical Technology Laboratory. UGM). adhesion test kit (Pharmaceutical Technology Laboratory, UGM), and VT 04 series viscosimeter (Rion Co, Ltd, Japan) were used in this study.

Materials

Temu mango rhizome (*Curcuma manga*) were collected from across Gunung Gondang, Margosari, Pengasih, Kulonprogo in February 2015 at a harvest age of 6 months, 70% ethanol (Brataco), chloroform (Merck®), methanol (Merck[®]), curcuminoids (Sigma[®]), silica gel 60 F₂₅₄ plate (Merck[®]), acetic acid (Merck[®]), distilled water, cellulose plate (Merck[®]), cetvl alcohol (Merck[®]), glycerin (Merck[®]), mineral oil (Sigma[®]), span 80 (Merck[®]), cera alba (Merck[®]), methylparaben (Sigma[®]), propylparaben (Sigma[®]), and 96% ethanol (Merck®) were used in the study.

Preparation of the temu mango extract

A total of 5 kg of temu mango rhizome powder was extracted by using the maceration method, dissolved in 10 L of 70% ethanol, and soaking for 24 h. The macerate was then separated with a vacuum Buchner, evaporated using a rotatory evaporator, and re-evaporated in a water bath until a liquid extract of temu mango rhizome was obtained.

Specification test of the temu mango liquid extract

Organoleptic

An organoleptical test was conducted by observing the color, odor, and shape of the specimen.

Qualitative testing of total flavonoids

Qualitative tests of total flavonoids were carried out using the thin-layer chromatography method with a mobile phase of acetic acid: water (3:1 v/v), and a cellulose stationary phase, and detection was performed using UV light at 254 and 366 nm and sprayed using AlCl₃.

Materials	F-1(%)	F-2(%)	F-3(%)	F-4(%)	F-5(%)	F-6(%)	F-7(%)	F-8(%)	F-9(%)	F-10(%)
Temu mango extract	0.135	0.135	0.135	0.135	0.135	0.135	0.135	0.135	0.135	0.135
Mineral oil	40	40	40	40	40	40	40	40	40	40
Cetyl alcohol	2	5	3	3.5	4.5	3.5	5	4.5	4	5
Glycerin	10	7	9.5	8.5	8	10	10	9.5	9	8.5
Beeswax	7	7	6.5	7	6.5	5.5	4	5	6	5.5
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Span 80	7	7	7	7	7	7	7	7	7	7
Aquadest	add 100									

Table I. The optimization results of the w/o lotion formula using the Simplex Lattice Design method with Design Expert[®] version 9.0.4.1 software.

Qualitative test for curcumin

The qualitative test of curcumin was carried out using the thin-layer chromatography method with the mobile phase composed of chloroform P: methanol P (4.75: 0.25 v/v), stationary phase silica gel 60 F₂₅₄, followed by detection using UV light at 254 and 366 nm.

Drying shrinkage

A drying shrinkage test was performed by weighing 1 g of liquid extract into a closed weighing bottle. Furthermore, it was heated at 105°C for 5 h in an oven (Komala & Haryoto, 2021). The drying shrinkage was obtained using the following formula:

Drying Shrinkage= $\frac{x(g) - y(g)}{y(g)}$ X 100%

X = Weight of the extract after drying Y = Weight of extract before drying

SPF value examination of the temu mango ethanol extract

The ethanol extract of temu mango was prepared by dissolving it in an ethanol solvent, taking as much as 0.2; 0.225; 0.25; 0.275; and 0.3 mL of the extract and diluting it with 70% ethanol up to 10 mL volume. The sample was measured at a wavelength of 290- 320 nm at each 5 nm increment and repeated twice; the SPF value was calculated using the Mansur method (Malsawmtluangi *et al.*, 2013) by applying the Mansur equation given below:

SPF= CF × $\sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$

CF = Correction factor, EE = Erythema effect spectrum, I = Solar intensity spectrum, Abs = Absorbance of the sample.

Formulation of w/o lotion from temu mango ethanol extract

Formulation of the w/o lotion

The w/o lotion formulation was prepared by heating the oil phase (mineral oil, span 80, cetyl alcohol, cera alba, propylparaben) at 65- 75°C until melted. The water phase (glycerin, water, methylparaben) was heated separately at 65- 75°C. The extract was mixed in boiling water and then sonicated and mixed with glycerin and propylparaben. The water phase was gradually added to the oil phase and stirred using ultraturrax until a homogeneous solution was obtained. The prepared lotion was added into a container and allowed to stand for 24 h (Table I).

Evaluation of w/o lotion from the ethanol extract of temu mango rhizome Viscosity

The viscosity test was performed using a Rion Viscosimeter VT 04 series. The viscosity results were recorded in units of dPa.s (Alam *et al.*, 2020).

pH Test

The pH test was conducted using a universal pH by applying lotion on universal pH paper (Arifin *et al.*, 2020).

Adhesive Power Test

The adhesion test was conducted by applying 0.1 g w/o lotion on the top of another glass object and then crushed with a load of 1 kg for 5 min. Then the glass object was mounted on the test device, the load weighing 20 g was released, and the time was recorded until the two glass objects were released (Arifin *et al.*, 2020).

Spreadability Test

The spreadability test was carried out by applying 0.5 g of the w/o lotion to center of a round

glass scale, and allowed to stand for 1 min. Furthermore, an additional load was added at the 2nd, 3rd, 4th, 5th, 6th, and 7th min. Each additional load of 50 g was added and allowed to stand for 1 min, after which the diameter of the lotion spread extent was recorded (Alam *et al.*, 2020).

Volume separation by centrifugation

The lotion was placed into two scaled test tubes to certain scale. The scaled test tubes containing the w/o lotion preparation were tested by centrifugation at 1500, 3000, and 4500 rpm for 30 min, observing every 5 min increment. The volume of separation was calculated using the following formula:

Separation(%)= $\frac{A}{B}$ 100%

A= the volume of emulsion that separates; B= the volume of whole emulsion

Activity evaluation of w/o lotion from temu mango ethanol extract

Determination of SPF value of w/o lotion

A total of 0.25; 0.5; 0.75; 1; and 1.25 g were added to a 25 mL volumetric flask, and then dissolved in a mixture of 96% ethanol and 1:1 chloroform up to 25.0 mL until dissolved. After being completely dissolved, the sample was filtered using filter paper. The filtrate was subjected to absorbance determination with a UV-Vis spectrophotometer every 5 nm in the wavelength range of 290- 320 nm (Malsawmtluangi *et al.*, 2013).

Determination of pigmentation percentage and erythema percentage

The effectiveness of sunscreen preparations can be determined by determining the percent erythema transmission (%Te) and percent pigmentation transmission (%Tp) as follows: (Saraha *et al.*, 2018).

Erythema % equation:

$$(Te) = \frac{\Sigma Ee}{\Sigma Fe} = \frac{\Sigma(TxFe)}{\Sigma Fe}$$

Ee= Erythema Energy; Fe = Erythema effectiveness factor

Pigmentation % equation:

$$(Tp) = \frac{\Sigma Ee}{\Sigma Fp} = \frac{\Sigma(TxFp)}{\Sigma Fp}$$

Ee= Erythema Energy; Fe = Pigmentation effectiveness factor

Statistical Analysis

All data are expressed as the mean \pm standard deviation (SD). Computerized data are described statistically with Microsoft Excel v.10.0 (Microsoft, US). The independent variable responses, which include viscosity, pH, adhesion, and spreadability, were analyzed by using Design Expert[®] version 9.0.4.1 software. The optimum formula and the response results were analyzed using a one-sample t-test with IBM statistics v.20.

RESULTS AND DISCUSSION

Characteristics of the temu mango ethanol extract

The ethanol extract of temu mango has a brown color, a distinctive smell of temu mango, and is viscous, with a drying shrinkage of $17.27 \pm 0.55\%$ and a moisture content of 1.67%. The water content test revealed that the liquid temu mango extract contained water that met the requirements for water content in extracts, which is not more than 30%. The water content in extracts that are too high can be a growing medium for bacteria and fungi that can damage the compounds contained in the extract (Ekramian *et al.*, 2021).



Figure 1. Chromatogram of flavonoid detection with a cellulose stationary phase and 15% acetic acid: water (3:1) mobile phase after spraying with AlCl₃. A. UV 366 nm before being sprayed with AlCl₃ B. UV 366 nm after being sprayed with AlCl₃

Based on the results of flavonoid detection using thin-layer chromatography (Figure 1), the spot color obtained under UV light 366 was greenish-yellow. However, after being sprayed with AlCl₃, no color change was found. The spot color in the flavonoid structure can be interpreted as follows; if the results of irradiation in UV light produce a light blue fluorescent color or yellowgreen fluorescence, then the compound is believed to contain flavonoids with flavone and flavanone types that do not contain 5-OH and flavanols without free 5-OH but with refined 3-OH (Panche *et al.*, 2016).



Figure 2. Chromatogram of curcuminoids with mobile phase chloroform P: methanol (4.75: 0.25 v/v) and stationary phase silica gel 60 F_{254} . (A) Visible light (B) UV 254 nm (C) UV 366 nm

Curcumin detection using a 1% curcuminoid comparator (Figure 2) presented three spots, with the same spot color and hRf price as the comparator used. The color of the sample spot and the comparator in visible light was yellow, with a spot of curcumin that displayed fluorescence at a wavelength of 366 nm. Curcumin displayed the highest Rf because curcumin contains more methoxy groups. Methoxy groups reduce the polarity of curcumin, hence curcumin elutes more easily than demethoxycurcumin and bisdemethoxycurcumin (Monton *et al.*, 2016).

Based on the Food and Drug Administration (FDA), the SPF value at 3% concentration is included in the medium SPF as it has an SPF value of 16.62 (Table II). The minimum sunscreen

protection value recognized by the FDA is 15. The SPF value at 3% concentration was selected as the concentration of active ingredients in the formulation of w/o lotion preparations. The 3% concentration of temu mango extract was then calculated as the concentration of extract used to manufacture the w/o lotion formula (100mL) with an SPF value of 16.62. Based on this calculation, the weight of the extract to be used in the formulation was set at 0.135 mg.

Table II. SPF value determination results for temu mango extract

Concentration (%v/v)	SPF Value
2%	11.58 ± 0.33
2.25%	12.66 ± 0.04
2.5%	14.61 ± 0.60
2.75%	16 ± 0.67
3%	16.62 ± 0.08

Formulation of the w/o lotion

The optimization of the w/o lotion formula from the ethanol extract of temu mango was performed by entering the properties and stability of the thirteen formulas as a response into the Design Expert software (version 9.0.4.1). The physical and stability properties entered as responses were spreadability, stickiness, and viscosity, which were tested on day 1 after manufacturing.

The results of the ANOVA analysis contained in the design expert displayed that the model for the response of stickiness and viscosity produces a p-value of <0.05, simplying that the results of stickiness and viscosity in all optimized formulas differ in the values of stickiness and viscosity. Meanwhile, the results of the lack of fit analysis of the response of spreadability, stickiness, and viscosity produced a p-value of >0.05, which suggests a lack of any significant difference between the observed data and the predicted data from the model.

The results of the superimposed contour plot diagram using the Design Expert software version 9.0.4.1 displayed that the optimum formula was a formula was 5% cetyl alcohol, 7% cera alba, and 7% glycerin with the highest desirability value of 0.923 (Figure 3). This value ranged from zero to one, the closer the value was to one, the higher the possibility of obtaining the desired response.



Figure 3: Overlay plot graph of the optimal formula

Table III. One-sample t-test results of the optimum formula of lotion w/o temu mango ethanol extract predicted by the software compared with the experimental results.

Parameter	Prediction	Experiment	Significance	Conclusion
Viscosity (dPa.s)	88.01	95.19 ± 3.06	0.056	Not significant
Adhesion (s)	0.68 s	0.70 ± 0.03	0.472	Not significant
Spreadability (cm ²)	13.47	13.20 ± 0.83	0.623	Not significant

The solution offered by the Design Expert version 9.0.4.1 for the optimum formula has a desirability value of 0.923, simplying that the ability to predict the physicochemical properties of the optimum formula was approximately 92.3%.

In addition to predicting the optimum formula, the Design Expert software can predict the value of the physical properties and stability of the optimum formula. The predicted values offered in each response were spreadability of 13.471 cm², stickiness of 0.68 s, and viscosity of 88.006 dPa.s (Figure 3). There exist no specific requirements for the standardized values of spreadability, stickiness, and viscosity of the w/o lotion preparations.

The superimposed gotten results in a yellow-colored zone that provides the optimal response. In that zone, it provides one prognosis of the optimum w/o moisturizer equation with an appealing quality esteem of 0.923, which contains 5% cetyl alcohol, 7% cera alba, and 7% glycerin. After obtaining the ideal equation and the expected physical parameters, it is critical to validate the rationality of the anticipated response with the test results.

Verification of the Optimization Results and Statistical Analysis

The predicted response value of the optimum formula obtained from the software was then compared with the response obtained in the experiment on day 1 after manufacture (week 0). Thus, the significance between the physical properties of the optimum formula and the physical properties of the optimum formula according to the software can be determined. The results of the one-sample t-test of the predicted response and the experimental response of the optimum formula of w/o lotion (Table III).

The results of the viscosity, stickiness, and spreadability responses (Table III) were not significantly different between the predictions and experimental findings. Thus, it can be concluded that the experimental results were in accordance with the predicted response.

Evaluation of the Physical Stability of Lotions

The physical properties test of the optimum lotion formula during storage from week 0 to week 4 indicated no change in color, odor,

shape, homogeneity, or pH. The w/o lotion has a yellowish-white color and a distinctive odor of temu mango extract, with a smooth and soft texture, it retained its homogeneity and pH from weeks 0 to 4.

Statistical analysis of the viscosity test of the optimum w/o lotion formula during storage from weeks 0 to 4 as per the ANOVA test with a confidence level of 95%. The results indicated a p-value of 0.475, meaning no change in the viscosity of the optimum formula during storage. Meanwhile, the results of statistical analysis for the response of adhesion and spreadability using the Kruskal-Wallis test showed that the p-value was <0.05, implying the presence of changes in stickiness and spreadability during storage. These changes in the values of stickiness and spreadability during storage can be attributed to changes in the room temperature during the experimental period.

Stability testing of the w/o lotion

Accelerated stability tests can be conducted under stress conditions such as temperature and mechanical influences. One of the mechanical separation tests is centrifugation. The centrifugation test can be employed for accelerated stability testing because it is related to gravity. This finding agrees with Stokes' law, where separation is influenced by gravity, and the increasing gravity accelerates the separation (Okafo et al., 2023). The results of the centrifugation test showed that the separation ratio at 1500, 3000, and 4500 rpm for 30 min was F = 1, indicating that all lotions were stabilized at a high speed.

Activity test of w/o lotion from temu mango ethanol extract

The effectiveness of sunscreen preparations can be determined by calculating the SPF value of the preparation. The SPF value describes the ability of sunscreen products to protect the skin from erythema (Ma & Yoo, 2021; Morocho-Jácome *et al.*, 2021). The SPF price obtained in a preparation state the number of times a person's natural skin resistance is multiplied; hence, it is protected from solar radiation without getting burned.

The results of the SPF value indicate that the additional ingredients used to prepare the w/o lotion had an SPF value of 2.29 ± 0.33 . According to the FDA, the SPF price of the w/o lotion from temu mango ethanol extract is included in the medium category because it has an SPF value of 15- 30 (Cvetkovska *et al.*, 2017; Miksa *et al.*, 2016).

The observation revealed that w/o lotion in the optimum formula resulted in 10.41% pigmentation and 10.95% erythema. Therefore, the w/o lotion from the temu mango ethanol extract formulation gave a percent erythema (%Te) and percent pigmentation (%Tp) value that can be categorized as that for a standard suntan protection formula.

CONCLUSION

Temu mango ethanol extract contains active compounds that can be used to prepare a UV-B protective sunscreen with an SPF value of 16.62. In addition, the SPF value is included in the moderate protection category, as per the FDA standards (15-30 SPF); the SPF value met the recommended value in tropical countries, such as Indonesia. The formulation of temu mango ethanol extract in the w/o lotion preparation was 3% extract concentration, 5% cetyl alcohol, 7% cera alba, 7% glycerin, 40% mineral oil, 0.2% methylparaben, 0.1% propylparaben, and 7% span 80, which vielded an SPF value of 15.06 ± 0.39, 10.41% pigmentation, 10.95% erythema, as well as moderate protection in accordance with the FDA standards.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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