

Determination of sun protection factor and antioxidant properties of cream formulation of kencur (*Kaempferia galanga* L) and temu kunci (*Boesenbergia pandurata* (Roxb.) Schlecht) rhizomes extract

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ABSTRACT

Kencur (*Kaempferia galanga*, L.) rhizome contain ethyl-p-methoxycinnamate (EPMS) which has sunscreen properties. Temu kunci (*Boesenbergia pandurata* (Roxb) Schlecht) rhizome contains flavonoid and essential oils with radical scavenging properties. The aims of this study were to obtain the optimum physical properties of sunscreen and antioxidant cream and to compare the sun protection factor (SPF) values and antioxidant activity of kencur and temu kunci rhizomes extract before and after formulated. This research used Simplex Lattice Design (SLD) model with 2 components of kencur extract and temu kunci extract. Based on the SLD model obtained optimum formula design, then the SPF values and antioxidant activity were studied by UV spectrophotometric method and DPPH method respectively. Based on SLD model obtained optimum formula that containing kencur : temu kunci extract 80%:20% (formula A) and 70%:30% (formula B). The SPF values of kencur extract, formula A and formula B were 4.505, 5.024 and 4.511 respectively. Antioxidant activity showed that the IC₅₀ of formula A, formula B, temu kunci extract, BHT and Vitamin E were 109.15 µg/mL, 95.23 µg/mL, 10.20 µg/mL, 22.33 µg/mL and 8.78 µg/mL respectively. Cream formula A and formula B have optimum physical properties. The SPF value of the optimum formulations were higher than SPF value of kencur extract. Antioxidant activity of that combinations were lower than temu kunci extract, BHT and Vitamin E.

Keywords: kencur, temu kunci, SPF, antioxidant, Simplex Lattice Design

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INTRODUCTION

Ultraviolet (UV) radiation can be divided into 3 regions: UVA (400-320 nm) UVB (320-290 nm) and UVC (290-200 nm). UVA and UVB have negative effects on the human skin. Long term exposure of sun radiation could make degenerative changes in the skin cells which leads to premature ageing, sunburns and skin cancers (Gajardo *et al.*, 2016). The compounds that have sunscreen and antioxidant activity can prevent or minimize the negative effects of solar radiation on the skin.

Kencur (*K. galanga*, L.) is a tropical plant from Zingiberaceae family. This plant has been found in South India and Southeast Asia such as Malaysia, Indonesia, and Thailand (Sirisangtragul, 2011). Based on the data of Gas Chromatography and Mass Spectrometry (GC-MS), the largest peak area which correspond to volatile oil of kencur rhizome was ethyl p-methoxycinnamate (EPMC) (31.77%) (Tewtrakul, 2005; Raina and Abraham, 2015). Ethyl p-methoxycinnamate (EPMC) has been used as sunscreen, analgesic and anti-inflammatory agent, as cyclooxygenase-2 (COX-2) inhibitor, and to treat fibrosarcoma in mico (Ekowati *et al.*, 2016). Based on Cakhyo (2010), purified kencur extract has medium protection to UV radiation which Sun Protector Factor (SPF) value was 4.68. SPF values of kencur rhizome increased from 6.26 to 6.47 after formulated into cream dosage form (Nurhayati, 2011).

Temu kunci (*B. pandurata* (Roxb) Schlecht) is one of the ginger plants that is found in South East Asia. The main chemical compounds in temu kunci ethanolic extract are flavonoid and essential oils (Chahyadi *et al.*, 2014). The antioxidant activity of temu kunci extract has been investigated by Nihlati (2011). This study showed that temu kunci ethanolic extract have strong antioxidant activity with IC₅₀ value was 10,36 µg / mL, while IC₅₀ values of vitamin E and BHT as positive controls were 8.27 µg / mL and 19.5 µg / mL respectively.

Simplex Lattice Design is one method to determine the profile of mixed effects on a parameter (Bolton, 1997; Satish, *et al.*, 2012). This study used the Simplex Lattice Design (SLD) method of two extract combinations so that two optimum formula designs were obtained. The physical properties of the cream that will be used as parameters in the SLD method are pH, viscosity and dispersion.

Natural cosmetics that have sunscreen and antioxidant activity should be developed, because it is safer to use long term. The aims of this study were (a) To obtain the optimum physical properties of sunscreen and antioxidant cream by Simplex Lattice Design (SLD) model; (b) to compare the sun protection factor (SPF) values and antioxidant activity of kencur and temu kunci rhizomes extract before and after formulated.

MATERIALS AND METHOD

Plants collection and extraction

The dry rhizomes of kencur and temu kunci were collected from Yogyakarta, Indonesia. This plants were identified at Laboratory of Botany and Genetics, Faculty of Teacher Training and Development, Universitas Muhammadiyah Purwokerto by microscopic analysis.

Preparation of kencur ethanolic extract

The 250 g of the rhizome powder of kencur was percolated in ethanol 96%, the percolate then concentrated with a rotary evaporator until the ethanol vaporised. The concentrate extract reheated at 55°C by waterbath until obtained a fixed weight. The concentrate extract then recrystallized to obtain purified extract.

Preparation of temu kunci ethanolic extract

The 296.5 g of temu kunci rhizome was macerated in ethanol 96% for 24 hours with 3 times. The macerate were filtered, collected, then concentrated with a rotary evaporator until the ethanol vaporised. The concentrate extract than reheated at 55°C by waterbath until obtained a fixed weight.

Formulation and physical properties evaluation

Base cream containing water and oil phases was prepared. The compositions and the amounts of ingredients are shown in Table I. In order to prepare the cream, different amount of ingredients were incorporated together, and then the required amount of the herbal extract was added.

Table I. Formulation of sunscreen and antioxidant cream

Components	Amount (% w/w)		
	F1	F2	F3
Kencur extract	4	0	2
Temu kunci extract	0	4	2
Paraffin liquidum	40	40	40
Cetyl alcohol	5	5	5
Tween 80	3.25	3.25	3.25
Span 80	1.75	1.75	1.75
Water	ad 100	ad 100	ad 100

Cream that has been made further examined its physical properties include organoleptic identification, pH measurements, viscosity measurements and spreadability.

Cream optimization by Simplex Lattice Design Method (SLD)

The physical properties of the cream, including pH, viscosity and spreadability, were tested using the Simplex Lattice Design (SLD) method. Based on the physical properties of the cream (pH, viscosity and spreadability), we got each SLD equation. The equation then calculated response (R) and R_{total} so as to get the optimum formula design.

Determination of sun protection factor of optimum cream formula

The 40 mg of the kencur extract was accurately weighed and transferred to 10 mL volumetric flask and the volume was adjusted with ethanol pro analysis, then diluted to 10 µg/mL. The same method was applied to the optimum cream formula to obtain solutions with concentration 10 µg/mL.

The absorption spectra of samples in solution were obtained in the range of 290 to 450 nm using 1 cm quartz cell, and ethanol as a blank. The absorption data were obtained in the range of 290 to 320, every 5 nm, and 3 determinations were made at each point. The SPF values were calculated by following equation :

$$\log \text{SPF} = \frac{\text{AUC}}{\lambda_n - \lambda_1} \times 2$$

Information :

SPF : Sun Protection Factor

AUC : Average absorbance between λ_n and λ_{n-1}

λ_n : The wavelength that results in absorption of 0.05

λ_1 : The wavelength at 290 nm

Antioxidant activity of optimum cream formula

Free radical scavenging activity of optimum cream formula measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH). In brief, 0.4 mM solution of DPPH in ethanol was prepared. This solution (1 mL) was added to 100 µL of optimum formula in ethanol at different concentration (20, 40, 60, 80, and 100 µg/ml), add ethanol up to 5 mL. Temu kunci extract, vitamin E and BHT as positive controls were made with concentrations of 5, 10, 15, 20 and 25 µg/mL.

The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 518 nm. by using spectrophotometer. The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using

Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation:

$$I\% = \frac{A_0 - A}{A_0} \times 100$$

Information :

I : DPPH inhibition (%)

A₀ : Absorbance of control sample

A : absorbance of a tested sample at the end of the reaction

RESULT AND DISCUSSION

Plants Collection and Extraction

The dry rhizomes of kencur and temu kunci were collected from Yogyakarta, Indonesia. This plants were identified at Laboratory of Botany and Genetics, Faculty of Teacher Training and Development, Universitas Muhammadiyah Purwokerto by microscopic analysis. Based on microscopic analysis showed that the rhizomes powder were Kencur (*K. galanga*, L.) and Temu Kunci (*B. pandurata* (Roxb) Schlecht). The percentage yield of kencur and temu kunci ethanolic extract were found to be 4.84% and 12.43% w/w respectively.

Cream optimization by Simplex Lattice Design Method (SLD)

Parameters used for determination of optimum formula of sunscreen and antioxidant cream were pH, viscosity and spreadability. The response of each physical property of the cream is given points with the total weight amount equal to one. The pH test was given 0.2 points, the viscosity with 0.5 points, and spreadability with 0.3 points.

The units of each response were different, it was necessary to find the normality value of the response assessment. Xmin value for pH was 6 and Xmax of 7. Xmin value for viscosity was 47 poise and Xmax was 17 poise. Xmin value for spreadability 44 cm² and Xmax of 30 cm². The normality values of physical properties of cream showed on Table II.

Table II. Normality physical properties of cream

Proportion (A:B)	pH (X-6) : (7-6)	Viscosity (X-17) : (47-17)	Spreadability (X-30) : (44-30)
100% : 0	0.45	0.867	0.933
90% : 10%	0.45	0.935	0.753
80% : 20%	0.45	0.969	0.594
70% : 30%	0.45	0.969	0.456
60% : 40%	0.45	0.9352	0.339
50% : 50%	0.45	0.865	0.243
40% : 60%	0.45	0.764	0.179
30% : 70%	0.45	0.626	0.115
20% : 80%	0.45	0.455	0.083
10% : 90%	0.45	0.248	0.071
0: 100% B	0.45	0.008	0.081

A : Proportion of kencur extract

B : Proportion of temu kunci extract

The R value could be calculated by multiplying N with each predetermined point. Determination of the optimum formula obtained from the largest total physical response cream. Total response value of physical properties of cream showed on Table III.

Table III. Total response value of physical properties of cream

Proportion (A:B)	R pH Nx0,2	R Viscosity Nx0,5	R Spreadability Nx0,3	R Total
100% : 0	0.09	0.433	0.280	0.803
90% : 10%	0.09	0.468	0.226	0.784
80% : 20%	0.09	0.485	0.178	0.753
70% : 30%	0.09	0.484	0.137	0.711
60% : 40%	0.09	0.468	0.102	0.659
50% : 50%	0.09	0.433	0.072	0.596
40% : 60%	0.09	0.382	0.053	0.526
30% : 70%	0.09	0.313	0.034	0.438
20% : 80%	0.09	0.227	0.025	0.342
10% : 90%	0.09	0.124	0.021	0.236
0: 100%	0.09	0.004	0.024	0.118

A : Proportion of kencur extract

B : Proportion of temu kunci extract

The cream formula with 100% kencur extract has a higher total response value than the other formula. Based on the data, the optimum formula was kencur extract with concentration of 50-100% and temu kunci extract with a concentration of 0-50%. The formula with a mixture of kencur and temu kunci extract with the ratio of 80%: 20% and 70%: 30% were selected because this study will be determined of both combination on sunscreen and antioxidant activity.

Determination of sun protection factor of optimum cream formula

Sunscreen is a compound that can absorb, scatter or reflect sunlight energy that comes on human skin. Sunscreen power in a substance can be expressed by the value of Sun Protector Factor (SPF). Sun Protector Factor (SPF) is the laboratory value for presenting the ability of a compound in UV absorption (Latha *et al.*, 2013). Donglikar and Shrada (2016) present that the higher the SPF value will provide a longer protective effect on sunlight and prevent sunburn. Based on SPF values, sunscreen compounds can be categorized into several categories which can be seen in Table IV (Schalka and Viktor, 2011).

Table IV. Categories of sunscreens based on the value of the SPF

Protection Level	SPF Value
Maximum	>50
High	30-50
Medium	15-30
Low	2-15

In this study, the purpose of measuring the SPF value is to determine the ability or effectiveness of kencur extract as a sunscreen to absorb, scatter or reflect sunlight energy. Measurement of SPF values was carried out on kencur extract and cream combination formula of kencur and temu kunci extract. The measurement results of SPF values of purified kencur extract and cream formula can be seen in Table V.

Table V. SPF Values of sunscreen and antioxidant cream

Formula	SPF Value	Protection Level
Kencur extract	4.505	Low
Formula A	5.024	Low
Formula B	4.511	Low
Formula C	6.368	Low

Formula A : Combination of kencur and temu lawak extract with ratio (80%:20%)
 Formula B : Combination of kencur and temu lawak extract with ratio (70%:30%)
 Formula C : Cream containing 100% of kencur extract

After kencur extract formulated into cream preparations, the sunscreen activity of the kencur extract was increased. This was due to the formation of cream-forming components having chromophores and auxochromes which result in a shift in wavelength due to increased ability to absorb the energy of sunlight thus affecting its effectiveness. This increase of SPF value was also possible because the emulgator used were Tween 80 and Span 80 which have a group of OH which that can absorb ultraviolet light so it will shift the peak of chromophore to higher wavelength (Nurhayati, 2011).

Creams with 100% of kencur extract have an extra level of protection with an SPF value of 6.368. While the cream with 80% of kencur extract and 20% extract of temu kunci have medium protection level with SPF value of 5.024 and cream with 70% of purified kencur extract and 30% extract of temu kunci also have medium protection level with SPF value of 4.511. UV protection capability decreases with decreased levels of purified kencur extract in cream formula, but when compared with SPF value of formula containing 100% of kencur extract, it can be concluded that the addition of antioxidant compound does not decrease the activity of sunscreen compound.

Ethyl *p*-methoxycinnamate and ethyl cinnamate were the main volatile compound in kencur rhizomes extract. Based on Athikomkulchai *et al.* (2007) study, the GC/MS results showed that the volatile oil from the rhizomes of kencur were cinnamate derivatives such as ethyl-*p*-methoxycinnamate (43.35%) and ethyl cinnamate (29.56%).

Other study of Gas Chromatography and Mass Spectrometry (GC-MS) showed that the largest peak area which correspond to volatile oil of kencur rhizome is ethyl *p*-methoxycinnamate (EPMC) (31.77%) (Tewtrakul, 2005; Raina and Abraham, 2015). Ethyl *p*-methoxycinnamate (EPMC) has been used as sunscreen, analgesic and anti-inflammatory agent, as cyclooxygenase-2 (COX-2) inhibitor, and to treat fibrosarcoma in mico (Ekowati *et al.*, 2016). Chemical structure of Ethyl *p*-methoxycinnamate and ethyl cinnamate showed in Figure 1.

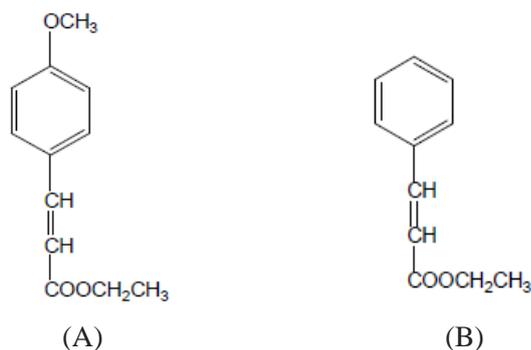


Figure 1. Chemical structure of main components of kencur extract : (A) Ethyl *p*-methoxycinnamate and (B) Ethyl cinnamate

Antioxidant activity of optimum cream formula

The antioxidant activity test was performed to find out how much antioxidant activity of cream's optimum formula. The amount of antioxidant activity is known by measuring the value of IC_{50} , which is the concentration of a substance that can cause 50% DPPH to lose its radical character. IC_{50} value inversely proportional to the antioxidant activity, the smaller the IC_{50} value of a sample showed better antioxidant activity. IC_{50} values of optimum cream formula showed on Table VI.

Table VI. Antioxidant activity of optimum cream formula

Preparations	IC_{50} ($\mu\text{g/mL}$)
Temu kunci extract	10.203 \pm 0.62
BHT	22.33 \pm 0.35
Vitamin E	8.786 \pm 0.52
Formula A	109.16 \pm 2.17
Formula B	95.24 \pm 2.89

Formula A: Combination of kencur and temu lawak extract with ratio (80%:20%)

Formula B: Combination of kencur and temu lawak extract with ratio (70%:30%)

IC_{50} value of temu kunci extract was 10.203 $\mu\text{g/mL}$ means at that concentration had 50% inhibition of DPPH free radical activity within 30 minutes. BHT as a comparator has an IC_{50} of 22.334 $\mu\text{g/mL}$, and vitamin E has an IC_{50} of 8.786 $\mu\text{g/mL}$. IC_{50} value of the cream formula A was 109.157 $\mu\text{g/mL}$, the antioxidant activity of this formula was 12 times lower than vitamin E and 5 times lower than that of BHT. While the B cream formula has an IC_{50} value of 95.238 $\mu\text{g/mL}$, the antioxidant activity of this formula is 11 times lower than vitamin E and 4 times lower than BHT. This is possible because of the low concentration of extract in the cream formula, ie 20% and 30%, in addition to possible interactions between the extract and the cream base, resulting in decreased antioxidant activity.

Flavonoids are large secondary metabolites found in rhizome of temu kunci. More than 51 flavonoid compounds from temu kunci have been isolated and their structure was confirmed. Some flavonoid compounds and their derivatives that have the potential as antioxidants in the ethanol extract of temu kunci rhizomes, including pinostrobin, pinocembrin, 2', 6' dihydroxy-4'-methoxychalcone, 2', 4'-dihydroxy-6'- methoxychalcone (kardamonin), (-) - panduratin A and (-) - 4-hydroxy panduratin A (Jaipetch *et al.*, 1982; Chahyadi *et al.*, 2014; Marliani *et al.*, 2013). Chemical structure of flavonoid compounds of temu kunci showed in Figure 2.

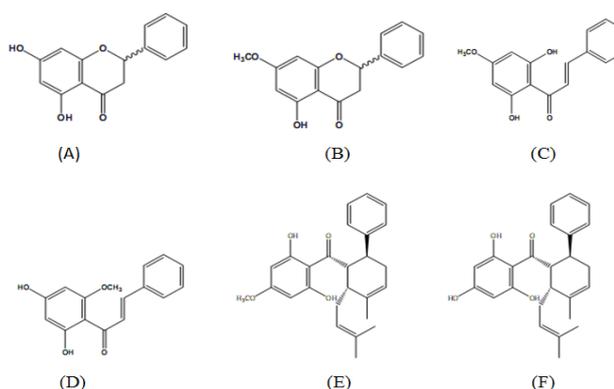


Figure 2. Chemical structure of flavonoid compounds of temu kunci : (A) pinocembrin; (B) pinostrobin; (C) 2', 6' dihydroxy-4'-methoxychalcone; (D) 2', 4'-dihydroxy-6'-methoxychalcone (kardamonin); (E) (-) - panduratin A; and (F) (-) - 4-hydroxy panduratin A

Antioxidants reduce free radicals by giving one or more electrons to free radicals to become normal molecules again. DPPH is used as a free radical model. DPPH free radicals will be captured by flavonoids. Flavonoids will be oxidized by free radicals DPPH produces more stable radical forms, namely radicals with low reactivity. Flavonoids donate hydrogen atoms from their aromatic rings to reduce toxic free radicals. This reaction produces new stable radical compounds and DPP-Hydrazine. Proton donation from antioxidants to DPPH radicals showed in Figure 3.

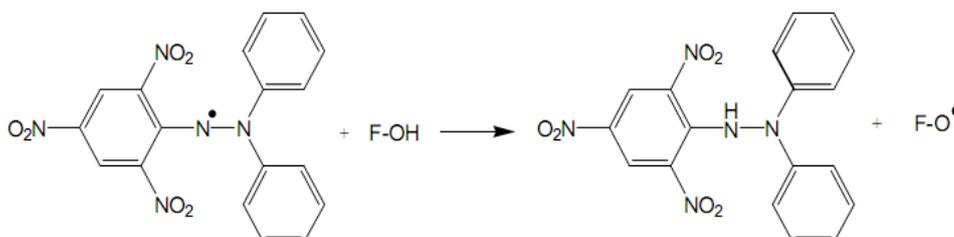


Figure 3. Proton donation from antioxidants to DPPH radicals (Mun'im *et al.*, 2008)

CONCLUSION

Cream formula A (80% kencur:20% temu kunci) and formula B (70% kencur:30% temu kunci) have optimum physical properties. The SPF value of the optimum formulations were higher than SPF value of kencur extract. Antioxidant activity of that combinations were lower than temu kunci extract, BHT and Vitamin E. Further research is needed to obtain a suitable cream base for both extracts to obtain a stable cream.

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