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SPF Test from *Baccaurea lanceolata* Muell.Arg Fruit Isolates

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Abstract

Not many researches on a fruit of limpasu (*Baccaurea lanceolata* Muell. Arg) were found, while the use by tribal hamlets in Kalimantan already became a daily routine before they went into the field with the way made powder and smeared on the face. Therefore, the research on active substances needs to be done. For this study, we prepared two isolates which coded as K2 and CE, K2 from qualitative test negative isolates phenolic groups was identified by spray $FeCl_3$, whereas qualitative test positive isolates were coded as CE and detected by a nitrogen-containing reagents dragendorf spray. The K2 isolate from SPF test resulted an SPF value of 3.2 at a concentration of 0.2 mg/ml, while CE isolate was given a value of SPF 5.0 at a concentration of 0.2 mg/mL. According to sensitivity skin test on rabbit skin, both K2 and CE isolated were given the value below 1, indicated the isolates were found to not irritate. This study proposes the potential from *Baccaurea lanceolata* Muell.Arg fruit isolates to be developed as a sunscreen for UV-protection.

Keywords : *Baccaurea lanceolata* Muell.Arg, SPF test, sensitivity skin test

INTRODUCTION

One of the utilization of natural ingredients for skin care by the tribe Banjar in South Kalimantan is from limpasu (*Baccaurea lanceolata*) fruit which frequently used topically on the skin for protecting the skin from sunburn. The use of limpasu as a protective skin from the sun can be tested out with several approaches to determine the value of a sunscreen with SPF (Sun Protection Factor).

Previous study were conducted on several species such as *B. angulata baccaurea* (better known as dayak blimbing) which performed antioxidant activity based on DPPH assay with IC_{50} value of 53.68 mg/100 (Jauhari, *et al.*, 2013), while this result correlated with another study by Ahmed, *et al.* (2015) who revealed that *B. angulata* methanol extract had several major phenolic flavonoids, with total carotenoid contents that greater in the fruit rather than the other part. Another related study was also revealed on *B. ramiflora* containing isolates 6'-O-vanilloylisota-chioside with antioxidant capacity (DPPH) 36.9 ppm (Yang, *et al.*, 2007). And *B. sapida* performed IC_{50} value for inhibition of lipid per oxidation by using ammonium thiocyanate with 0.84 mg/mL, iron ion chelating capacity of fruit *B.sapida* 0.47 mg/mL (Prakash, 2012).

MATERIALS AND METHODS

Sample and Reagent

The fruit of limpasu (*baccaurea lanceolata*) from South Kalimantan was prepared as much as 30 kg. The other materials that used for this study were 70% ethanol, n-hexane, ether, ethyl acetate, methanol, distilled water, reagents dragendorf, FeCl3 1%, p.a N-hexane, ethyl acetate p.a, methanol p.a, biocream® 7. The animal object that prepared in this study was mice and rabbits.

Isolation

Limpasu fruit was dried and macerated with 70% ethanol, later fractionated with n-hexane, ether, ethyl acetate and methanol constitutively. The ethyl acetate fraction, then proceeds with isolation using PTLC (Preparative Thin Layer Chromatography) with the mobile phase n-hexane p.a and ethyl acetate p.a.

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SPF Test

The isolate from limpasu fruit was made into various series of concentration with methanol p.a. as solvent. The absorbance spectra of the sample was determined by UV spectrophotometer using wavelength of 290-320 nm. The higher the SPF score, the more protection from the material offered against UV radiation causing sunburns. According to FDA in 2011, materials with SPF value of 2-14 can be claimed to prevent sunburn, meanwhile above 15 can be claimed to reduce the risk of skin cancer and early skin aging.

Sensitivity Skin Test

The sample was obtained by base Biocream® then applied to the skin of rabbits for observation in 72 hours. First irritation index value was determined by adding up the score of erythema and edema. The earliest irritation index calculation was done by calculating the average score of the observations at 24, 48 and 72 hours.

Based on Hayes (2001), index irritation was scored as below:

- 0 – 1: almost doesn't irritation
- 1 – 2: mild irritation
- 2 – 5: medium irritation
- Up 5: heavy irritation

RESULTS AND DISCUSSION

The maceration of dried limpasu fruit resulted 300 grams of viscous extract, then after fractionation with n-hexane, ether, ethyl acetate, and methanol gave filtrate as much as 95, 50, 20, and 50 grams respectively and 85 grams of insoluble macerate. Ethyl acetate fraction was found out as the most active fraction (the data not shown), thus ethyl acetate fraction was isolated further using preparative TLC with the mobile phase n-hexane p.a.: ethyl acetate p.a (4: 1 v/v), which was isolated in 8/16 Rf die isolates with K2 and RF 4/16 code that isolates the CE code, then tested qualitatively with the spray reagent. K2 isolates qualitative test beneficial $FeCl_3$ whereas explicit isolates Dragendorf CE.

Based on the qualitative test using $FeCl_3$ for K2 isolate, the TLC plate showed a spontaneous reaction to the reagent spray, indicated that the isolate was not able to be concluded into specifically group containing phenolic, however after being heated, the K2 isolated showed patches of black, which showed that the isolates did not contain phenolic groups but only -OH groups at most, possibly a class of terpenes containing OH groups. Meanwhile in CE isolate, after reaction with Dragendorf reagent showed spontaneous reaction which suspected that the isolate might contain several nitrogen atoms. The supported data from absorbance (data not shown) revealed that either K2 isolates or CE isolates had λ max below 254 nm which indicated the compound which contained in the isolates were possibly from class of terpenes.

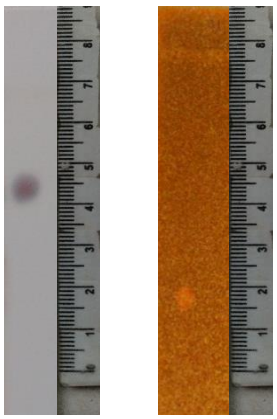

Figure 1. Qualitative test isolates K2 and CE

Table 1. (A) isolates SPF values K2 with three unusual concentrations. (B) The value of SPF CE isolates with three distinctive concentrations

Concentration mg/ml	SPF value
0,1	1,5
0,15	1,7
0,2	3,2

(A)

Concentration mg/ml	SPF value
0,1	2,5
0,15	3,2
0,2	5,0

(B)

SPF test was conducted on K2 and CE isolates at concentration of 0.1 mg/mL, 0.15 mg/mL, and 0.2 mg/mL with SPF value of each shown in Table 1. The highest SPF value was found in CE isolate although with the same concentration between isolates K2 and CE dies 0.2 mg/mL.

On the sensitivity test of K2 isolate that conducted on rabbits at a concentration of 0.2 mg/mL in the base show the average below 1 at 48 and 72 hours of observation, which demonstrated this isolate could be used maximally for 24 hours to be protected from the sun and light sensitive air. A value of 1 is also interpreted that this isolate was relatively not irritated.

Meanwhile, on the sensitivity test from CE isolate conducted on rabbits at a concentration of 0.2 mg/mL in the base shows the average below 1 at the 72-hour observation, showing that the isolate was better to be used maximum for 48 hours to be safe at sensitive sun light and air. A value of below 1 is showed the isolate was found be not relatively irritant.

Table 2. The sensitivity skin test of K2 isolate with the concentration of 0.2 mg/mL (the highest SPF value) on the skin of rabbits

Rabbit	Observation in hours			Average
	24	48	72	
Control rabbit	0	0	0	0
Rabbbit I	0	I	I	0.66
Rabbit II	0	I	I	0.66
Rabbit III	0	I	I	0.66
Avarage rabbit I ,II, III				0.66

Table 3. The sensitivity skin test of CE isolate with the concentration of 0.2 mg/mL (the highest SPF value) on the skin of rabbits

rabbit	Observation in hours			average
	24	48	72	
Control rabbit	0	0	0	0
Rabbit I	0	0	I	0.33
Rabbit II	0	0	I	0.33
Rabbit III	0	0	I	0.33
Avarage rabbit I ,II, III				0.33

CONCLUSION

These study revealed that K2 and CE isolates gave the SPF value of 3.2 5 respectively at a concentration of 0.2 mg/mL. On the sensitivity skin test on rabbit, both K2 and CE isolates gave the average value below 1, which indicated that the isolates were found to not relatively irritate.

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