Trad. Med. J., September-December 2020
Vol. 25(3), p 135-139
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 23-09-2019
Revised : 20-09-2020
Accepted : 23-12-2020

# *In-vitro* and *In-vivo* Determinations of Sun Protection Factors (SPF) of Skin Lotions Containing Mountain Papaya Fruit and Mangosteen Peel Ethanolic Extract

**Heru Sasongko[1,2*], Natasyha Advaita[1], Ratih Guswinda Lestari[1], Karimah Umar Aidid[1]**
[1] Department of Pharmacy Faculty of Mathematics and Natural Sciences Sebelas Maret University, Surakarta, Central Java, Indonesia
[2] Department of Pharmacy School of Vocational Sebelas Maret University, Surakarta, Central Java, Indonesia

## ABSTRACT

Indonesia is a high sun exposure country. Exposure to ultraviolet (UV) causes various kinds of skin disorders such as erythema, sunburn, aging, and cancer. Mountain papaya fruit (*Vasconcellea pubescens* A.DC.) and mangosteen peel (*Garcinia mangostana* L.) contains metabolite compounds that can protect the skin from sunlight because of its antioxidants activity. The purpose of this study to determine whether the combination of the mountain papaya fruit and mangosteen peel extracts in skin lotion can be used as sun protectors through the *in-vitro* and *in-vivo* study. The experiment was done by extracting the mountain papaya fruit and mangosteen peel through the maceration method. The extracts were formulated into skin lotion in three different formulas with the ratio of mountain papaya fruit extract: mangosteen peel extract as follows F1(1:1), F2(1:3), and F3(3:1). I*n vitro* test was done by using UV-VIS spectrophotometry to determine the SPF value and *in vivo* test was used erythema-induced rats by exotera beam light. The result of *in vitro* test gained a high enough SPF value for all three formulas F1=23,23; F2=21,70 and F3=28,64 and the result of *in vivo* test showed that all three formulas did not indicate the existence of erythema value. It can be concluded that three skin lotion formulas containing mountain papaya fruit and mangosteen peel ethanol extract have the effect of sun protection.
**Keywords:** antioxidant; mountain papaya; mangosteen; sun protection

## INTRODUCTION

Indonesia is a high sun exposure country which its major population works outdoors (Sineke, 2016). It is the reason for most people to use sun protectors for protecting their skin from the sun's UV rays (Widyastuti *et al.,* 2015). Sun's ultraviolet rays (UV) A and UV B can cause various disorders that affect the skin such as erythema, hyperpigmentation, sunburn, skin cancer, and premature aging because of the wrinkled skin (Zaidi *et al.,* 2019). Nowadays, most people choose to use sunscreen, especially those that contains natural ingredients (Kyaw *et al.,* 2018). The use of natural ingredients for cosmetics is considered safer, affordable, and effective than the use of products derived from synthetic chemical compounds.

Mountain papaya fruit (*Vasconcellea pubescens* A.DC.) and mangosteen peel (*Garcinia mangostana* L.) are several types of natural ingredients that are often used for health. Papaya mountain or carica fruit is often found in Dieng Plateau (Sasongko *et al.,* 2018). In previous studies, it is known that mountain papaya contains antioxidant compounds including flavonoids which can counteract free radicals (Laily *et al.,* 2012; Simirgiotis *et al.,* 2009). The in vitro study of mountain papaya fruit extract shows that it has an effect as a sun-protector (Advaita *et al.,* 2018). Mangosteen fruit is often used in medical health because of its potential antioxidant activity (Ovalle-Magallanes *et al.,* 2017; Supiyanti *et al.,* 2015). Mangosteen fruit is a widely studied part of the plant because of its high phenolic content (Azima *et al.,* 2017; Zadernowski *et al.,* 2009). Empirically, mangosteen fruit is consumed orally by the major community, but on the other hand, mangosteen peel can also be used topically. Mangosteen fruit is rich in phenolic acid, xanton, anthocyanin, and tannins, including α-mangostin, β-mangostin, γ-mangostin, and gartanin (Zadernowski *et al.,* 2009). According to Mu'awanah, the flavonoid is also known for having chromophore groups which are classified as conjugated aromatic groups and having a role as binding metal ions that can prevent the effects of UV rays from the sun (Mu'awanah *et al.,* 2016).

Based on the potential of mountain papaya fruit (*Vasconcellea pubescens* A.DC) and mangosteen peel (*Garcinia mangostana* L.), this study will be conducted to investigate the value of

***Corresponding author : Heru Sasongko**
**Email : heru_sasongko@staff.uns.ac.id**

Table I. Skin lotion formula of papaya mountain fruit ethanol extract (EEPM) and mangosteen peel ethanol extract (EEMP)

| Ingredients | Formula 1 (%) | Formula 2 (%) | Formula 3 (%) |
|---|---|---|---|
| EEBK | 10 | 5 | 15 |
| EEKM | 10 | 15 | 5 |
| Stearic acid | 2.5 | 2.5 | 2.5 |
| Carageenan | 1 | 1 | 1 |
| Liquid parafin | 7 | 7 | 7 |
| Gliseryn | 5 | 5 | 5 |
| TEA | 1 | 1 | 1 |
| Benzoic acid | 0.2 | 0.2 | 0.2 |
| Chocholate essense | 0.5 | 0.5 | 0.5 |
| Aquades | Ad 100 | Ad 100 | Ad 100 |

sun protection factor (SPF) of skin lotions containing papaya mountain fruit and mangosteen peel extract through *in vitro* and *in vivo* study. The results obtained are expected to be used for information on the development of cosmetic products.

## METHODOLOGY

### Materials and Tools

Papaya mountain fruit (*Vasconcellea pubescens* A.DC.) taken from Dieng District, Wonosobo Regency, Central Java, and mangosteen peel (*Garcinia mangostana* Linn) from Surakarta, Central Java. Stearic acid, carrageenan, liquid paraffin, TEA, glycerin, benzoic acid, chocolate essences, 70% technical ethanol, and distilled water obtained from Pharmacy Faculty of FMIPA Sebelas Maret University. White male Wistar rats (Sprague Dawley) were used for *in vivo* test which purchased from the "White Mouse" Surakarta weighing 150-200 grams and aged 2-3 months. The equipment used was UV-Vis spectrophotometer (Genesys®), analytic scales (Precisa®), oven (Memmert®), homogenizer (IKA®), horizontal rotary evaporator, UV lamp (Exotera®), and other glass equipment.

### Extraction of papaya mountain fruit and mangosteen peel

Ten kilos of each papaya mountain fruit (*Vasconcellea pubescens* A.DC.) and mangosteen peel (*Garcinia mangostana* Linn) were cleaned and cut into small pieces. The sample was dried using an oven at temperature 50$^{o}$C. Papaya mountain fruit and mangosteen peel were extracted by 70% ethanol solvents through the maceration process at room temperature (25$^{o}$C). Stired it up for 15 minutes using a stirring rod. Maceration was carried out for 3x24 hours and evaporated using a rotary evaporator to obtain viscous extract (Sasongko and Sugiyarto, 2018).

### Formulation of Skin Lotion

The lotion contains the combination of mountain papaya fruit ethanol extract (EEPM) and mangosteen peel ethanol extract (EEMP) was made into 3 different formulas ratio of extract concentrations as follows 50%: 50%, 25%: 75%, and 75%: 25% as shown in Table I. Stearic acid and liquid paraffin as the oil phase were put into Erlenmeyer. The samples EEPM and EEMP, glycerin, TEA, carrageenan solution, and residual water were dissolved in water and mixed into the water phase. The oil phase and water phase were heated and stirred at 50°C for ± 10-15 minutes until separated homogeneously. The stirring process was carried out until both preparations were homogeneous and reached 40$^{o}$C temperature. Next, benzoic acid and chocolate essences were added and stirred until homogeneous.

### *In Vitro* Test (SPF value)

Determination of the sunscreen effectiveness was carried out using UV-Vis spectrophotometer instrument. The lotion was diluted 4000 ppm for each formula. Each formula was read at wavelengths of 290-320 nm with a distance interval of 5 nm. Aquades were used as blanks, then the absorbance value was used to calculate the SPF value (Wulandari, 2017). Calculation of SPF values is done using the following formula:

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

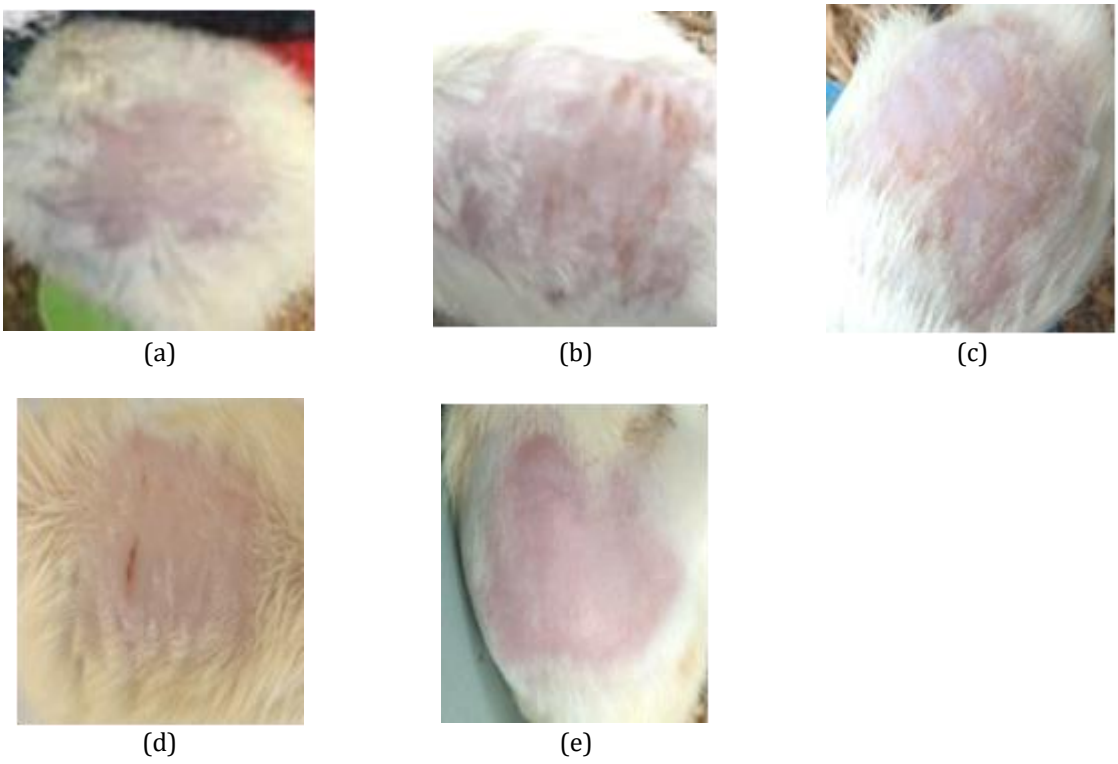


Figure 1. Erythema on rats' back skin (a) Positive control (Parasol SPF 33); (b) Negative control (base lotion); (c) Lotion EEPM-EEMP 1:1; (d) Lotion EEPM-EEMP 1:3 and (e) Lotion EEPM-EEMP 3:1

CF = Correlation factor (10); EE = Reduction of erythema; I = Solar ray simulation spectrum; Abs = Readable absorbance value

***In Vivo* Test**

The *in vivo* test was carried out by observing the effects of erythema in animals test. All procedures for handling these animals test received approval from the Sebelas Maret University ethics committee number 525/IV/HREC/2018. All rats were shaved 4x4 cm long, then applied lotion formula on the back surface rats. The sample test was left in contact for 1 hour then radiated by UV B Exoterra for 1x24 hours (Shovyana and Zulkarnain, 2015). Animal tests were divided into 5 groups (positive control, negative control, and three treatment groups), each group consisted of 4 rats. Parasol SPF 33 used positive controls and basic lotions used negative controls.

## RESULT AND DISCUSSION

The effectiveness of skin lotion was determined by *in vitro* test using the UV spectrophotometry method which produced absorbance values. The absorbance value shows the value of the rays protection factor (SPF) after entered into the calculation formula. The SPF value is a value that shows the ratio of the minimum amount of UV energy needed to cause erythema of the skin (Minimal Erythema Dose / MED) after being smeared with sunscreen. MED is the amount of UV radiation needed forming the erythema which can be measured by a UV spectrophotometer (Mitsui, 1997). The results of the *in vitro* SPF test values are shown in Table II.

Sunscreen or similar cosmetics used on the face and neck should have a minimum SPF value of 15 (Wasitaatmadja, 1997). If a sample is known to have SPF value <2 indicates that the sample is not a sunscreen. SPF value 2 - 11 shows that sunscreen has minimal protection. SPF value 12-30 shows moderate protection of sunscreen, and SPF values greater than 30 shows high protection against UV radiation (Mitsui, 1997; Mu'awanah *et al.,* 2016). *In vitro* test in Table II shows that formula 1, formula 2, and formula 3 had moderate protection against UV radiation. Formula 3 contains 15% papaya mountain fruit and 5% mangosteen peel extract which has the highest SPF value of 28.64 ± 0.025.

*In vivo* test of skin lotion containing the combination of mountain papaya fruit ethanol extract (EEPM) and mangosteen peel ethanol extract (EEMP) was done for testing the potential

Table II. SPF Value and The Ability of Skin Lotion Protection

| Group | Replication of SPF Test | | | Average of SPF | Protection Category |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| Skin lotion base | 8,75 | 8,75 | 8,75 | 8,75± 0,00 | Minimal |
| Formula 1 (EEPM-EEMP 1:1) | 23,19 | 23,22 | 23,27 | 23,227 ± 0,04 | Medium |
| Formula 2 (EEPM-EEMP 1:3) | 21,69 | 21,78 | 21,67 | 21,7 ±0,075 | Medium |
| Formula 3 (EEPM-EEMP 3:1) | 28,62 | 28,64 | 28,67 | 28,643 ±0,025 | Medium |

of skin lotion formulas as sun protection by comparing formulas with positive controls (Parasol SPF 33) and negative controls (base lotion). The parameters used were the extent of erythema produced after radiation, then plotted in erythema scores. The results of the test can be seen in Figure 1. The results showed that skin containing a combination of mountain papaya fruit and mangosteen peel extract in formulas 1, 2, and 3 had a protective effect on sunlight UV radiation. All formulas had a score of 0 erythema as well as positive controls, while negative controls still showed erythema scores. The negative control showed a large area of erythema and high erythema score. This showed that formula skin lotion has a protective effect against UV radiation.

Irradiation of UV rays on the skin increases collagenase activity and contributes to the formation of wrinkles through degradation of collagen in the extracellular matrix of the skin (Fisher *et al.,* 2002). Phenolic content, especially flavonoids found in mountain papaya fruit and mangosteen peel has an antioxidant effect because it has a chromophore group that can absorb UV A and UV B rays thereby reducing its intensity on the skin (Svobodová *et al.,* 2003). The α-mangostin compounds found in mangosteen inhibit the formation of skin wrinkles which are characterized by the erythema formation due to UV B induction (Im *et al.,* 2017).

## CONCLUSION

Skin lotion containing the combination of papaya mountain fruit and mangosteen peel ethanol extract has an effect as a sun protector. In vitro tests carried out with UV-VIS spectrophotometry produced SPF values for F1 = 23.23; F2 = 21.70 and F3 = 28.64. The results of the in vivo test showed that the three formulas did not cause erythema values.

## ACKNOWLEDGEMENT

Great appreciation and acknowledgment to Kementerian Riset Teknologi dan Pendidikan Tinggi for funding and Sebelas Maret University for supporting this research.

## REFERENCES

Advaita, N., Lestari, R.G., Aidid, K.U. & Sasongko, H., 2018, 'In vitro evaluation of sun protection factor of *Vasconcellea pubescens* fruit extract', *International Conference On Pharmaceutical Research And Practice*, *Proceedings,* Indonesia Islamic University Jogjakarta, December 15, 2018.

Fisher, G.J., Kang, S., Varani, J., Bata-Csorgo, Z., Wan, Y., Datta, S. &Voorhees, J.J., 2002, 'Mechanisms of Photoaging and Chronological Skin Aging', *Arch. Dermatol.* 138, 1462–1470.

Im, A.-R., Im, A.-R., Kim, Y.-M., Kim, Y.-M., Chin, Y.-W., Chin, Y.-W., Chae, S. & Chae, S., 2017, 'Protective effects of compounds from *Garcinia mangostana* L. (mangosteen) against UVB damage in HaCaT cells and hairless mice', *Int. J. Mol. Med*. 40, 1941–1949.

Kyaw, M.S., Aye, M.M., Grinnell, M. & Rabach, M., 2018, 'Traditional and ethnobotanical dermatology practices in Myanmar', *Clin. Dermatol. Complementary Dermatology* 36, 320–324.

Laily, A.N., Suranto, S. & Sugiyarto, S., 2012, 'Characterization of *Carica pubescens* in Dieng Plateau, Central Java based on morphological characters, antioxidant capacity, and protein banding pattern', *Nusant. Biosci*. 4.

Mitsui, T., 1997, *New Cosmetic Science*, Elsevier.

Mu'awanah, I.A.U., Setiaji, B. & Syoufian, A., 2016, 'Pengaruh Konsentrasi Virgin Coconut Oil

(VCO) Terhadap Stabilitas Emulsi Kosmetik dan Nilai Sun Protection Factor (SPF)', *BIMIPA* 24, 1–11.

Ovalle-Magallanes, B., Eugenio-Pérez, D. & Pedraza-Chaverri, J., 2017, 'Medicinal properties of mangosteen (*Garcinia mangostana* L.): A comprehensive update', *Food Chem. Toxicol*. 109, 102–122.

Sasongko, H., Efendi, N.R. & Sugiyarto, 2018, 'The ethanolic extract of mountain papaya (*Vasconcellea pubescens* A.DC.) fruit against lipid peroxidation of rat liver tissues', *AIP Conf. Proc*. 2019, 50001.

Sasongko, H. & Sugiyarto, S., 2018, 'Pengaruh Pemberian Ekstrak Daun Karika (*Vasconcellea pubescens* A.DC.) Terhadap Nilai SGPT dan SGOT pada Tikus Jantan yang Diinduksi Paracetamol', *JPSCR J. Pharm. Sci. Clin*. Res. 3, 70–75.

Shovyana, H.H. & Zulkarnain, A.K., 2015, 'Physical Stability and Activity of Cream W/O Etanolik Fruit Extract Mahkota Dewa (*Phaleria macrocarph* (scheff.)Boerl) as a Sunscreen', *Maj. Obat Tradis*. 18, 109–117.

Simirgiotis, M.J., Caligari, P.D.S. & Schmeda-Hirschmann, G., 2009, 'Identification of phenolic compounds from the fruits of the mountain papaya *Vasconcellea pubescens* A. DC. grown in Chile by liquid chromatography–UV detection–mass spectrometry', *Food Chem*. 115, 775–784.

Sineke, F.U., 2016, 'Penentuan Kandungan Fenolik dan Sun Protection Factor (SPF) dari Ekstrak Etanol dari Beberapa Tongkol Jagung (*Zea mays* L.)', *Pharmacon* 5.

Siti Azima, A.M., Noriham, A. & Manshoor, N., 2017, 'Phenolics, antioxidants and color properties of aqueous pigmented plant extracts: *Ardisia colorata* var. elliptica, *Clitoria ternatea*, *Garcinia mangostana* and *Syzygium cumini*', *J. Funct. Foods* 38, 232–241.

Supiyanti, W., Wulansari, E.D. & Kusmita, L., 2015, 'Test of Antioxidant Activity and Determination of Total Anthocyanin Content in Rind of Mangosteen (*Garcinia mangostana* L)', *Maj. Obat Tradis*. 15, 64–70.

Svobodová, A., Psotová, J. & Walterová, D., 2003, 'Natural phenolics in the prevention of UV-induced skin damage, A review', *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czechoslov*. 147, 137–145.

Wasitaatmadja, S.M., 1997, *Penuntun ilmu kosmetik medik*, 3, 58–59, Penerbit Universitas Indonesia, Jakarta.

Widyastuti, W., Fratama, R.I. & Seprialdi, A., 2015, 'Pengujian Aktivitas Antioksidan dan Tabir Surya Ekstrak Etanol Kulit Buah Naga Super Merah (*Hylocereus costaricensis* (F.A.C. Weber) Britton & Rose)', *Sci. J. Farm. Dan Kesehat*. 5, 69–73.

Wulandari, S.S., 2017, 'Aktivitas Perlindungan Tabir Surya Secara In Vitro dan In Vivo dari Krim Ekstrak Etanol Daun Soyogik (*Saurauia bracteosa* DC)', *Pharmacon* 6.

Zadernowski, R., Czaplicki, S. & Naczk, M., 2009, Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*), *Food Chem.* 112, 685–689.

Zaidi, Z., Hussain, K. & Sudhakaran, S., 2019, 'Diseases Due to Ultraviolet Radiation, in: Zaidi, Z., Hussain, K., Sudhakaran, S. (Eds.), Treatment of Skin Diseases: A Practical Guide', *Springer International Publishing, Cham*, pp. 171–184.