

Safety Study of Purple Sweet Potatoes Leaves Extract (*Ipomoea batatas* (L.) Lamk) Antin-3 Variety as a Sunscreen Active Ingredient on Human Skin

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ABSTRACT

Background: Ultraviolet (UV) radiation intensively has known as a major cause of photoaging and photodamage on skin. Anthocyanin is one of flavonoid group, which has a chromophore and conjugated double bond, absorbing electromagnetic radiation in UV wavelength. Ethanolic leaves extract of (*Ipomoea batatas* (L.) Lamk) Antin-3 variety contains considerably high anthocyanins so that it can be a potential sunscreen active ingredient. However, the safety effect of ethanolic extract of (*Ipomoea batatas* (L.) Lamk) Antin-3 variety on human skin is still unclear. Objective: The objective of this study is to evaluate the safety (irritation and allergic effect) of ethanolic leaves extract of (*Ipomoea batatas* (L.) Lamk) Antin-3 variety on human skin through a patch test. Materials and Methods: Ethanolic young and old leaves extract of (*Ipomoea batatas* (L.) Lamk) Antin-3 variety. Each of them is on a 500, 1000 and 5000 ppm on *vaselinum album* base. The patch test was applied on the back skin on seven of adult females who are included in inclusion criteria (as a subject study) for 24 hours and they were observed for four days. Results: There are two out of seven subjects indicated irritation which understandably were caused by the old leaves 1000 and 5000 ppm. Conclusions: The old leaves vary in the phytochemical compound compared to the young leaves which have more potential risk to irritate and cause allergies to the human skin. Anthocyanin is a major content in young leaves.

Keywords: Young leaves, old leaves, ethanolic leaves extract of Antin-3, skin irritation and allergy.

INTRODUCTION

Ultraviolet radiation which potentially causes skin damage is divided into two, namely UV A and UV B. UV A is on (320-400 nm) wavelength, while UV B is on (290-320 nm) wavelength. An increasing of the UV wavelength shows a positive relationship with the depth of its radiation to the human skin. Excessive UV B exposure could cause an acute skin damage such as photoaging and photodamage. For a long-term effect, when it is not addressed immediately, it tends to be accumulative and potentially triggering the weakening immunity of the skin. Moreover, skin cell mutations, which could result in skin cancer in the end, could occur².

UV A exposure could damage the skin through triggering an appearance of free radicals which could block nutrition diffusion, deactivate the enzymes, oxidize fats (intracellular, cell membrane and intercellular) and damage the DNA, resulting in premature aging and precancerous cells. UV A and UV B exposures, at the same time and in relatively long duration, also take account in causing some diseases associated with sun sensitivity (photosensitive)². Using sunscreen is important to protect skin from the danger of the UV radiation. Sunscreen is designed as UV light filter and reflection which is formulated in form of cream, lotion, oil and gel².

Sunscreen could be formulated from some chemical materials such as *amino benzoic acid* (PABA) and derivate PABA such as *cinnamates* and *benzophenones*, as well as natural material such as flavonoid (*anthocyanin*). The use of chemical material would be effective at relatively a high concentration, as much as 26%, but at this high concentration, it could trigger a sensitivity on the skin².

Nowadays, the utilization of natural ingredients becomes a smart option as it has a little side effect. In addition, the use of natural resources is believed as one action of environment preservation. Flavonoid (*anthocyanin*) based on its structure, contains chromophore clusters and conjugated double bonds which can absorb the UV radiation and later decrease the intensity of the radiation energy when reaching the skin³.

Ipomoea batatas (L.) Lamk, also known as purple sweet potatoes, with variety of Antin-3 contains relatively very high anthocyanin, as much as 150,67 mg/100 g (b/b)²³. Ethanol extract of the leaves of *Ipomoea batatas* (L.) Lamk with variety Antin-3 has 80.43% antioxidant activity, which is the same as a pure vitamin C⁵.

The extract of Antin-3 leaves are suitable for a sunscreen material as the combination effect of some antioxidant could be useful in minimizing the skin damage caused by the UV radiation¹³. However, it is necessary to prove that

Table 1: Patch Test Evaluation¹⁴

No	Result	Explanation
1	No abnormalities: no irritation	-
2	Weak Erythema (doubtful)	(?)
3	Erythema, infiltration or edema, papule: positive, weak	+
4	Erythema, infiltration or edema, papule, vesicles: positive, strong	++
5	Bula: positive, very strong	+++

Table 2: Irritation symptoms score^{8,10}

Erythema	Edema
No Erythema	No Edema
Little erythema (d<25 mm)	Very light edema (peripheral and enlargement hardly seen)
Erythema clearly seen (d = 25,1-30 mm)	Peripheral edema with clear boundary (t<1 mm)
Medium erythema (d = 30,1-35 mm)	Medium edema (upper peripheral ±1 mm)
Acute erythema (wound found)	Acute edema ((t>1 mm, widening to exposures area)

Explanation:

d = diameter

t = thickness

the extract of Antin-3 leaves is safe for the human skin. Antin-3 leaves, during harvest season, have two categories based on the age including young leaves and old leaves. Different age of plants would affect the amount and the complexity of the phytochemical component contained in those plants¹¹.

This research aims to see if the young and old leaves of Antin-3 could potentially irritate and cause allergy on the human skin used as sunscreen material, it is important to make sure that this leaves extract as the main ingredient would not cause skin irritation and allergy as it is a crucial element of a safety procedure¹⁷.

Abnormalities on skin caused by a particular substance which directly makes a contact with the skin are called as a dermatitis contact. Probability of dermatitis contact of Antin-3 leaves observed in this research are types of irritations and allergies⁹. Additionally, this research employs human as the subject of the research in order to obtain a real effect on human²².

MATERIALS AND METHOD

Research Preparation

This research is a safety study of Antin-3 leave extracts through patch testing method. In addition, under supervision of dermatovenereology certified by Good Clinical Practice (GCP) and ethical clearance from dr. Soetomo hospital, this research attempted to employ human as the main subject of the experiment.

Research Subject

Research subject has met inclusion criteria involving some adult females with healthy skin which are referenced by a dermatovenereology. The participants who agree to take a part had signed some informed consents and met exclusion criteria (atopy history, pregnancy, and medication affecting skin reaction)¹⁴.

There are seven participants in this research which are considered as an ideal number as this research is a pilot study which becomes a basic authentication research if the extract of Antin-3 leaves causes an irritation or allergy on the skin. The result of this study will be used for another research in the future. Besides, there has been a research regarding an irritation test of ethanol extract of mangosteen rind (*Gracinia mangostana L.*) to six research participants¹⁰.

Research Sample

Materials

This research involves 3 parts of young and old Antin-3 leaves of purple sweet potatoes, ethanol 96%, and Aquadest.

Sample preparation

The Antin-3 leaves of purple sweet potatoes are washed using flowing water. The next step is air-drying which avoids the use of direct sunshine. Next, the dry leaves are ground into powder, then, finally to be extracted through maceration method using ethanol 96% as a solvent. The macerate obtained, then, are condensed using vacuum rotary evaporator with 40°C temperature¹⁶.

The extract of the young and old Antin-3 leaves are divided into three concentrations, which are 500 ppm (50mg extract in 100 g of *Vaselineum album*), 1000 ppm (100 mg extract in 100 g *Vaselineum album*) and 5000 ppm (500 mg extract in 100 g *Vaselineum album*)⁴.

Testing method

20 mg of each of those concentrations are put in finn-chamber with 8 mm diameter. *Vaselineum album* is used as a *blanko* in this research, while the negative control is only finn chamber and the patch, so that there are about 8 patches for each subject¹⁴.

The part of the skin subjected in this research is the left upper part of the back skin which should be clean and dry. This location is considered as an ideal target since the back area is large and less risky for an excessive movement. The application is done for 24 hours²¹.

The patch is numbered based on the concentration of the young Antin-3 leaves. It was done as the following system: concentration 500 ppm (1), 1000 ppm (2), 5000 ppm (3) and extract of old Antin-3 leaves with concentration 500 ppm (4), 1000 ppm (5) and 5000 ppm (6). *Vaselineum album* is in number 7 and finn chamber is in number 8.

Observation Result

Observation result of this safety study was done after the patch was opened (24 hours application). The observation was done fourth times: the first, second, third and fourth day (24 hours, 48 hours, 72 hours and 96 hours)^{9,14}.

The safety study of this extract has be done through human-24-hours-patch test method. 24 hours patch test method illustrates an accurate safety profile and offers a

Table 3: Irritation index calculation.

Subject	Patch Number							
	1	2	3	4	5	6	7	8
1	0	0	0	0	0,29	0,29	0,29	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0,86	0,86	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0



a



b

Picture 1: a. Patch application sample b. Result of observation



Picture 2: Antin-3 Leaves.

comfortable method for patients and examination⁸. During the application period, the back skin must not make a direct contact with water^{10,14}.

The patch is opened after being applied for 24 hours. The former location of the fin chamber application is marked. Observation results on the first, second and third day (24 hours, 48 hours and 72 hours) aim to see potential reactions of the irritation. The observation is continued on the fourth day (96 hours) to see the possibility of the allergic reaction. During the observation process, the back skin, which is marked, may be washed without soap^{9,14,21}.

The following table shows the result of the evaluation.

Explanation:

Erythema: reddish skin condition

Edema: swollen skin (locally)

Papule: small solid lesion appearing on the surface of the skin (< 1 cm)

Vesicles: protruding skin with a clear boundary containing serous fluid with diameter <1 cm

Bula: protruding skin with a clear boundary containing serous fluid with diameter >1 cm

The next step is scoring, as follows:

From that scoring, it can be obtained the irritation index calculation as follows:

$$\frac{(\text{erythemascore}_{24+48+72\text{hours}}) + (\text{edemascore}_{24+48+72\text{hours}})}{\text{Number of participants}}$$

Number of participants

The proportion result of irritation index to irritation degree is used to determine the irritation category: no irritation (0,0), very little irritation (0,1-0,4), little irritation (0,41-1,9), medium irritation (2,0-4,9), acute irritation (5,0-8,0)^{8,10}.

RESULT

Extract of Antin-3 leaves:

Performance of the young leaves of Antin-3 is (2,52 g / 41,51 g) x 100 % = 6,07%. Performance of the old leaves of Antin-3 is 1,68 g / 23,34 g) x 100 % = 7,19 %

Irritation test:

DISCUSSION

Antin-3 Plants

Antin-3 variety (MSU 03028-10) is the result of a research done by Agriculture Research and Development Agency researchers, stating its root contains relatively high anthocyanin around 150,67 mg/100 g (bb) (Yusuf, et al., 2003). From some varieties of the purple sweet potatoes (*Ipomoea batatas* (L.) Lamk) observed, it was found that the secondary metabolite found in leaves is higher than the one found in the root part¹². This statement becomes a main reference for other researchers in using the leaves part

Table 4: Observation Result of Subject 1.

Patch Number	24 hours	48 hours	72 hours	96 hours
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	?	?	-	-
6	?	?	-	-
7	?	?	-	-
8	-	-	-	-

Table 5: Observation Result of Subject 4.

Patch Number	24 hours	48 hours	72 hours	96 hours
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	+	+	-
6	-	+	+	?
7	-	-	-	-
8	-	-	-	-

of Antin-3, besides its utilization is not optimal yet.

Simplicia of Antin-3 leaves

Antin-3 leaves dried through air-drying method without using direct light of the sun because of the flavonoid (anthocyanin) is unstable to heat. Freeze-drying on purple sweet potatoes' leaves (*Ipomoea batatas (L.) Lamk*) results in higher flavonoid compared to the air-drying under room temperature 40:8¹⁶. However, air-drying under room temperature is considered as more appropriate for this research due to its efficient cost compared to freeze-drying. Moreover, the extract of Antin-3 leaves will be mass-produced as an active ingredient for sunscreen products.

Young and Old leaves of Antin-3

Antin-3 leaves used in this research were obtained in harvest period which is 4-5 months old. This decision was obtained as all secondary metabolite components are perfectly formed in the roots and leaves in that period¹¹. Young leaves of Antin-3 dominated with purple and tender for its texture and the old leaves have dark green colour, thicker and harder than the young leaves.

Ethanol extract 96 % of the young and old Antin-3 leaves

Yield percentage of the young Antin-3 leaves is lower than the old Antin-3 leaves. The yield percentage of the extract is comparable to the amount of secondary metabolite compound that can be extracted and it is depend on the mechanical process during the extraction, type of solvent and the amount of secondary metabolite components¹⁶.

Potential reaction of irritation and allergy from the old leaves extract of Antin-3

From the result of the observations, it was obtained that there are two out of seven subjects who indicated irritations with a very little irritation score (0,1-0,4) for the patch number 5 and 6 containing the old leaves extract of Antin-3 with 1000 ppm and 5000 ppm concentration.

Additionally, one of those two subjects indicated a weak allergic reaction from patch number 5 and 6 containing 1000 ppm and 5000 ppm of the old leave extract of Antin-3. This result is convinced as the irritation reaction did not occur at 24 hours application, but at 48 hours and 72 hours. Specifically, for patch number 6, the irritation reaction kept appearing until 96 hours application.

The older Antin-3 leaves the more complex the secondary metabolite compound are¹⁰ Phytochemical screening of 70% ethanol extract of the purple sweet potatoes (*Ipomoea batatas (L.) Lamk*) found some alkaloids, antraquinones, coumarins, flavonoids, saponins, tannins and phenolic acids¹³. Based on the Material Safety Data Sheet (2012), it is revealed that saponins compound in plants could trigger the occurrence of skin irritation¹⁰.

The young Antin-3 leaves, dominated by purple as shown by its roots' colour, is predicted to contain flavonoid (anthocyanin) which is the highest secondary metabolite found in the young Antin-3 leaves. The flavonoid concentration of the young Antin-3 leaves dried through freeze drying method could potentially reach 3,193±0,438 %. Therefore, the young Antin-3 leaves used in this research do not cause irritation and allergy reaction¹.

CONCLUSION

The young leaves of Antin-3 are deep purple resembling its root's colour which indicates the existence of the main secondary metabolite namely flavonoid (anthocyanin) so that the young leaves of Antin-3 with 500 ppm, 1000 ppm and 5000 ppm are not significantly proved causing skin irritation and allergy.

The old leaves of Antin-3 with 500 ppm concentrations do not cause irritation and allergy, while at 1000 ppm and 5000 ppm concentration indicate a very little irritation and weak allergy. The higher concentration of the old leaves of Antin-3 used, the higher saponin concentrations attached to the skin.

However, the young leaves of Antin-3 extract could be processed for formulation stage of sunscreen products.

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