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RESEARCH ARTICLE

Sunscreen activity on fruit skin extract of Annatto (*Bixa orellana* L.) *in vitro*

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Abstract

Background/Objectives: Indonesia is one of the countries that are included in the high category of sun exposure. This fact can have one of the fatal consequences on the skin which can be addressed by the usage of sunscreen. One of the natural ingredients that can be used as sunscreen is the skin of the annatto fruit. **Methods/Statistical analysis**: The sample was extracted using 95% ethanol by the maceration method. Phytochemical screening is carried out on phenolics, flavonoids, tannins, alkaloids, saponins, and terpenoids. The phenolic and flavonoid contents were determined using the spectrophotometer method. The sunscreen potential is determined based on the calculation of the percent of Erythema Transmission (% Te) and the percent of Transmission Pigmentation (% Tp) measured at a wavelength of 292.5 - 372.5 nm. Measurement of the value of sunscreen uses the spectrophotometric method at a wavelength of 290 - 400 nm to determine % Tp, % Te, and the Sun Protection Factor (SPF) value. **Findings/novelty/applications:** The fruit skin extract of annatto contains phenolic, flavonoids, tannins and terpenoids compounds. The total phenolic content was 193.05 $\pm$ 1.22 mg GAE / g extract, and total flavonoids amounting to 138.03 $\pm$ 2.24 mg QE / g extract. From the research results, it was obtained that the value of% Tp,% Te was produced with a concentration of 200 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm respectively for the value (% Te;% Tp) of (18.56%; 45, 25 %) Fast Tanning category, (3.81%; 19.80%) standard suntan category, (1.20%; 10.08%) Ultra-Protection category, (0.30%; 1.64%) sunblock category, (0.09%; 1.42%) sunblock category. Furthermore, for the results of the SPF value in a row is 4.52 (moderate); 8.41 (maximum); 13.10 (maximum); 16.21 (ultra); and 21.12 (ultra).

Keywords: Fruit skin of annatto; extract; phenolic; flavonoids; sunscreen

1 Introduction

Indonesia is one of the countries that is included in the high category of sun exposure. If excessive sun exposure occurs, the epidermal tissue of the skin is not sufficiently able to resist these negative effects. The spectrum of sunlight that has an adverse effect on the skin is the ultraviolet rays called UVB and UVA [(1)]. Both of these ultraviolet rays work synergistically so that prevention or protection is needed to reduce the adverse

effects on the skin [2]. Protection against these two rays is very important because they can cause cancer of the skin [3]. One of the ingredients used is sunscreen. Sunscreen is a preparation that can protect the skin from the influence of ultraviolet rays emitted by the sun so that it can prevent skin disorders due to sun exposure [4].

Annatto is a very useful plant. It is known that the content in annatto plants such as phenolic, flavonoids, beta carotene, terpenoids, and carotenoids [5]. All parts of this plant can be used, starting from the roots, stems, seeds and even fruit skins. However, most of what is used so far is limited to the leaves and seeds, while the skin is only thrown away. Even though, the skin contains ingredients that can be used as sunscreen. The flavonoid compounds contained are thought to be efficacious as an active ingredient in sunscreens.

The effectiveness of sunscreens can be determined by calculating the percent erythema transmission (% Te), percent pigmentation transmission (% Tp) and the SPF value. This research was conducted to determine the sunscreen activity of the fruit skin extract of Annatto *in vitro* using a spectrophotometer method.

2 Materials and Methods

2.1 Tools and materials

The tools used in this study were glassware, Whattman filter paper, rotary evaporator, water bath, oven, porcelain dish, analytical scales, sieve and UV-Vis spectrophotometer. The materials used in this study were the annatto fruit skin extract and 95% ethanol.

2.2 Sample preparation of Annatto fruit skin

Fresh plant material *Bixa orellana* is obtained from the Salatiga area, Central Java, Indonesia. This experiment was carried out in the Biology laboratory of STIFAR Yayasan Pharmasi Semarang. The sample is taken and then washed, the seeds and skin are separated. Furthermore, the skin is taken and cut into thin strips, dried until dry, then blended and sifted with no. 40.

2.3 Extraction of Annatto fruit skin

Extraction of annatto fruit skin powder was carried out by the maceration method with 95% ethanol solvent [6]. A total of 100 grams of dried fruit skin are macerated with 1,000 mL of ethanol% for 5 days. The results of maceration were concentrated using a rotary evaporator.

2.4 Phytochemical screening

The screening for active compounds was carried out on the annatto rind extract qualitatively using color reagents. Screening is carried out to identify metabolite compounds secondary [7].

2.5 Total Phenolic content

Total phenolic content was determined using the Folin Ciocalteu method [8]. A total of 0.1 mL of each extract solution was put in the test tube, then added 0.1 mL Folin Ciocalteu reagent 50%. Mix the divortex, then add 2 mL of 2% sodium carbonate solution. Then the mixture was incubated for 30 minutes. The absorbance is read λ 750 nm. Total phenolic content expressed as the acid equivalent error in mg GAE / g extract.

2.6 Determination of total Flavonoids content

Total flavonoid content fruit skin extract annatto determined according to the method of [9]. A total of 1 mL each - each extract solution was included in a test tube, then added 2 mL of 2% aluminum chloride. The mix is divortexed, and read absorbance at λ 415nm. The total flavonoid content is stated as the inner quercetin equivalent mg GAE / g extract.

2.7 Erythema percent value

The transmittance value at various wavelengths then calculated the percentage of erythema transmission by determining the erythema transmission value is T.Fe. determined the amount of erythema flux that is transmitted by the sunscreen material (Ee) is calculated by the formula:

$$Ee = \Sigma T.Fe$$

Then % erythema transmission is calculated by the formula:

% erythema transmission = Ee / ΣFe (10)

T = Transmission value

Fe = Erythema flux

Ee = ΣT. Fe = the amount of erythema flux transmitted by the extract at a wavelength of 292.5 - 317.5 nm.

2.8 Percent of transmission pigmentation

The percent value of transmission pigmentation was calculated by determining the value of transmission pigmentation is T.Fp. then calculated the amount of pigmentation flux that is continued by the sunscreen material (Ep) which is calculated by the formula

$$Ep = \Sigma T.Fp \quad (11)$$

% pigmentation transmission is calculated by the formula

% transmission pigmentation = = Ee / ΣFp

T = transmission value

Fp = pigmentation flux

Ep = ΣT.Fp = the amount of erythema flux transmitted by the extract at a wavelength of 322.5- 372.5 nm

ΣFp = the total amount of UV light energy that causes pigmentation.

2.9 Determination value for Sun protective factor (SPF)

The ethanol extract of the Annatto skin was weighed. Then it is diluted with 95% ethanol to its concentration (200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm). The UV-vis spectrophotometer is calibrated in advance. A test absorption curve was created in a cuvette with a wavelength between 290-400 nm. Then the mean absorption was determined at 5 nm intervals. The absorbance results of each concentration were recorded and then the SPF value was calculated.

The SPF value is obtained by calculating the area under the absorption curve (AUC) from the absorption value at a wavelength of 290-400 nm. Furthermore, the AUC value is calculated using the following formula:

$$AUC = Aa + Ab/2 \times dPa - b \quad (1)$$

Aa = absorbance at the wavelength a nm

Ab = absorbance at the wavelength b nm

dPa-b = the difference in wavelengths a and b

The total AUC value is calculated by adding up the AUC value for each wavelength segment. The SPF value of each concentration is determined using the following formula:

Log SPF = AUC / $\lambda n - \lambda 1$ (12)

λn = the largest wavelength (with A> 0.05 for extracts and A> 0.01 for preparations)

$\lambda 1$ = the smallest wavelength (290 nm)

This study used a comparator benzophenone as a positive control with a concentration of 10 - 50 ppm. Preparation of Benzophenone solution by weighing as much as 10 mg dissolved with ethanol in a 100 ml volumetric flask to obtain a concentration of 100 ppm. Furthermore, it was diluted to obtain 5 series of concentrations, namely 10, 20, 30, 40, and 50 ppm.

3 Results and Discussion

3.1 Phytochemical screening

Color reaction test or phytochemical analysis is one way to determine the content of secondary metabolites in a plant. Phytochemical analysis was carried out on the annatto flower peel extract qualitatively and visually observed a change in color. Results of phytochemical screening of annatto fruit skin extract in Table 1.

A qualitative phytochemical screening test was carried out on the fruit skin extract annatto. The purpose of screening is to know the content of secondary metabolites. The screening carried out in this study included testing for phenolic, flavonoids, tannins, alkaloids, saponins, and terpenoids. The results of phytochemical screening showed that the fruit skin extract of the annatto contains phenolic, flavonoids, tannins and terpenoids.

Table 1. Results of phytochemical screening of Annatto fruit peel extract

No	Compound group	Results
1.	Phenolic	+
2.	Flavonoids	+
3.	Tannin	+
4.	Alkaloids	-
5.	Saponins	-
6.	Terpenoids	+

3.2 Total Phenolic and Flavonoids content

The total phenolic and flavonoid content in the fruit skin of the annatto is shown in the Figure 1.

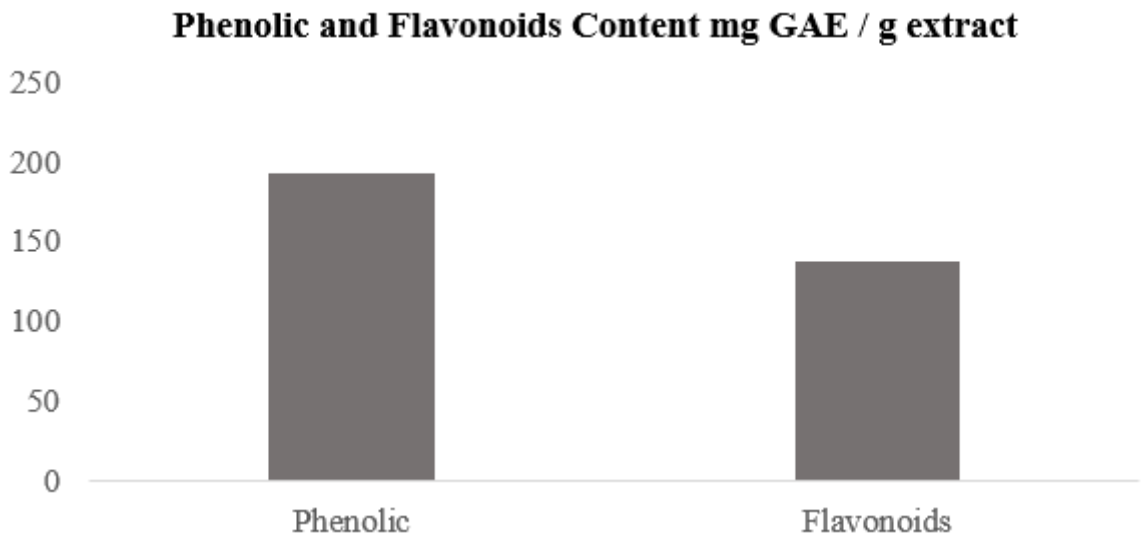

Fig 1. The Total Phenolic and Flavonoids content from the fruit skin of annatto

The calculation results show that the phenolic and flavonoid content annatto rind extract 193.05 ± 1.22 mg GAE / g extract, and total flavonoids amounting to 138.03 ± 2.24 mg QE / g extract. Based on Figure 1 it is known that the extract annatto fruit skin contains total phenolic content high enough, namely 193.05 mg GAE/g, this is normal phenolic compounds including a number of compounds which generally has an aromatic ring with one or more hydroxyl groups [13]. One of the phenol groups that are quite abundant in nature are flavonoids. The flavonoid content of fruit skin extract of annatto is also quite large.

3.3 Sunscreen activity

The sunscreen activity test was carried out by measuring the sample solution at a wavelength of 290 - 400 nm. UV-B rays are a group of harmful rays that can cause damage more quickly and more easily than UV-A rays [14]. UV B rays have 1000 times the activity of sunburn compared to UV A rays [15]. The radiation effect of UV B rays actually causes skin cancer when exposed to excessive radiation [16].

The effectiveness of solar water can be determined by calculating the SPF value as well as the percent erythema transmission (% Te) and the pigmentation percent transmission (% Tp) [17]. A compound is said to have the potential for good sunscreen activity if the material can produce a small% Te or% Tp value with the optimum concentration used. This shows that these compounds are able to block UV rays that pass through the skin so that they can prevent skin damage due to sunlight. The values of% Te and% Tp are categorized into several as shown in Table 2 [18].

Table 2. Sunscreen rating

% Te	% Tp	Category
<1	3 - 40	Sunblock
1 - 6	42 - 86	Ultra Protection
6 - 12	45 - 86	Suntan
10 - 18	45 - 86	Fast Tanning

The results obtained for the percentage value of erythema transmission (% Te) and the percentage value of transmission of pigmentation (% Tp) of the skin fruit extract of Annatto can be seen in Table 3 at a concentration of 200 ppm, 400 ppm, 600 ppm, 800 pp and 1000 ppm. These results indicate that the skin fruit extract of Annatto at a concentration of 200 ppm reaches the fast tanning category, while at a concentration of 400 ppm it reaches the standard suntan category. Furthermore, for a concentration of 600 ppm it reaches ultra-protection. At a concentration of 800 ppm and 1000 ppm it reaches the category of sunblock. This indicates that the skin fruit extract of Annatto has sunscreen activity.

Table 3. The results of testing the Sunscreen activity of the skin extract of Annatto

Concentration (ppm)	% Te	% Tp	Category
200	18.56	45.25	Fast Tanning
400	3.81	19.80	suntan standard
600	1.20	10.08	Ultra Protection
800	0.30	1.64	sunblock
1000	0.09	1.42	sunblock

Furthermore, the SPF value is tested. The SPF assessment refers to the classification Table 4. Categories for the SPF value range from minimum, moderate, extra, maximum to ultra-protection [19]. The data on the results of SPF determination can be seen in Figure 2. The results obtained show that the skin fruit extract of annatto with a concentration of 200 ppm is in the moderate level category, the concentration of 400 ppm and 600 ppm is in the maximum category, the concentration of 800 ppm and 1000 ppm is in the ultra-category.

Table 4. SPF value category

SPF	Category
2 - 4	Minimal
4 - 6	Moderate
6 - 8	Extra
8 - 15	Maximum
≥ 15	Ultra

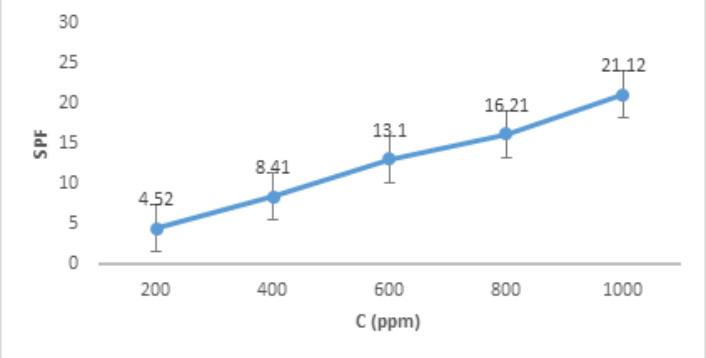

Fig 2. The results of the SPF test of the fruit skin extract of Annatto

The preparation is said to provide protection if it has an SPF value of 2-100 [20]. Several factors that can affect the determination of the SPF value include the use of different solvents, the type of emulsion, the combination and concentration of sunscreen, the effects and interactions of component carriers such as emulsifiers, esters, carrier interactions with the skin, the addition of active ingredients and the pH system [21]. The results of the SPF value can be increased with an increase in the phenolic compounds contained in an extract [22].

The comparator sunscreen used is a benzophenone compound. This compound was chosen because it is able to absorb UV B rays and UV A rays which have a short wavelength but have high energy. Benzophenone SPF measurement results can be seen in Table 5 . Benzophenone compounds have very good photostability capabilities, so they function as UV light filters.

Table 5. SPF value of Benzophenone

C(ppm)	SPF value
10	11.95
20	18.13
30	24.31
40	30.17
50	35.3

The data obtained from the determination of the SPF value of the fruit skin extract of annatto showed that the equivalent results obtained when compared with the benzophenone ratio turned out that the protective power of benzophenone was greater than the sample. The fruit skin extract of with a concentration of 600 ppm is equivalent to a concentration of 10 ppm of benzophenone. Furthermore, the fruit skin extract of the annatto with a concentration of 800 ppm is equivalent to a concentration of 20 ppm of benzophenone. And fruit skin extract of annatto with a concentration of 1000 ppm or equivalent to a concentration of 30 ppm benzophenone.

The sunscreen activity of the annatto rind extract is due to the phenolic compounds present in it. According to [23] the content of phenolic compounds is proven to protect the skin from damage caused by the effects of UV radiation.

4 Conclusion

The fruit skin extract of annatto contains phenolic, flavonoids, tannins and terpenoids compounds. The total phenolic content was 193.05 $\pm$ 1.22 mg GAE / g extract, and total flavonoids amounting to 138.03 $\pm$ 2.24 mg QE / g extract. The phenolic contents of fruit skin extract of annatto have sunscreen activity.

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