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RESEARCH ARTICLE

Total flavonoid content and *in vitro* study on the sunscreen activity of extracts of leaves of *Elaeocarpus floribundus* blume

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Abstract

Background: *Elaeocarpus floribundus* blume leaves have traditionally been used to treat a variety of diseases; its hot water infusion is used as a gargle to treat inflamed gums and relieve rheumatoid arthritis. Furthermore, previous research has shown that its methanol extract contains a high level of total phenolic content. **Objective:** To determine the total flavonoid content (TFC) of *E. floribundus* leaves extracts and to conduct preliminary research on their sunscreen activity. **Method:** This plant's dried-bulk leaves were macerated in three different solvents, *n*-hexane, ethylacetate, and methanol, in that order. The TFC of all extracts was determined using a colorimetric method with $AlCl_3$ as the reagent and quercetine as the standard. In contrast, sunscreen activity was assessed by determining the value of the Sun Protection Factor (SPF) using a UV-Vis spectrophotometer and 3-benzophenone as the positive control. **Result:** The results indicated that the *n*-hexane, ethyl acetate, and methanol extracts contained 35.58 ± 1.87 mgQE/g extract, 60.59 ± 0.53 mgQE/g extract, and 149.28 ± 0.89 mgQE/g extract, respectively. With SPF values of 45.470; 44.791; 43.754; 38.861 and 21.102 for tested concentrations of 1000; 800; 600; 400 and 200 μ g/mL, respectively, methanol extract demonstrated the highest potential for sun protection activity. However, this activity was not more potent than 3-benzophenone, which had an SPF value of 22,647 at a concentration of 50 μ g/mL. **Conclusion:** The methanol extract of *E. floribundus* blume's leaves had the highest total flavonoid content and the greatest potential for sun protection among the tested extracts.

Introduction

Elaeocarpus floribundus Blume is a species of plant belonging to the genus *Elaeocarpus* that typically grows in India, Burma, Thailand, Vietnam, Malaysia, and Indonesia on lowland hills and mountains (Wuart, 2006). An infusion of its leaves and stem bark has traditionally been used as a mouthwash to treat inflamed gums. A decoction of its leaves is used to treat diabetes and hypertension in Mauritius. The fruit extract is also used as an anti-ageing agent and has been found to be beneficial for the skin (Roslim, Khumairoh, & Herman, 2016; Wuart, 2006). Moreover, the leaves are also used to treat rheumatism, and the

fruit can be used to treat dysentery and diarrhoea (Zaman, 2016).

There have been reports about the chemical components that make up the leaves of this plant. Several flavonoids have been isolated from the leaves, including: including myricetin, myricitrine, mearnssetine, and ellagic acid (Zaman, 2016) The total phenolic content of the leaves and stem bark was determined using a colorimetric method. When compared to methanol extract of stem bark, the total phenolic content of methanol extract of leaves was 503.08 ± 16.71 mg GAE (gallic acid equivalent)/g DW (dried weight). The Ethylacetate extract of its leaves

demonstrated strong antioxidant activity, with an IC50 value of 9.37 ± 0.06 g/mL (Utami et al., 2013).

Antioxidants are molecules that are able to easily give their electrons to free radical molecules, which allows the free radical molecules to become stabilised and prevents unwanted oxidation processes from occurring in cells (Bubols et al., 2013). The consumption of foods and drinks that are high in antioxidants can help prevent a variety of diseases that are brought on by exposure to UV radiation. According to the findings of previous studies, it is established that the class of active antioxidant compounds known as phenolics and flavonoids possess the capability to shield the skin from the damaging effects of UV radiation (Nagula & Wairkar, 2019). These findings prompted the authors to conduct research on the total flavonoid content (TFC) and an *in vitro* study of the sunscreen activity of extracts from the leaves of *E. floribundus* Blume.

Methods

Leaves of *E. floribundus* Blume were collected from Desa Kotaringin, Kecamatan Mempura, Kabupaten Siak Sri Indrapura, Provinsi Riau, Indonesia. This plant's herbarium was identified by a plant taxonomist at Riau University's Botany Laboratory, Department of Biology.

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Determination of total flavonoid content.

For the determination of TFC, a colorimetric method with $AlCl_3$ as the reagent, was used. The value of TFC was expressed in terms of mg Quercetin Equivalent (QE) per gram of extract, with quercetin serving as the standard. The microplate reader set to a wavelength of 430 nm was utilized in order to carry out the analysis of the tested solutions of each extract (Liu, Song, & Zhang, 2017). This test was carried out a total of three times.

In vitro sunscreen activity assay

For the purpose of this study, a UV-Vis spectrophotometer was utilized to determine the sun protection factor (SPF) value. This was accomplished by measuring the absorbance of various extract

concentrations obtained at a wavelength with a range of 290-320 nm (at intervals of 5 nm) and using ethanol as a blank (Dutra et al., 2004). As a positive control, the authors used 3-benzophenone with a concentration that was tested at 50 µg/mL, and carried out this evaluation three times.

The absorbance value of the extract at each wavelength was then multiplied by the constant value of $EE \times I$ (Table I). After that, the obtained value was added up. The sum result is then multiplied by the correction factor of 10 to get the SPF value of the extract for each tested concentration based on this formula (Dutra et al., 2004) :

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

CF - Correction Factor (10)

EE - Erythremal Effect

I - Intensity

Abs - Absorbance of the tested solution

Table I: Value of EE x I for SPF formula

Wavelength (λ) (nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Results

For the determination of TFC, a colourimetric method that: uses $AlCl_3$ as its reagent, analyses the sample with a UV-Vis spectrophotometer and uses quercetin as its standard was developed. The n-hexane extract produced 35.579 1.872 mgQE/g extract, the ethylacetate extract produced 60.599 0.535 mgQE/g extract, and the methanol extract produced the highest amount of TFC with a value of 149.283 0.886 mgQE/g extract (Figure 1).

Regarding the *in vitro* evaluation of the sunscreen activity, n-hexane extract only demonstrated the ultra protection category with an SPF value of 18.354 when tested at the highest concentration possible, which was 1000 µg/mL. The ethyl acetate extract provided ultra protection of sunscreen activity with SPF values ranging from 20.509 to 44.018 when tested at concentrations of 1000; 800; 600 and 400 µg/mL. However, at the lowest concentration of 200 µg/mL, the extract only showed maximum protection with an SPF value of

10.028. Nevertheless, each and every concentration of methanol extracts showed an ultra-protection category with SPF values ranging from 21.102 to 45.470 (see Table II). The positive control, 3-benzophenone,

demonstrated an ultra-protection category with an SPF value of 22.647 for a concentration of 50 g/mL that was tested.

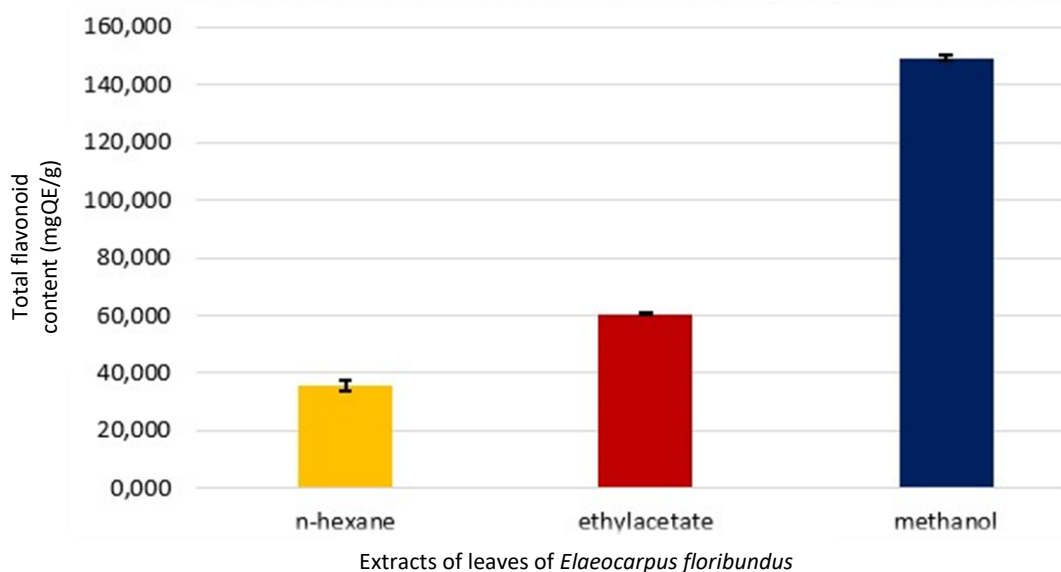


Figure 1: Total flavonoid content of n-hexane, ethylacetate and methanol extracts of leaves of *Elaeocarpus floribundus*

Table II: Sun protection factor (SPF) values of n-hexane, ethylacetate and methanol extracts of leaves of *Elaeocarpus floribundus*

Tested Concentrations (µg/mL)	n-hexane extract		Ethylacetate extract		Methanol extract	
	SPF value	Category*	SPF value	Category*	SPF value	Category*
1000	18.354	Ultra protection	44.018	Ultra protection	45.470	Ultra protection
800	14.867	Maximum protection	39.236	Ultra protection	44.791	Ultra protection
600	11.280	Maximum protection	30.842	Ultra protection	43.754	Ultra protection
400	7.312	Extra protection	20.509	Ultra protection	38.861	Ultra protection
200	3.637	Minimum protection	10.028	Maximum protection	21.102	Ultra protection

*Category classification used is the same as Sami, Nur, and Martani (2015)

Discussion

Flavonoids are a class of polyphenolic compounds that can stop a variety of different oxidation reactions from happening. Flavonoids have antioxidant properties as they can donate electrons to free radical compounds (Bubols et al., 2013). A colourimetric approach can be used to ascertain the number of flavonoids that are

present in the plant. The formation of complexes between $AlCl_3$ and flavonoid compounds at ortho-hydroxy ketone groups is the basis for measuring the total flavonoid content of a substance. Quercetin is a compound that is a member of the flavonols group and is capable of reacting with $AlCl_3$ to form those specific complexes (Liu, Song, & Zhang, 2017). The solution of

yellow-coloured complexes was then analysed with a UV-Vis spectrophotometer.

Determination of the calibration curve of quercetin resulted in a regression equation of $y = 0.0094x + 0.0237$ with R^2 of 0.9563. Based on this equation, TFC of n-hexane, ethylacetate and methanol extracts of leaves of *Elaeocarpus floribundus* were obtained as presented in Figure 1. The methanol extract showed the highest level of TFC with a value of 149.283 ± 0.886 mgQE/g extract, followed by ethyl acetate extract with TFC value of 60.599 ± 0.535 mgQE/g extract, while n-hexane extract exhibited the lowest TFC level with value of 35.579 ± 1.872 mgQE/g extract.

In terms of the potential activity of a sunscreen when tested in vitro, the methanol extract showed the highest level of potential. All concentrations of this extract that were put to the test showed activity in the ultra protection category, with an SPF value ranging from 21.102 to 45.470 (Table II). It was discovered that the highest possible concentration of TFC resulted in the greatest possible sunscreen activity.

Flavonoids are compounds with antioxidant and photoprotective activity that can be very useful for lowering the amount of UV radiation that is absorbed by the skin, preventing premature aging of the skin, and protecting the skin from damage caused by exposure to UV light (Cefali et al., 2016; Nagula & Wairkar, 2019).

Conclusion

The methanol extract of the leaves of *E. floribundus* blume demonstrated the highest level of TFC and also resulted in the most potential sunscreen activity when compared to the other extracts.

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