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In-vitro PHOTOPROTECTIVE ACTIVITIES OF DIFFERENT SOLVENT EXTRACTION OF *Hibiscus sabdariffa*

(*In-vitro* Aktiviti Foto Perlindungan Bagi Ekstrak Pelarut yang Berbeza dari *Hibiscus sabdariffa*)

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Abstract

In recent years, plant extracts have been considered as potential sunscreen resources owing to their antioxidant activity as well as capability to absorb ultraviolet (UV) rays in the UVA region. In the present study, the UV protective factor of three different extracts of *Hibiscus sabdariffa* (HS) namely ethanol (HS-ETOH), ethyl acetate (HS-EA), and hexane (HS-HEX), all of which are rich in flavonoids and other phenolic components were assessed by using UV-Vis spectrophotometer to estimate their corresponding *in-vitro* Sun Protection Factors (SPF). The results revealed that both extracts of HS-ETOH and HS-EA showed characteristic absorption bands in the UVA and UVB regions, while the HS-HEX extract was observed to absorb in the UVB and UVC regions under similar concentration. Pertinently, the highest SPF (17.53±0.22) was obtained in the HS-ETOH extract, inferring its potential as an active component for sunscreen in pharmaceutical and cosmeceutical preparations.

Keywords: *Hibiscus sabdariffa*, sun protection factor, sunscreen, UV-spectrophotometry

Abstrak

Bahan-bahan semulajadi yang telah diekstrak daripada tumbuh-tumbuhan baru-baru ini telah dipertimbangkan potensinya untuk menjadi sumber perlindungan matahari bagi penyerapan sinar ultraviolet dalam lingkungan UVA dan aktiviti antioksidan. Dalam kajian ini, faktor perlindungan UV bagi tiga ekstrak yang berbeza iaitu ekstrak etanol (HS-ETOH), ekstrak etil asetat (HS-EA), dan ekstrak heksana (HS-HEX) daripada *Hibiscus sabdariffa* (HS) yang kaya dengan komponen seperti flavonoid dan fenolik telah dinilai dengan menggunakan spektrofotometer UV-Vis dan dikira dengan Faktor Perlindungan Matahari (SPF). *In-vitro* SPF daripada ekstrak HS telah dianalisis dengan menggunakan pengukuran penjerapan terhadap pelarut yang berbeza semasa proses pengekstrakan. HS-ETOH dan HS-EA telah menunjukkan ciri jalur penyerapan bagi kawasan UVB dan UVA manakala HS-HEX di kawasan UVC dan UVB pada kepekatan yang sama. HS-ETOH mencapai faktor perlindungan matahari yang paling tinggi SPF (17.53±0.22). Ia menunjukkan kemungkinan untuk menggunakan ekstrak ini sebagai perlindungan matahari dalam persediaan farmaseutikal dan kosmeseutikal.

Kata kunci: *Hibiscus sabdariffa*, faktor perlindungan matahari, pelindung matahari, UV-spektrofotometri

Introduction

Oxidative stress and inflammatory responses induced by ultraviolet radiation (UV) can result in a variety of harmful effects on skin, which include premature photoaging, induction of immunosuppression, skin carcinogenesis [1] and even skin cancer [2]. The harmful effects of UV rays on humans can be divided into three regions: ultraviolet A (UVA – from 320 nm to 400 nm); ultraviolet B (UVB – from 290 nm to 320 nm) and ultraviolet C (UVC – from 200 nm to 290 nm). UVC radiation is filtered by the atmosphere before reaching Earth but radiation from UVB may still pass through the ozone layer and is responsible for skin damage through sunburn. Furthermore, UVA radiation can reach the deeper layers of epidermis and dermis, and the damaging effects provoke premature skin aging [2]. The problem has been further exacerbated by the fact that the ozone layer surrounding the Earth's atmosphere, which shields us from the harmful effects of UV, has become considerably thinner than it was 50 years ago. This has resulted in higher penetration of all types of UV radiation. The unfortunate outcome has been associated with increased hazardous compound emissions, i.e., chlorofluorocarbons into the atmosphere, which is brought about by increased industrialization activities. In view of such circumstances, skin protection by using creams with high sun protection factor (SPF) [1] may prove to be essential for reducing the harmful effects of UV exposure. Therefore, concerted efforts in developing sunscreen formulations that effectively protect the human skin against UVA-induced and UVB-induced damages have merit scientific and commercial pertinence.

Among the active compound sources described to exhibit sun-protective properties against UV-induced damages are those extracted from plants. It has been indicated that plants produce various antioxidants as well as compounds that quench and protect against molecular damage caused by reactive oxygen species (ROS). The most important group of compounds includes flavonoids which are known for their ability to scavenge UV-induced radicals, a feature which also indicates that flavonoids make excellent UV-absorbing screens [3]. *Hibiscus sabdariffa* (HS), also known as Roselle from the Malvaceae family [4], is among the many plants known for being high in polyphenols [5] and the calyces are inherently rich in antioxidants. So far, HS calyce extracts were mainly studied to combat degenerative diseases and act as local herbal medicines for their diuretic, choleretic, febrifugal and hypotensive, blood thinning effects as well as stimulating intestinal peristalsis [6]. Studies utilizing extracts of HS as active ingredients in sunscreen preparations have yet to be reported and the feasibility of using HS for such purpose remains to be seen.

To date, only a limited number of plant extracts and oils exhibiting photo-protective action were used in formulations to manufacture sunscreens [1]. However, the current selection of plant extracts, containing sun-protective ingredients for preparing sunscreens, are rather limited and insufficient to satisfy the increased consumer demands for cosmetics formulated by using products obtained from natural resources. Herein, the study aims to evaluate another potential source of sun-protective compounds, i.e. HS, extracted by using solvents, ethanol, ethyl acetate and hexane. The extracts of HS were evaluated for their photo-protective potential by using a UV-Vis spectrophotometer.

Materials and Methods

Hibiscus sabdariffa L.

Roselle (*Hibiscus sabdariffa)* was purchased in February, 2017 from the Federal Agricultural Marketing Authority (FAMA) in Rengit, Malaysia. The plant was authenticated by an expert and deposited at the Herbarium Unit of Institute of Bioscience, in Universiti Putra Malaysia.

Chemicals and reagents

All reagents used were of analytical grade and used as-received without further purification. Distilled water was used for preparing solutions. Solvents, ethanol, methanol and ethyl acetate were purchased from Sigma Aldrich (Milwaukee, WI, USA).

Preparation of HS extracts

The calyces were washed with tap water three times and air-dried for 7 days prior to being grinded into coarse powder. HS extracts, containing high quantities of antioxidant rich compounds, were prepared according to the method by Zhen et al. with some minor modifications [8]. HS powder (10 g) was transferred into a 200 mL round-bottom flask, containing 100 mL of solvent (ethanol, ethyl acetate and hexane) and shaken by using an orbital

shaker for 12 hours at temperature 40 °C. The mixture was filtered through a Whatman filter paper (No. 4) and the residues were further extracted with a fresh batch of solvents (100 mL) and filtered once again. The supernatants of the extracts were combined and concentrated in a rotary evaporator (IKA RV 10 Digital V, German) at 45 °C and lyophilized.

Screening of potential of UV-protective effect and sun protection factor value *in-vitro*

For determining of the maximum absorption wavelength (λ_{max}), the dried extracts were diluted in the extraction solvent, to have the final concentration as 1 mg/mL. Subsequently, the spectrophotometric scanning was performed at wavelengths between 260-400 nm, with intervals of 5 nm. The readings were performed using 1 cm quartz cell, and ethanol used as blank [7]. Meanwhile the calculation of SPF was obtained according to the equation 1 developed by Mansur [9]:

$$SPF_{spectrophotometric} = CF \times \Sigma EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (1)$$

where EE (λ) is defined as erythemal effect spectrum, I (λ) is solar intensity spectrum, Abs (λ) is absorbance of sunscreen product, CF is correction factor (= 10). The values of EE x I are constants. The value was determined by Sayre et al. [10] and shown in Table 1.

Table 1. Normalized product function used in the calculation of SPF

Wavelength (nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1.0000

Results and Discussion

SPF is a quantitative measurement of sunscreen formulation effectiveness. To be effective in preventing sunburn and other skin damages, a sunscreen product should have a wide range of absorbance between 290 nm and 400 nm. The potential of HS-EtOH, HS-EA, and HS-HEX extracts as UV-sunscreen agents was measured by the extract and solution absorbance in UVA range and UVB range. Figure 1 shows the spectrophotometric absorption profile of the HS extracts with different solvent extractions. From the data analysis, HS-EtOH and HS-EA showed characteristic absorption bands in both regions, UVB (290 nm – 320 nm) and UVA (320 nm – 400 nm) at 1 mg/mL concentration, suggesting a possible photo-protective potential. Furthermore, HS-EtOH extracts showed a higher absorbance at UVA wavelength of 330 nm at 1.73 and 355 nm at 1.01. The effect of different solvents, in which the extracts are dissolved and emollients have upon the wavelength of maximum absorbance and upon the UV absorbance of several sunscreen chemicals, alone or in combination; thus, interfering those of UVA and UVB regions is well known and documented [11, 12]. This protective effect against sunrays could be advantageous to an extract as it could enhance the effect of anti-wrinkles activity.

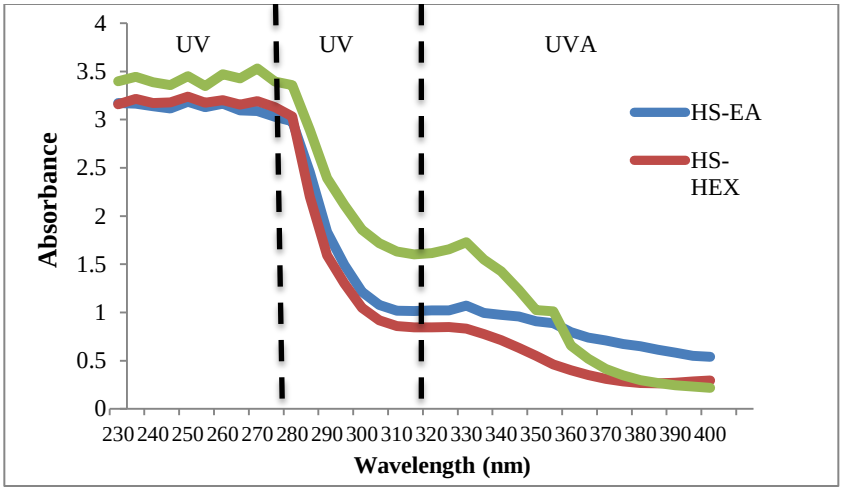

Figure 1. UV-Spectrum Profile of ethanol (HS-ETOH), ethyl acetate (HS-EA) and hexane (HS-HEX) extracts of *Hibiscus sabdariffa* (230-400 nm)

The SPF in vitro value was determined by the spectrophotometric method developed by Mansur et al. through using the UVB region [9], which was considered to be the region of greatest incidence during the day in which people are exposed longer [2]. This in vitro assay is a simple, reliable, quick, inexpensive and a validated technique, which has been widely used to determine the sunscreen potential of several, natural and synthetic products or formulations [13, 14]. In Figure 2, it can be observed that HS-EtOH extract showed 4 higher SPF value (17.53±0.22), followed by HS-EA extract (12.26±0.15). The lowest SPF value was obtained with HS-HEX extract at 10.74±0.13, respectively, with same concentration (1 mg/mL). This may due to the presence of flavonoids, flavones, phenolics acids as well as other phytoconstituents in the HS-EtOH and HS-EA extracts. Flavonoids and phenolic compounds were reported as functional components in plant and fruits, which play an important role in the management of inflammation and erythema, due to solar radiations [15]; thus, it can be concluded that high amount of phenolic compound and flavonoids could be the cause for their high SPF [16]. The antioxidant property of the flavonoids and phenolic compounds further potentiates the UV protection or photo-protection activity. Antioxidants provide endogenous photo-protective activity and are essential for the protection and maintenance of skin health. Further, flavonoids can also modulate enzyme activity and effect cell division [17].

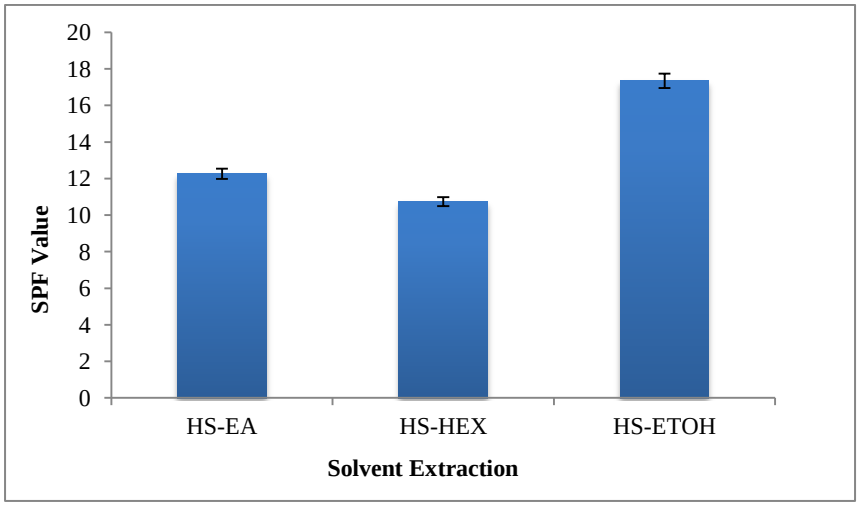

Figure 2. Sun Protection Factor (SPF) value of ethanol (HS-ETOH), ethyl acetate (HS-EA) and hexane (HS-HEX) extracts of *Hibiscus sabdariffa*

Conclusion

The study demonstrated the ethanolic and ethyl acetate extracts of *Hibiscus sabdariffa* have photoprotective properties, suggesting that these solvents were efficient in extracting high concentrations of photoprotective active compounds in HS calyces. The high SPF values obtained for HS-EtOH extracts (17.53±0.22), as well as strong absorptions in UVA region for HS-EtOH (330 nm and 355 nm), were good indications that HS-EtOH extracts would be beneficial as photo-protective additives in sunscreen formulations. Considering the favorable results for the HS- EtOH extracts, *in-vivo* experiments to validate efficacy of both extracts for application in sunscreen formulations may prove necessary.

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