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Original Article

Preliminary Evaluation of Photoprotective Potential in Flowers of *Osbeckia octandra* (L.) DC. for Development of Herbal Sunscreen Formulations

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Abstract

Introduction: Excessive exposure to ultraviolet (UV) radiation of the solar spectrum is often linked with the onset of conditions like inflammation, photoaging, immunosuppression, hyperpigmentation, and photocarcinogenesis. Although synthetic sunscreens have emerged as protectants against this harmful UV radiation, there is an increasing demand for sunscreens of herbal origin, which are believed to have low side-effect profiles in comparison to their synthetic counterparts. In order to cater for this need, the present study aimed at developing herbal sunscreen formulations from flowers of *Osbeckia octandra* DC, a purple-coloured wildflower widely distributed across different geographical regions in Sri Lanka.

Methodology: The UV filtering potency and subsequently, the sun protection factor (SPF) in the methanolic extract of *O. octandra* was initially determined. Thereafter this extract was incorporated into the aqueous cream base at 25%, 50%, and 75% (w/w) and the SPF values and photostability of the prepared formulations were evaluated.

Results: The initial SPF value of the crude extract (39.91 ± 1.93) had hardly changed even after incorporating it at 75% into the aqueous cream base. This 75% formulation surpassed the other two formulations and the commercial sunscreen (positive control) in terms of the higher SPF and broader spectrum of UV absorption. Its SPF value altered only slightly during the storage for 21 days in light or dark conditions and was photostable.

Conclusion: Our preliminary observations demonstrated the appropriateness of *O. octandra* for the formulation of herbal sunscreens at the commercial stage.

Keywords: Herbal sunscreens, *Osbeckia octandra*, Photoprotection, Sun protection factor, UV radiation

Introduction

Life on earth would not be possible without the sun, however; excessive and prolonged exposure to solar radiation is associated with several dermatological problems in humans. The ultraviolet (UV) component of the solar spectrum accounts for electromagnetic radiation with short wavelengths and high energy and is responsible for conditions such as erythema, inflammation, hyperpigmentation as well as photo aging and photocarcinogenesis. Solar UV radiation is divided into three categories; UV-C (100–280 nm), UV-B (280–315 nm), and UV-A (315–400 nm), out of which UV-B and UV-A account for the aforementioned deleterious effects on human skin whereas most devastating UV-C radiation gets completely filtered by the stratospheric ozone layer, hence does not reach earth [1]. However, anthropogenic activities have led to a substantial depletion of the stratospheric ozone level and resulted in a significant increase of the UV radiation reaching the earth and consequently the related dermatological problems [2]. The harmful effects of solar UV radiation on human skin could be avoided or minimized by an innovative approach termed "photoprotection", which involves various strategies such as avoidance of sun, protection of skin with clothing, and topical or oral application of sunscreens [3].

Topical sunscreens are widely used as protectants against photodamage and are comprised of active ingredients with the ability to reflect or absorb UV rays and thereby reduce the penetration of UV radiation in the skin [3]. However, the presence of multiple chemicals in sunscreen products could trigger allergic responses [4] and particularly the oxybenzone, which is frequently present in synthetic sunscreens has been reported to produce contact and photo contact allergy reactions, implemented as a possible endocrine disruptor and has been linked to Hirschsprung's disease [5]. In view of these adverse effects associated with synthetic sunscreens along with the emergence of the concept of "green cosmetics", plant extracts and phytochemicals are receiving increased attention as natural alternatives for synthetic chemical ingredients in sunscreen products [2].

In the quest for natural photoprotective agents, flavonoids would be ideal candidates due to their high UV absorption capability, direct and indirect antioxidant properties, and ability to modulate several signaling pathways [6]. Among the different classes of flavonoids, anthocyanins are considered the main pigments accountable for the colors of many fruits, vegetables, and flowers which are red, blue, and purple [6,7]. On this basis, colorful flowers could be studied as potential sources of natural sunscreen agents. Thus, the present study is carried out to evaluate the photoprotective activity in *Osbeckia octandra* DC., one of the colorful and common wildflowers in Sri Lanka.

Osbeckia octandra DC.,is native to Sri Lanka and is found as a small shrub in the moist mid and low-country regions of Sri Lanka [8]. The color of the flower varies from pink to mauve or purple. The plant belongs to the family Melastomataceae from which different anthocyanins such as pelargonidin, cyanidin, peonidin, delphinidin, and malvidin glycosides or acyl glycosides have been isolated [9]. Further, *Osbeckia* species are rich in anthocyanin and thus display potent antioxidant activity [10]. In addition, significant antioxidant activity and a high phenolic content were detected in leaf decoctions prepared from *O. octandra* grown in Sri Lanka [11]. Based on this literature evidence, we hypothesized that the flowers of *O. octandra* possess desirable features to be used in the development of natural sunscreen formulations with high UV absorbance capacity and hence, a high sun protection factor (SPF). Initially, the UV-filtering potential of the methanolic extract of the flowers of *O. octandra* was determined, thereafter, this extract was incorporated at different percentages to formulate herbal sunscreens, and the *in vitro* photoprotective potency of these sunscreen formulations was evaluated in this study.

Methodology

Plant Material and preparation of extract

Flowers of *O. octandra* were collected from Pasyala, Gampaha District -Western Province of Sri Lanka in 2020 and identified by the author (MN), a botanist. The plant material was authenticated by comparison with the herbarium specimens at the National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka. A voucher specimen (MN_20_05) was deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka.

The flowers were washed with running water to remove the dust and thereafter dried in shade (30 °C) for four days until a constant weight was obtained. Dried plant materials (3.2 g) were cut into small pieces and extracted in 250 mL of methanol (Sigma-Aldrich, Germany). The extract was evaporated into dryness with the use of a rotary evaporator (HS-2005V-N, South Korea).

Evaluation of UV filtering potential in the methanolic extract of *O. octandra* (MEFO)

The UV filtering potential of the extract was determined following the method described by Napagoda et al. [2] which involved measuring the UV absorption of the extract (1 mg/mL in methanol- denoted as "MEFO") followed by calculating the sun protection factor (SPF) according to the Mansur equation given below [12].

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

where EE (λ) – erythemal effect spectrum; I (λ) – solar intensity spectrum; Abs (λ) – absorbance of sunscreen product; CF – correction factor (=10)

In order to assess whether the initial SPF value of this extract would change under different storage conditions, the extract was exposed to direct sunlight and also stored in darkness separately for 21 days, and subsequently, the SPF value was calculated on the 7th, 14th and 21st day. The experiment was conducted in triplicates.

Qualitative analysis for the presence of phenolic compounds in the MEFO

The qualitative tests for the detection of phenolic phytochemical compounds were carried out for the MEFO (1 mg/mL) as per the standard methods [13,14].

Test for phenolics

The extract (1 mL) was treated with 3-4 drops of ferric chloride solution and the appearance of a bluish-black color indicated the presence of phenolics

Test for flavonoids

The extract (1 mL) was treated with a few drops of NaOH. The appearance of intense yellow color which turns colorless upon the addition of diluted HCl was an indication of the presence of flavonoids.

Additionally, the extract (1 mL) was treated with a few drops of lead acetate solution and the formation of a yellow-colored precipitate was an indication of the presence of flavonoids.

Test for anthocyanin

To determine the presence or absence of anthocyanins, 2 N HCl (2 mL) was added to the flower extract (2 mL). The appearance of a pink-red color that turns purplish-blue after the addition of diluted ammonia was considered an indicator of the presence of anthocyanin.

Determination of total flavonoid content

The method described by Khodaie et al. [15] and modified by Liyanaarchchi et al. [16] was employed for this purpose. The MEFO (1 mg/mL;500 µL) was treated with $NaNO_2$ (150 µL)

and incubated for 5 min and further incubated for 6 min after the addition of 10% $AlCl_3$ (150 µL). Into this reaction mixture, NaOH (2 mL) was added, and the absorbance was measured at 510 nm. A standard curve was plotted following the same procedure using 0.02-1 mg/mL of quercetin solutions in methanol. The total flavonoid content of the extract was calculated according to the calibration curve, y=0.0081x-0.0025 (R^2=0.9956) and was expressed in terms of quercetin equivalence in (QUE)/g dry weight (DW) of extract. The experiment was performed in duplicate.

Formulation of herbal sunscreens and determination of the UV filtering potential

The methanolic extract (i.e. the residue after evaporating methanol) of *O. octandra* was mixed with an aqueous cream base at 25%, 50%, and 75% (w/w). This resulted in three different sunscreen formulations. The UV absorbance measurements of the prepared formulations were obtained, and the SPF was determined on the 7th, 14^{th,} and 21st day after storing under light and dark conditions separately.

A commercial herbal sunscreen formulation containing *Aloe*, Sandlewood, *Ficus* was used as the positive control whereas the aqueous cream base was used as the negative control. The experiment was performed in triplicate.

Determination of Photostability

The photostability of the sunscreen formulations was determined by the method described by Gonzalez et al. [17] and modified by Liyanaarachchi et al. [16]. In brief, 50 mg of the sample was applied on a 25 cm^2 area of a stainless steel plate, which corresponds to an area density of 2.0 mg/cm^2 [18]. The plates were exposed to natural sunlight for five hours (from 9.30 a.m. to 2.30 p.m.). Control plates of each formulation which have not been exposed to sunlight were also prepared for UV absorption measurements.

The UV absorbance of each sample was determined in the 290–400 nm range. The commercial herbal sunscreen product and the aqueous cream base were used as the positive control and negative controls respectively. The experiment was performed in duplicate. A curve between absorbance versus wavelength was drawn and the area under the curve (AUC) for total UV spectrum (290–400 nm), UV-A1 (340–400 nm), UV-A2 (320–340 nm), and UV-B (290–320 nm) spectra were calculated for each of the exposed and non-exposed samples. The following equation was used to determine the AUC index (AUCI) where AUCI = $AUC_{exposed}$/ $AUC_{non\text{-}exposed}$, and if the AUC index≥0.8, the sunscreen formulation was considered to be photostable [16, 17].

Results

The high SPF value, a broad spectrum of UV protection, and photostability are accepted as features of an ideal sunscreen [19]. These parameters were evaluated in the present study.

UV filtering potential in the MEFO

Figure 1(A) shows the UV absorbance of the methanol extract of *O. octandra* at the range of 260-400 nm. This indicates high UV absorbance throughout the range of 290–350 nm with the maximum absorbance within the UV- B range.

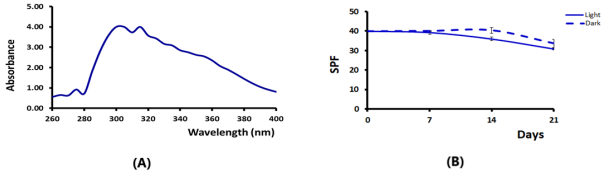

Figure 1: UV filtering potential in methanolic extract of *O. octandra*
(A) Absorption of UV radiation between 260–400 nm
(B)Variation of SPF at different time intervals under light and dark conditions

The SPF of the MEFO was recorded as 39.91±1.93. This value varied only slightly

over 21 days as evident from Figure 1 (B). Nevertheless, out of the two samples, the variation of SPF value was higher in the sample that was exposed to sunlight.

Analysis of the presence of phenolic compounds in the MEFO

Positive results were obtained for all the qualitative phytochemical tests indicating the presence of flavonoids, particularly, anthocyanins in the flower extract while the total flavonoid content in this extract was determined as 20.93 mg (QE)/g. Phenolic secondary metabolites such as flavonoids have been considered effective shielding materials and the anthocyanins present in flower petals are reported to have the ability to effectively screen harmful UV-B radiation [20]. Therefore, the detection of anthocyanin in the flower extract could be correlated to the high UV absorbance observed in Figure 1 (A).

Formulation of herbal sunscreens and evaluation of UV filtering potential

All the sunscreen formulations prepared from the flower extract appeared brown. The color intensity varied depending on the percentage of the extract incorporated in the formulation (Figure 2).

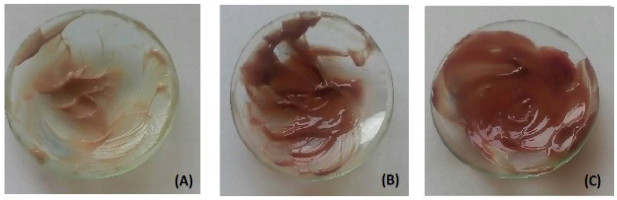

Figure 2: Sunscreen formulations prepared with (A) 25% (B) 50% and (C)75% of the methanolic extract of *O. octandra*

Figure 3 illustrates the UV absorbance pattern versus wavelength of sunscreen formulations. In general, all three formulations exhibited the highest UV absorbance in the UV-B range, similar to the original crude extract. However, the UV absorbance was much more conspicuous in the formulation comprised of 75% of the extract than the other two counterparts. On the otherhand, the commercial sunscreen product, which was the positive control, was not capable of absorbing UV radiation effectively, either in UV-B or UV-A regions of the solar spectrum. Moreover, the UV absorbance was negligible in the aqueous cream base which was used as the negative control, indicating an insignificant contribution from the cream base to the high UV absorbance of the formulations prepared from it.

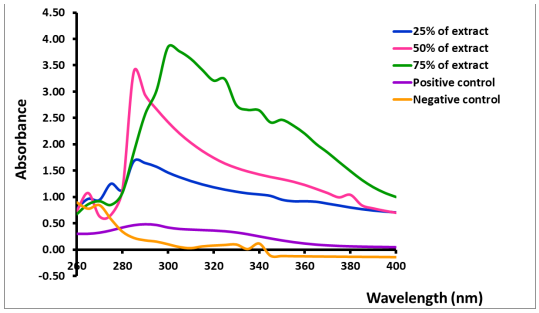

Figure 3: Absorption of UV radiation between 260–400 nm by sunscreen formulations of *O. octandra* extract, positive control, and negative control

The initial mean SPF values for the formulations with 25%, 50%, and 75% of the flower extract were recorded as 14.54±0.47, 26.53±0.54, and 36.89±0.52 respectively. This is indicative of the capability of these products to provide good photoprotection from UV radiation. The photoprotective potency in all three formulations outclassed that of the commercial sunscreen product used as the positive control. The SPF value calculated from the absorbance data for the positive control was 5.25±0.93 although it was mentioned as 15 on its label and only about three months have passed since its manufacturing date. This agrees with previous observations that the labelled SPF value of a sunscreen product may not always display its actual SPF value [2, 21].

The variation of SPF values in the three formulations over 21 days did not follow the same pattern (Figure 4). Only a slight reduction of SPF value was observed with time in the formulation with 75% extract under both conditions, in

contrast to the increment of SPF value in the other two formulations when exposed to light. The increment in the SPF value was most prominent in the formulation containing 50% of the extract. On the other hand, the change in the SPF value was insignificant in the positive control.

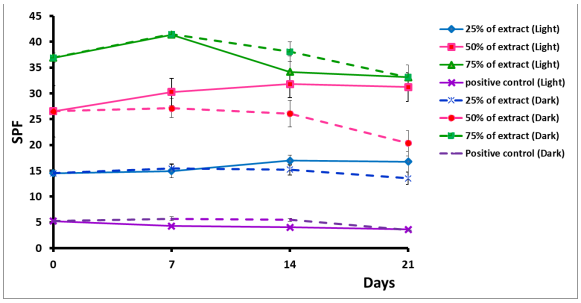

Figure 4: Variation of SPF at different time intervals in various strengths of sunscreen formulations of *O. octandra* extract compared with positive control, stored under light and dark conditions

Determination of Photostability

Table 1 presents the AUC index of the different formulations, the positive control, and the negative control. The calculation of the AUC index provided a better insight into the photostability of the prepared sunscreen formulations. The formulations with an AUC index greater or equal to 0.8 were considered photostable [17] and based on these criteria, the formulation comprised 50% of the extract exhibited photo instability throughout the UV range, unlike the formulations with 25% or 75% extract. Yet, its AUC index in the UV-A2 region was better than that of the formulation with 25% extract. The AUC index was almost 0.8 for the formulation containing 75% of the extract. Moreover, the formulation with 25% of the extract was photostable in the total UV range, UV-B, and UV-A1 regions, although this ability was lost in the UV-A2 region. Despite having fairly low UV absorbance values, the positive control exhibited photostability throughout the UV region. On the other hand, the negative control completely lacked photostability, thus the cream base did not contribute to the photostability of the prepared formulations.

Table 1: Area under the curve (AUC) index of the herbal sunscreen formulations and the positive and negative controls

Sample	AUC Index			
	Total spectrum	UV-B	UV-A1	UV-A2
25% of extract	0.87	0.99	0.76	0.17
50% of extract	0.61	0.65	0.56	0.59
75% of extract	0.78	0.78	0.78	0.78
Positive control	0.87	0.89	0.87	0.82
Negative control	0.10	0.11	0.08	0.09

Discussion

Sri Lanka is a country located within the equatorial belt, thus receiving a high amount of solar radiation throughout the year, which makes people highly vulnerable to UV-induced skin damage due to extensive exposure to the intense solar radiation [2]. Therefore, the formulation of herbal sunscreen products with high SPF values would be highly beneficial. In this respect, the formulations developed from *O. octandra*, particularly, the formulation containing 75% of the extract are of great significance not merely due to the high SPF value, but also due to its photostability and broad-spectrum sunscreen activity. Normally, sunscreen products are categorized according to their SPF values as minimal (SPF < 12), moderate (SPF 12-30), and high (SPF ≥ 30) sun protection products [22]. Our observations demonstrated that the formulation comprised of 75% extract has the potency of a high sun protection product. On the other hand, most commercial sunscreen products are highly effective against UV-B, but not against UV-A, even though the ideal sunscreen should provide good protection throughout the whole UV spectrum [19]. However, the formulation composed of 75% extract displayed a broad spectrum of sun protection by efficiently absorbing the UV radiation between 290–350 nm which covers both UV-B and UV-A regions of the solar spectrum. Another challenge in the cosmetic industry is the development of sunscreens with photostability, as most of the UV filters in sunscreens may degrade or destroy

over time or once exposed to the sun [2]. The photostability displayed by the formulation composed of 75% extract further confirms its suitability to be developed as a potent topical sunscreen on a commercial scale in this aspect too. Prompted by these preliminary observations, our investigations are now focused on enhancing the efficacy of this product via nanotechnological approaches, while further experiments are warranted to evaluate the stability on a long-term basis, possible cytotoxic effects of this formulation *in vitro*, as well as with more clinical facet to ascertain its safety on the normal human skin.

Conclusion

The preliminary findings of this study reveal that the sunscreen formulations prepared from flowers of *O. octandra*are highly effective as herbal cosmetics. Especially the formulation containing 75% methanolic extract possesses strong and broad-spectrum UV-filtering ability, high SPF, and photostability highlighting its potential to be developed into herbal cosmetic products of commercial value.

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Conflicts of interest

The authors declare that they have no competing conflict of interest.

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