RESEARCH ARTICLE

DEVELOPMENT OF HERBAL SUNSCREEN FORMULATIONS FROM THE LEAVES OF SRI LANKAN MEDICINAL PLANTS, *HIBISCUS FURCATUS* WILD. AND *OLAX ZEYLANICA* LINN.

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ABSTRACT

Excessive exposure to ultraviolet (UV) radiation in the sunlight has many deleterious consequences on human skin. In order to alleviate these harmful effects, sunscreen formulations are widely used. Although most of these synthetic sunscreens are highly effective, the adverse effects associated with these products should not be neglected. The current trend, therefore, is to explore natural sources to develop novel sunscreen formulations that are efficacious and more human-friendly. This study was conducted to formulate herbal sunscreens using two Sri Lankan medicinal plants used as remedies for dermatological conditions, *hibiscus furcatus* wild (nabiriththa) and *olax zeylanica* linn. (mella) and to evaluate their efficacy. Initially, aqueous-methanolic extracts were prepared from the leaves of the two plants, followed by the preparation of sunscreen formulations from each extract by incorporating different concentrations of the extracts into an aqueous cream base. Thereafter, the uv absorbance and sun protection factor (spf) were obtained. The sunscreen formulations containing 75% of each extract displayed high UV absorbance and the highest spf values. The photostability profiles indicated that some formulations prepared from *o. zeyalnica* were more effective than the formulations developed from *h. furcatus*. Therefore, this preliminary study demonstrated the suitability of *h. furcatus* and *o. zeylanica* extracts towards the development of commercial herbal sunscreens.

Keywords: *Herbal sunscreens, Hibiscus furcatus, Olax zeylanica, Sun Protection Factor*

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1. INTRODUCTION TO SINGULARITY FUNCTIONS

Ultraviolet (UV) radiation belongs to the electromagnetic spectrum that contributes to approximately 8-9% of the total light emitted by the sun [1]. UV radiation is subdivided according to the wavelength into three categories; UV-A (320–400 nm), UV-B (280–20 nm), and UV-C (200–280 nm) and is responsible for various deleterious effects on the human skin. These effects can be either acute or chronic such as sunburn (erythema), wrinkling, and hyperplasia as well as photoaging and photocarcinogenesis [2]. In order to prevent or reduce these harmful dermatological effects caused by UV radiation, various cosmeceutical formulations comprised of chemicals that can absorb or reflect UV radiation have been introduced into the market [3]. These UV-barring cosmeceuticals are also known as sunscreens.

Sunscreens with high capacity in absorbing or reflecting UV radiation are generally considered to be highly effective. The efficacy of a sunscreen formulation is measured by its Sun Protection Factor (SPF), which is defined as the ratio of sun exposure that skin can tolerate before burning or minimal erythema is apparent with and without sunscreen protection [4]. Based on the SPF value, sunscreen products are usually categorized as minimal (SPF < 12), moderate (SPF 12-30), and high (SPF ≥ 30) sun protective products [5]. Although synthetic chemical sunscreens are widely used, they may become allergenic to people who have very sensitive skin. As a result, nowadays, more emphasis is given towards the development of sunscreens of herbal origin, which are believed to be less irritant and more adjustable to the skin [6]. Thus, the present study is aimed to develop herbal sunscreens using two medicinal plants available in Sri Lanka; *Hibiscus furcatus* Wild. (common name: nabiriththa) and *Olax zeylanica* Linn (common name: mella).

Figure 1: Plants used in the preparation of herbal sunscreens *H. furcatus* (B) *O.zeylanica*

H. furcatus (Family: Malvaceae) is a large scrambling or climbing shrub (Figure 1-A) which grows in the warmer parts of India, Sri Lanka, and in some other Asian countries. This plant is used in traditional medicine of Sri Lanka to treat wounds, ulcers, abscess, pustules, gangrene and cysts [7]. *O. zeylanica* (Family: Olacaceae) is a commonly found tree (Figure 1-B) in the moist low country in Sri Lanka. The leaves are consumed as salads and used in the treatment of wounds, snake bites and urinary tract infections [7]. In an earlier study, Napagoda et al. [8] measured the UV barring property of the aqueous-methanolic extracts of leaves of *H. furcatus* and *O. zeylanica*. They reported remarkably high SPF values of 29.4 ±0.40 and 24.5 ±0.47 respectively. Thus, the present study is focused to prepare herbal sunscreen formulations from the same plant extracts and to evaluate the UV absorptive potential of each formulation to determine their suitability to develop commercial herbal sunscreens.

2. MATERIAL AND METHODS

2.1 Preparation of plant extracts

Leaves of *H. furcatus* were collected from Pasyala in the Gampaha district, Western Province of Sri Lanka while leaves of *O. zeylanica* were collected from Devalapola in the Gampaha district, Western Province of Sri Lanka in 2017. The plant materials were identified by the author (MN), a botanist, and authenticated by comparison with the herbarium specimens at the National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka. Voucher specimens, MN_2017_012 (*H. furcatus*), and MN_2017_07 (*O. zeylanica*) were deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka.

The plant materials were thoroughly washed and dried in shade for six days. Dried leaves were powdered using a domestic grinder (Singer, model KA-MIXEE). Then the powdered materials (15 g) were extracted in 350 mL of 70% methanol water. The extracts were evaporated to dryness using a rotary evaporator (BÜCHI, R-114, Germany).

2.2 Determination of total flavonoid content (TFC) and total phenolic content (TPC)

The total flavonoid content (TFC) of each extract was determined by the aluminium chloride colorimetric method as described by Khodaie et al.[9] with slight modifications. In brief, 500 µL of the crude extract (1 mg/mL) was added to a test tube containing 5% $NaNO_2$ (150 µL) and incubated at room temperature for 5 min. After the incubation period, 10% $AlCl_3$ (150 µL) was added, vortexed, and incubated for 6 min. Thereafter, NaOH (2 mL) was added and the final volume was made up to 5 mL using distilled water. The absorbance of the reaction mixture was measured at 510 nm. 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 as in terms of quercetin equivalence in (QUE)/ g dry weight (DW) of leaf materials.

The phenolic content of each extract was determined using Folin Ciocalteu (FC) colorimetric method as described by Alhakmani et al. [10] with slight modifications. Gallic acid was used as a standard and total phenolic content was calculated from the calibration curve. Here, 500 µL of the extract (1 mg/mL) was mixed with 10% of freshly prepared FC reagent (2.5 mL) and 7.5% of Na_2CO_3 (2.5 mL). The final volume was made up to 10 mL using distilled water. Then the reaction mixture was incubated in dark at room temperature for 2 hrs and thereafter the absorbance was measured at 765 nm. The total phenolic content of extracts was calculated according to the calibration curve, $y=0.0041x + 0.0197$ ($R^2=0.9845$), and was expressed as in terms of gallic acid equivalence (GAE) /g dry weight (DW) of leaf materials.

2.3 Formulation of herbal sunscreen products and evaluation of UV-filtering capability

Sunscreen formulations were prepared by incorporating each extract at different concentrations (i.e., 25%, 50%, 75%) separately into an aqueous cream base comprised of cetostearyl alcohol, white soft paraffin wax, and sodium lauryl sulphate.

The UV filtering capability and the variation of SPF over time were determined following the method described by Napagoda et al. [8]. Briefly, the UV absorption of

each formulation (1 mg/mL) was measured between 260 and 400 nm at 5 nm intervals using the UV-Visible spectrophotometer (Shimadzu, UV_ 1800), and the SPF value was calculated according to the Mansur equation [11]. All the formulations were exposed to direct sunlight for 21 days and UV absorption was measured on the 7^{th}, $14^{th,}$ and 21^{st} day and subsequently, SPF values were calculated.

A commercially available herbal sunscreen product (containing *Aloe*, Sandalwood, *Ficus* as active ingredients) was used as the positive control and the aqueous cream base was used as the negative control. The experiment was conducted in triplicate.

2.4 Determination of photostability of the formulations

The photostability of the formulations was determined following the method described by Gonzalez et al. [12] with slight modifications. Each formulation (50 mg) was applied evenly on a 25 cm^2 area of a stainless-steel plate, corresponding to an area density of 2.0 mg/cm^2 [13]. The plates were dried for 15 min under dark conditions and thereafter exposed to natural sunlight for the same length of time 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 exposed and non-exposed formulations were diluted in distilled water to reach a final concentration of 0.2 mg/mL and thereafter the UV absorbance of each sample was determined in 290-400 nm range. A commercial sunscreen product was used as the positive control while an aqueous cream base was used as the negative control. The experiment was performed in duplicate. The average absorbance value was considered to draw a curve between absorbance versus wavelength. The area under the curve (AUC) for total UV spectrum (290–400 nm) as well as 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 AUC index was determined from the equation, $AUCI = AUC_{exposed}/AUC_{non\text{-}exposed}$, and if the AUC index ≥ 0.8, the sunscreen formulation was considered to be photostable [12].

3. RESULTS AND DISCUSSION

3.1 Total phenolic and total flavonoid content

The total phenolic content in the aqueous- methanolic extract of *H. furcatus* was determined as 41.74 ± 5.27 mg (GAE)/g while the flavonoid content was found as 8.83 ± 1.57 mg (QE)/g. Similarly, the extract of *O. zeylanica* also contained high phenolic content with 43.30 ± 12.23 mg (GAE)/g while the flavonoid content was observed to be 5.00 ± 1.22 mg (QE)/g.

3.2 Photoprotective potential in sunscreen formulations

The high SPF value, broad-spectrum of UV protection and photostability are accepted to be some features of an ideal sunscreen [14]. Therefore, in this study, the sunscreen formulations prepared from the leaves of each plant (Figure 2) were evaluated using the above parameters.

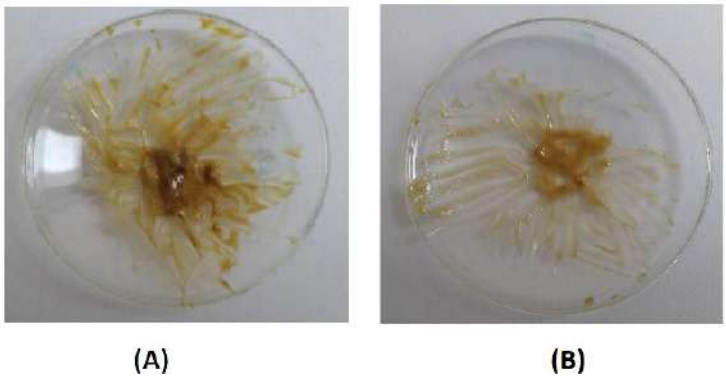

Figure 2: Sunscreen formulations prepared with aqueous-methanolic extracts of (A) *H. furcatus* (B) *O. zeylanica*

3.3 UV-filtering potential

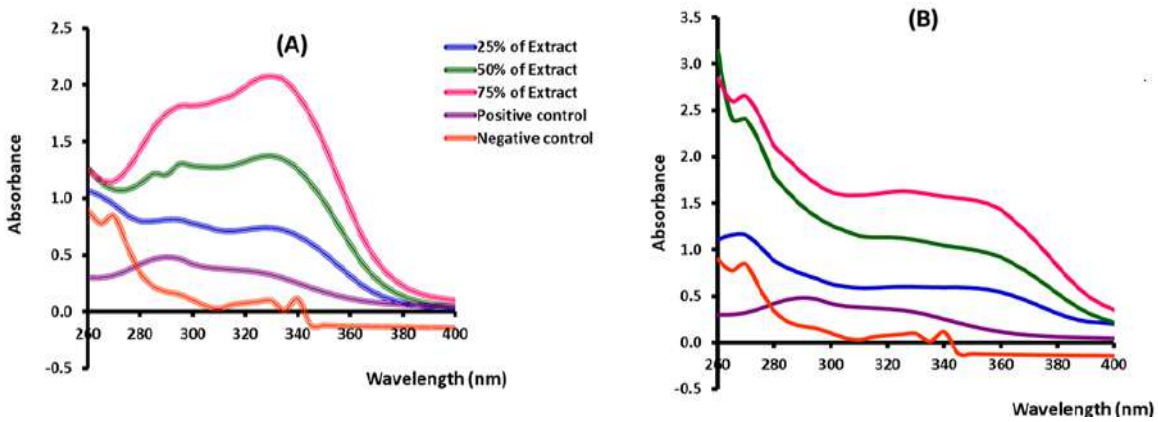

Figure 3: Absorption of UV radiation between 260–400 nm by herbal sunscreen formulations developed from (A) *H. furcatus* (B) *O. zeylanica* (n=3)

As indicated in Figure 3-A, a broad-spectrum of UV absorbance was observed for all the prepared from *H. furcatus* extract. In comparison to the two counterparts, more conspicuous UV absorbance was detected in the formulation comprised of 75% of the extract. This formulation displayed its maximum absorbance at 340 nm while retaining high absorbance values in the range of 290-350 nm covering both UV-B and UV-A regions. However, all three formulations prepared from *O. zeylanica* exhibited their maximum UV absorbance in the UV-C range. Here again, the highest UV-filtering potential was observed in the formulation containing 75% of the extract and it maintained a relatively high UV absorbance throughout the UV-B and UV-A regions of the solar spectrum (Figure 3-B). Interestingly, the UV absorption potency was very low in the commercial herbal sunscreen product (positive control) and it was not capable of absorbing UV radiation effectively, either in UV-B or UV-A regions. Moreover, the UV absorbance was insignificant in the negative control which was used as the cream base to prepare the sunscreen formulations. This indicates that the contribution of the cream base was negligible to the observed high UV absorbance in the formulations prepared using it.

3.4 Variation of SPF values in the sunscreen formulations

Figure 4 illustrates the variation of SPF values with the storage time. The initial SPF values of the formulations consist of 25%, 50%, and 75% of *H. furcatus* were observed as 7.83, 13.25, and 19.04 respectively. Interestingly the SPF values of all three formulations hardly changed during the monitored time period (Figure 4-A). In the case of *O. zeylanica*, the initial SPF values were recorded as 6.43, 12.74, and 16.79 for formulations consist of 25%, 50%, and 75% of the extract respectively.

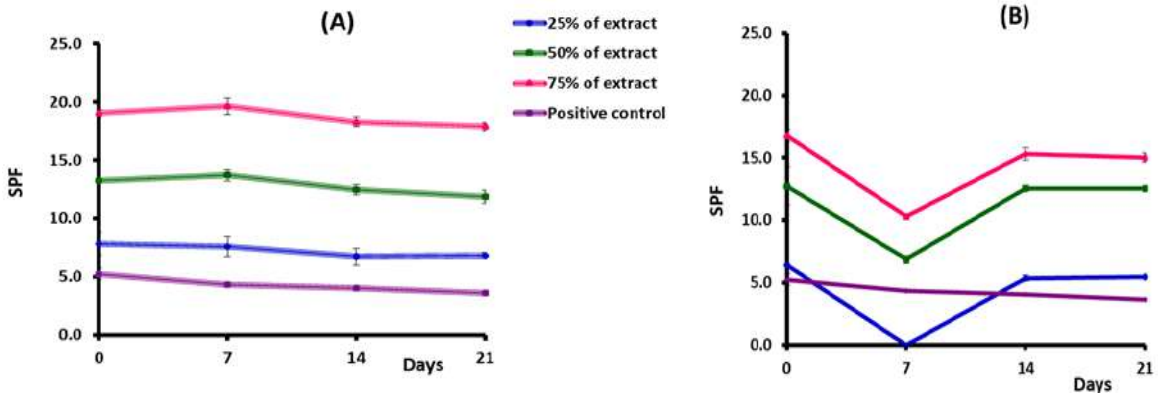

Figure 4: Variation of SPF at different time intervals in herbal sunscreen formulations developed from (A) *H. furcatus* (B) *O. zeylanica* (n=3)

These values decreased in the first week, recovered in the second week, and stayed constant then onwards (Figure 4-B). The initial SPF values were slightly higher in all three formulations prepared from *H. furcatus*, in comparison to the sunscreens developed from *O. zeylanica*. The photoprotective potency in all the formulations developed in this study outclassed that of the commercial herbal sunscreen product (SPF = 5.25) used as the positive control. However, the variation of the SPF value of positive control was insignificant during the observed time period.

3.5 Determination of photostability

Table 1 presents AUC index of the different formulations, the positive control, and the negative control. The calculation of 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 as photostable [12] and based on these criteria, except the formulation prepared from 25% of the *H. furcatus* extract, all the other formulations developed in this study displayed photostability throughout the UV range. The formulation with 25% of the *H. furcatus* extract was photostable only in the UV-B, and UV-A1 regions, and this ability was lost in the UV-A2 region. On the other hand, the photostability was much prominent in the formulations prepared from *O. zeylanica*. Interestingly, the negative control completely lacked photostability, thus the cream base has not contributed 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 control

Sample	AUC Index			
	Total spectrum	**UV-B**	**UV-A1**	**UV-A2**
H. furcatus				
25% of extract	0.76	0.83	0.81	0.69
50% of extract	0.94	0.95	0.92	0.94
75% of extract	1.74	1.42	1.50	2.36
O. zeylanica				
25% of extract	2.00	2.03	2.02	1.97
50% of extract	1.77	1.78	1.80	1.75
75% of extract	1.26	1.26	1.26	1.25
Positive control	0.87	0.89	0.87	0.82
Negative control	0.10	0.11	0.08	0.09

Terrestrial plants have developed several adaptive mechanisms to reduce the penetration of UV radiation into the leaf tissues [1]. The epidermis of leaves is viewed as a selective filter of sunlight that could absorb much UV radiation while transmitting visible light to be utilized for photosynthesis in the underlying mesophyll tissues [15]. The presence of phenolic compounds like flavonoids which are capable of the interception of UV-B in relatively high concentrations in the epidermal layer and in the leaf hairs is one of the morphogenic responses of plants to solar UV radiation [1]. It would be an innovative approach to exploit the UV-barring properties in these natural molecules to develop herbal cosmeceuticals. For example, Darmawan et al. [16] showed that a mixture of tengkawang butter and lignin could provide effective UV protection and the natural sunscreen formulations with high SPF can be prepared. Similarly, there are several reports on preparation of natural sunscreen formulations using lignin, silymarin and other phenolic compounds without the addition of any physical or chemical UV-filters [17;18] Therefore, the present study focused on the preparation of sunscreen formulations from leaf extracts makes a significant contribution to this contemporary concept of "green cosmetics".

Most of the active ingredients in commercially available sunscreens are active only against UV-B radiation [19]. Hence the cosmetic industry is facing a major challenge in developing UV-A radiation barring sunscreens. The herbs we examined in this study manifested a way to prepare broad-spectrum sunscreens with both UV-B and UV-A filtering potential. *H. furcatus* was more prominent than *O. zeylanica* in this regard. Moreover, the formulation consists of 75% of each extract displayed the highest UV absorbance in comparison to the other two formulations, and the negative and positive controls. This shows a trend where the protection offered by the sunscreen formulation from UV radiation increases with the extract concentration. In addition, the formulations with 75% of the extract also recorded the highest SPF values. Moreover the SPF values of the formulations comprising 75% of the plant extract were comparable or even superior to the SPF values of topical herbal sunscreen formulations developed by other researchers, for example; the herbal sunscreen cream containing extracts of *Terminalia arjuna*, *Tinospora cordifolia* and *Gycyrrhiza glabra* (SPF =24.35) [20] , the formulation containing sesame oil, *Hippophae rhamnoides* and *Elaeagnus angustifolia* (SPF value = 16.03) [21] and the herbal creams prepared from flower extract of *Nyctanthes*

arbortristis (SPF=10.21) or *Tagetes erecta* (SPF= 8.67) [22]. Interestingly, these SPF values were superior to the commercial sunscreen product that was used as the positive control. Another drawback of most of the commercially available sunscreens is photo instability [8]. However, *H. furcatus* and *O. zeylanica* preparations provided a solution to the photo-instability problem too. Based on these credible findings, experiments are underway to enhance the UV-barring capability of these herbal formulations using a nanotechnological approach and to evaluate the possible cytotoxicity effects, which would further qualify them to be developed as commercial sunscreens.

CONCLUSION

The preliminary findings of this study reveal that the formulations prepared from aqueous-methanolic extracts of leaves of *H. furcatus* or *O. zeylanica* are potential candidates for the development of herbal sunscreen formulations. Particularly, the formulation containing 75% of the aqueous-methanolic extract of *H. furcatus* possesses a strong broad-spectrum UV-filtering ability, high SPF, and photostability and offers a great promise towards the development of herbal cosmetic products of high commercial value.

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