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Evaluation of *invitro* sun screen activities of salt marshy plants *Suaeda monoica, Suaeda maritima* and *Halosarcia indica*

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ABSTRACT

The objective of this study was to evaluate the sun protective activities of three Sri Lankan salt marshy plants, Suaeda monoica, Suaeda maritime and Halosarcia indica (Family: Chenopodiacea) invitro using UV spectroscopic technique and Mansur equation. Methanolic extracts of each of the plant species were made and Sun Protection Factor(SPF) values were ascertained (which is an index of sun protection activity) using a concentration of 2.0 mgmL^{-1}. Methanol soluble fraction of Dermatone® (2.0 mgmL^{-1}) was used as the reference agent. Phytochemical profile of each of the extracts was determined using standard procedures.The results showed that the methanolic extract of S. monoica, S. maritima, H. indica exhibited a SPF value of 15.55, 10.83 and 8.63 respectively whilst Dermatone® had a SPF value of 34.23. Phytochemical analysis revealed the presence of flavonoids, tannins, phenols and polyphenols in all the three extracts. On the other hand, alkaloids were present in S. monoica and S. maritima and steroids in S. monoica and H. indica. It is concluded that the salt marshy plant, S. monoica, exhibits moderate sun protective activity and a safe, effective and affordable sun screen formulation may be developed from this plant. In comparison, sun protective activities of S.maritima and H. indica were mild.

Key words: *Suaeda monoica, Suaeda maritima, Halosarcia indica, sun protection factor, sun screen,UV-B rays*

INTRODUCTION

Our life ultimately depends on sun light. It consists of ultraviolet rays(UV-R) which are electromagnetic radiations with wavelengths between 100 and 400nm (1,2). It is categorized in to three types: UV-A(320-400 nm), UV-B (280-320 nm) and UV-C (200-280 nm) (1,2). UV light in small amounts is beneficial to humans as it is known to promote skin cell regeneration and stimulation of hormone production, synthesis of vitamin D and melanin pigment(3). However, over exposure to UV radiation, especially, UV-B radiation, induce devastating biological effects which are detrimentalto the health and well-being of the individual (1,2,4). Importantly, the risk of harmful effects due to exposure of UV-B radiation is increasing day by day due to thinning and creation of holes in the stratospheric ozone layer resulting from chemical reactions between ozone and chloroflurocarbons (chemicals in refrigerators and spray can propellants) (4,5). Ozone layer is the natural filter for UV rays (5).

Some of the biological effects of UV-B radiation are acute: these include inflammation, erythema (sun burns), hyperpigmentation (tanning), thickening of epidermis, burning, development of brown and red spots, local immunosuppression and irritation (1,2,4,5,6,,7,8,9,10,11).These acute effects are generally short lived and

reversible(9,10). Photoageing of skin [rough texture, sagging, dry and leathery appearance and wrinkling(both fine and coarse)]and skin cancers are the chronic effects of exposure to sun's UV radiation (1,2). In this regard, it is noteworthy that UV-B radiation is involved in about 65% of skin cancers(6,9,10,11). It is also of interest to note that increased exposure to UV-B raysdelays amphibian metamorphosis and induce severe malformations(12), and is also claimed as one of the causative factors attributable for their global decline(12).

Absolute avoidance of sun would eliminate the development of aforementioned deleterious effects of UV-B rays (11). Unfortunately, contemporary life styles make this an impracticable alternative for most individuals which has led to the search for better approaches(11). Application of topical sunscreen formulations (which absorb, scatter or reflect suns radiation) is one such approach. Currently, several sun screen formulations are available in the market in the form of lotions, oils, creams, ointments, waxes, butters, sprays or gels(1,2,5,6,13,14).Some of these sun screen formulations contain synthetic ingredients(such as titanium dioxide, zinc oxide, avobenzone, oxybenzone) (5.13.14.15) and other natural ingredients (such as flavonoids, polyphenols, tannins) (16,17,18). However, although, synthetic sunscreens are fast acting, efficacious and provide broad spectrum UV protection, their safety is doubtful: as they are known to induce contact/irritant dermatitis, hypersensitivity allergies, whitening, melanomas or skin cancer (5,6,7,18). Moreover, they are relatively expensive, often stain clothing(13,17,18) and some are claimed to increase the risk of giving birth to underweight babies when applied during the pregnancy period(8). Conversely, herbal sunscreens are relatively cheap/affordable and safe and are noncomedogenic(8,13,17). In addition, these are claimed to impair the likelihood of developing skin cancer(18). As such, there is a big demand for herbal sunscreens and development of novel herbal sunscreens which are cheaper, safer and efficacious is desirable.

In this regard, we haveinitiated a research program to ascertain the sunscreen potential of Sri Lankan salt marshy plants with a view to develop topical herbal sunscreen formulations. These plants are normally found in arid habitats where the ambient temperatures are high and are exposed to relatively high levels of UV-B radiation(19,20) and there is strong evidence that UV light induces accumulation of UV light absorbing flavonoids and other phenolics in their dermal tissues(21). So far, we have investigated and demonstrated remarkable sun protective activity of a Sri Lankan salt marshy plant, *Salicornia brachiata* (Family: Amaranthaceae; formally family chenopodiaceae) (22).
In this study, we evaluated the sunscreen potential of another threesalt marshy mangrove associate herbs, namely, *Suaeda monoica* Forssti ex J.F. Gmel, *Suaeda maritima* (L) Dumort and *Halosarcia indica* willd. which are found growing close to our previously investigated halophyte herb, *Salicornia brachiata*(L.).All these plants are small, annual, succulent and bushy with much branched and numerous jointed linear babulor shoots with simple obscure leaves, belonging to the Family Chenopodiaceae. These plants are distributed alone the tropical coasts of the Indian Ocean (20,23,24,25,26,27). Of the three plants studied in this investigation, both *S. monoica*(23,24) and *S. maritima*(25,26) are used in traditional medicine for treatment of hepatitis. In addition, *S. monoica* is used as an ointment to promote wound healing(23,24). Experimentally, *S.monoica* is shown to possess antiviral and hepatoprotective activities (24) whilst *S. maritima* is shown to have antiviral,antibacterial, hepatoprotective, antioxidative activities(25,26) and the ability to inhibit nitric oxide production, expression of i NOS enzyme, and IL-6 and TNF α (proinflamatory cytokines) production in LSP stimulated BV-2 microglial cells *in vitro*(26). On the other hand, bio activities of *H. indica* are less known but are used as an animal feed and eaten as a cooked vegetable, particularly in times of food scarcity (28).

MATERIALS AND METHODS

Collection and identification of*S. monoica, S. maritima* and *H. indica*

Plants were collected from Mannar (geographical coordinates: 8.8667° N, 80.0667° E), Sri Lanka in March 2015 and were identified by Dr. Sampath Seneviratne, Department of Zoology, University of Colombo, Sri Lanka. Voucher specimens of aerial parts of *S. monoica* (BLCS/Pharm/ 02),*S. maritima* (BLCS/Pharm/ 03)and *H. indica* (BLCS/Pharm/ 04) were deposited in the Pharmaceutical Chemistry Skill Lab, Department of Pharmacy, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahera, Sri Lanka.

Preparation of methanolic extracts of *S. monoica, S. maritima* and *H. indica*

The succulent areal parts of each plant species were thoroughly washed in running tap water and were then oven dried at 40^{o}C until a constant weight was obtained. The areal parts were then cut into small pieces using a razor blade. Ten grams of cut pieces of each of these plants were macerated for 7 days in 100 mL of distilled methanol (Sigma-Aldrich Company, St. Louis, USA). The resulting extracts were filtered separately through double layered muslin cloth and the filterates were evaporated to dryness. Yields obtained for *S. monoica, S. maritima* and *H. indica* were 10%, 10.2% and 9.8% respectively. These products were stored in airtied bottles at 4^{o}C until use.

Phytochemical analysis

The methanolic extracts of *S. monoica*,*S. maritima* and *H. indica* were subjected to qualitative analysis for saponins, alkaloids, tannins, flavonoids, phenols, steroids, glycosides and diterpenes using standard procedures.

***In vitro* evaluation of sun protection factor**

Each of the solid products obtained from *S. monoica, S. maritima* and *H. indica* was redissolved in methanol (ACS reagent, 99.8% purity from Sigma-Aldrich) to prepare solutions of 2.0 $mgmL^{-1}$ from each plant species. In addition, Dermatone® was dissolved in methanol to obtain a solution of 2.0 $mgmL^{-1}$. Absorbance of UV radiation by the methanol extracts of *S. monoica*,*S. maritima*, *H. indica* and Dermatone® were determined (at 23°C with an equilibration time of 1h) in 1cm quartz cells, in triplicate, using a UH 5300 Hitachi spectrophotometer from 290 to 320 nm, at 5 min intervals taking methanol as the blank. Sun Protection Factor (SPF) values were then determined using the Mansur equation [9,16,29] given below.

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

Where EE – erythemal effect spectrum: I –Solar intensity spectrum: Abs- Absorbance of sunscreen product: CF-correction factor (=10). The values of EEx I are constant and predetermined.

Statistical Analysis

The results are given as mean ± SEM. Statistical comparisons were made using χ^2-test. Significance was set at $P<0.05$.

RESULTS

The results are summarized in Tables 1,2 and 3. As shown in Table 1, the reference agent, Dermatone® exhibited markedly high absorbance value (range: 3.1 to 3.6). The absorbance values shown by methanolic extracts of *S.monoica* (range: 1.5- 1.7) and *S.maritima* (range: 1.0-1.2) were moderate whilst of *H. indica* was weak (range: 0.8-1.0). As indicated in Table 2, the computed SPF values for Dermatone®, *S.monoica, S.maritima* and *H.indica* are 34.23, 15.55,10. 82 and 8.63 respectively. This SPF value of *S.monoica* was significantly higher than *S.maritima* (by 43%) and *H.indica* (by 80%), and significantly lower (by 55%) than Dermatone®.

As shown in Table 3, phytochemical screening showed the presence of flavonoids, tannins, phenols and polyphenols in all three plant extracts. Alkaloids were present in extracts of *S. monoica* and *S. maritima*. Steroids were present in *S. monoica* and *H. indica*. In contrast, the extracts of all the three plants did not show the presence of saponins, glycosides and diterpenes.

Table 1: Absorbance of 2.0 $mgmL^{-1}$ methanolic extracts of *Suaeda monoica, Suaeda maritima* and *Halosarcia indica* and Dermatone® (mean ± SEM)

Wavelength (nm)	EE x I	*Suaeda maritima* Extract	*Suaeda monoica* Extract	*Halosarcia indica* Extract	Dermatone®
290	0.015	1.26 ± 0.0028	1.71 ± 0.0086	1.02 ± 0.0007	3.18 ± 0.0170
295	0.0817	1.17 ± 0.0034	1.61 ± 0.0091	0.94 ± 0.0010	3.33 ± 0.0349
300	0.2874	1.10 ± 0.0027	1.55 ± 0.0094	0.88 ± 0.0006	3.13 ± 0.0172
305	0.3278	1.06 ± 0.0021	1.53 ± 0.0079	0.85 ± 0.0012	3.61 ± 0.0849
310	0.1864	1.05 ± 0.0020	1.55 ± 0.0079	0.83 ± 0.0012	3.56 ± 0.1009
315	0.0839	1.06 ± 0.0015	1.59 ± 0.0084	0.83 ± 0.0017	3.48 ± 0.0404
320	0.018	1.07 ± 0.0013	1.63 ± 0.0078	0.82 ± 0.0015	3.64 ± 0.1251

EE - Erythemal effect spectrum : I – Solar intensity spectrum

Table 2: Sun protection factor (SPF) of 2.0 $mgmL^{-1}$ methanolic extracts of *Suaeda monoica, Suaeda maritima* and *Halosarcia indica* and Dermatone®

	Sun Protection Factor (SPF)
Suaeda monoica	15.55
Suaeda maritima	10.84
Halosarcia indica	8.63
Dermatone®	34.23

Table 3: Chemical screening of methanolic extracts of *Suaeda monoica, Suaeda maritima* and *Halosarcia indica*

	Suaeda monoica	***Suaeda maritima***	***Halosarcia indica***
Alkaloids	+	+	-
Saponins	-	-	-
Tannins & Phenols	+	+	+
Flavonoids	+	+	+
Glycosides	-	-	-
Diterpenes	-	-	-
Steroids	+	-	+

+ = present; - = absent

DISCUSSION

This study examined the sun screen potential (in terms of SPF) of three Sri Lankan salt marshy plants (halophytes), namely, *S.monoica, S.martima* and *H.indica in vitro*. The efficiency of a sun screen agent is usually expressed by SPF value (5,11,15,21), which is simply a ratio of the time required to produce sun burn/ erythema with and without a sunscreen application (5,11,15,21). Moreover, higher the SPF value, more effective is the agent as a sunscreen/sun protective (5,11,15,21). Most organizations (15) and dermatologists (13,15) recommend to apply a topical sun screen formulation having a SPF value between 15-20, preferably, year round, to protect the skin against harmful UV-B rays (5,11,13,15). More importantly, a sunscreen with a SPF value of 15 is claimed to protect against 93% of UV-B rays and no available sunscreen is capable of protecting 100% UV-B rays (14)

In this study, SPF values were assessed using UV absorption spectroscopy (290-320nm) technique and Mansur equation (9,16,29). 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/formulations (2,6,7,9,16,22,29). Moreover, this *in vitro* assay bypasses the variability and ethical issues encountered with using animals and humans as experimental subjects (30). As SPF value is known to vary with several factors such as type of solvent, concentration of the test materials, temperature and time of equilibration , quality of spectrophotometer, type of cuvette used (6,30, 31), a methanolic extract of the plants having a concentration of $2mgmL^{-1}$, equilibration time of 1h, an ambient temperature of 23^oC, high quality 1cm quartz cells and a spectrometer was used as reported by other investigations (4,6,7,8,30,31).

The results unequivocally showed that 2mg/mL methanolic extract of *S.monoica* exhibited a moderate sunscreen activity (SPF=15.55) whilst extracts of *S.maritama* (SPF=10.83) and *H.indica* (SPF=8.63) showed mild sunscreen activity. This is a novel finding. In SPF ratings, SPF values 2-12, 12-30 and $\geq$30 are considered as having respectively minimum, moderate and high sun protective activity (11). Further, SPF value of 15.55 of *S. monoica* suggests that this plant extract can protect the skin against 93% of harmful UV-B rays (14). However, the sun protective activity of *S.monoica* is about 55% lower than Dermatone®, the reference agent used, which is a synthetic sun screen containing 3% ensulizole, 7.5% octinoxate 9.8% zinc oxide(32). Nevertheless, SPF value of *S. monoica* was comparable to Himalaya®, commercially available sun screen which has been used as a reference agent by other workers (31). Furthermore, several herbal extracts which are claimed to have a high potential to be developed as sun screens either have lower SPF values (7,10) or comparable SPF values (10,31) to what has been reported for the three salt marshy plants reported here. In contrast, in a previous study, we have showed that *Salicornia brachiata*, which is another salt marshy plant, which is found in close proximity to three plants investigated in this study had a SPF value of 30:89 (22). Thus, the low and moderate SPF values, evident in this study, is an unexpected finding: since salt arid coastal habitats where the ambient temperatures are high are exposed to relatively high levels of UV-B radiation (19,20); UV light is shown to induce UV absorbing flavonoids and other phenolics (21); flavonoids, tannins, phenols and polyphenols were found to be present in extracts of all the three plants investigated in this study; and several field studies have demonstrated an increase in UV absorbing compounds when leaves are artificially exposed to UV light(33).

It is well recognized that UV-B rays involve the production of variety of free radical molecules such as $^{\cdot}O2$, $^{\cdot}OH$, $HO_2^{\cdot}$ in the human skin (1,2,9,18). Free radicals are linked with UV induced photodamage of skin and sun screen activity of many herbal sun screens are attributed to their antioxidant activity (1,2,9). Further, commercially available sunscreens are, often, enriched with antioxidants like vitamin E (2,17). Interestingly, strong antioxidant activities are shown to be present in salt marshy plants (25,26,33) possibly because they are frequently exposed to oxidative stress. Accordingly, it is very likely that sunscreen activities evident in this study are mediated mainly by antioxidant activity. In plants, antioxidant activities are mainly triggered by flavonoids, tannins, and phenolics (16,21,25) which were shown to be present in all the three plant extracts. This is suggestive that the sun protection activities seen in this study are also mediated via these phytoconstituents. Good correlations are shown between SPF value and phenolic content of medicinal plants (21). This is an additional support for our notion. Sun protection

activities of the three plants tested were different. This may be ascribed to differences in these phytoconstituent between the three plant species which needs to be experimentally evaluated. Nevertheless, such explanations are given by other investigators as well (21,25,33).

CONCLUSION

In conclusion, this study shows, for the first time, *in vitro* sun protective activity of three Sri Lankan salt marshy plants, namely, *S. monoica* (moderate), *S. maritima* (mild) and *H. indica* (mild). Considerable potential exists to develop a cheap, safe and effective topical sunscreen from *S. monoica*.

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