

In vitro sun screening activity of salt marshy plant *Salicornia brachiata*

W.D. Ratnasooriya¹, R.N.Pathirana¹, A.S.Dissanayake², B.L.C.Samanmali³ and P.K.Desman³

¹. Department of Basic Sciences, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahera, Sri Lanka.

². Department of Chemistry, Faculty of Science, University of Ruhuna, Matara, Sri Lanka.

³. Department of Pharmacy, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahera, Sri Lanka.

Abstract- At present, there is a need and demand for the development of safe, cheap and effective topical herbal formulations as sun screens. In this context, this study was initiated to evaluate the sunscreen potential of a Sri Lankan salt marshy plant, *Salicornia brachiata* Roxb (Family: Amaranthaceae; formally Family: Chenopodiaceae) *in vitro* using a UV spectroscopic technique and Mansur equation. Sun Protection Factor (SPF) value was ascertained (which is an index of sun protection activity) using a methanolic extract having a concentration of 2.0 mgmL⁻¹. Methanol soluble fraction of Dermatone® (2.0 mgmL⁻¹), a well known sunscreen cream, was used as the reference agent. The results showed that the plant extract has a markedly high absorbance values (3.0-3.2) at 290-320 nm range with a SPF value of 30.89 (which is a novel finding) whilst the reference agent exhibited a SPF value of 34.23. Phytochemical analysis of the extract showed the presence of flavonoids, tannins, phenols and steroids. It is concluded that a safe, cheap and efficacious topical sun screen formulation could be developed from salt marshy plant *S. brachiata*.

Index Terms- *Salicornia brachiata*, Sunscreen, Sun Protection Factor, Photoprotection

I. INTRODUCTION

Sunlight is mandatory for life. It contains ultraviolet rays (UV-R) belonging to three categories: UVC (200-280 nm), UVB (280-320 nm) and UVA (320-400 nm) [1,2]. In fact, sun is the main source of UVR [1,2]. In humans, overexposure to UVR is shown to produce deleterious effects (both acute and chronic) on the skin, the largest organ of the body [1,2,3]. Importantly, the risk of harmful effects due to the exposure to UV-radiation is increasing day by day due to depletion/thinning of the stratospheric ozone layer [3]. The acute biological effects due to UV radiation, especially, UVB includes inflammation, erythema (sunburn), hyperpigmentation (tanning), local immuno suppression and irritation [1,2,3,4,5,6,7,8]. These acute effects are generally short lived and reversible [7,8]. Photoageing of skin (rough texture, wrinkling) and photo carcinogenesis of skin are the chronic effects of overexposure to sun's UV rays [1,2,7]: the most common cancer is skin cancer [4]. In USA alone about 10,000 die from malignant skin melanoma every year [4]. It is noteworthy that UVB radiation is involved in about 65% of skin cancers [4,7]. It is also of interest to note that increased exposure of UV-B radiation has delaying effects on amphibian metamorphosis and induce severe malformations (such as

scoliosis, ectromelia, amelia, eye opaqueness, brachygnathia), and has been claimed as a contributory factor for the global decline of amphibians [9]. However, there are some positive impacts of UV radiation on the well-being of individuals such as promotion of skin cell regeneration, stimulation of hormone production, synthesis of vitamin D and melanin synthesis [10].

Dermatologists now strongly recommend to apply topical sunscreen formulations (which absorb, scatter or reflect suns radiation) with a sun protection factor (SPF) value of 15 (threshold value) or greater, preferably year round, to protect the skin against harmful UV rays, especially, UVB rays [11]. Currently, several sunscreen formulations are available in the market in the form of creams, oils, gels, ointments, lotions or sprays [1,2,4,11,12]. Some of these sunscreen formulations contain synthetic ingredients (such as zinc oxide, titanium dioxide, avobenzone) and others contain natural herbal ingredients (such as polyphenols, flavonoids)[5,13,14]. However, the safety of using synthetic sunscreens are doubted although some are fast acting, provide broad spectrum of UV protection and highly efficacious: these include the development of contact and/or irritant dermatitis, hypersensitivity, allergies, whitening, melanomas, skin cancers [4,5,15]. Further, synthetic sunscreens are relatively expensive and often stain clothing [11]. Yet another point of particular concern is that it is claimed that mothers with high blood levels of certain synthetic sunscreen ingredients are more likely to give birth to underweight babies [6]. In contrast, herbal sunscreens are claimed to be ecofriendly, relatively cheap and safe, devoid of undesirable side effects and are noncomedogenic [6,11,15]. What is more, is that these are effective even following chronic exposure [11,15] and has the potential to impair skin cancer formation by disrupting multiple pathways which are involved in carcinogenesis [11,15].

At present, there is an imperative need to develop novel sunscreen formulations which are consumer friendly, cheap, safe and highly efficacious, preferably from herbal sources [11,15]. In this context, this study was launched to investigate the sunprotection potential of salt marshy plant, *Salicornia brachiata* (Family: Amaranthaceae; formally Family: Chenopodiaceae) by evaluating the SPF value *in vitro* using UV spectroscopic technique and Mansur equation [7,12,16]. *S.brachiata* is an annual, succulent and bushy wild growing tropical halophytic herb with much branched and jointed shoots. Its leaves are obscure. Flowers are minute, solitary, axillary or terminate [17,18]. It is found in salt marshes of India and Sri Lanka [18]. In traditional medicine this plant is claimed to be used in the treatment of itches, toothache, rheumatism, constipation, obesity, cancer and diabetes [2,19,20]. Experimentally, methanolic

extract of *S.brachiata* has shown to possess antibacterial, anticancer and antioxidant activities [21].

II. MATERIALS AND METHODS

2.1 Collection and identification of the plant

Few plants were collected from Mannar (geographical coordinates: 8.8667° N, 80.0667° E), Sri Lanka in March 2015. The plants were identified by Dr. Sampath Seneviratne, Department of Zoology, University of Colombo, Sri Lanka. Voucher specimen of aerial parts of plant (BLCS/Pharm/ 01) was deposited in the Pharmaceutical Chemistry Skill Lab, Department of Pharmacy, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahera, Sri Lanka.

2.2 Preparation of the methanolic extract of *S.brachiata*

The succulent areal parts of the plant were thoroughly washed in running tap water and were then oven dried at 40°C until a constant weight was obtained. The dried leaves were then cut into small pieces (approximately 2-3mm pieces) using a razor blade. Ten grams of these were macerated for 7 days in 100 mL of distilled methanol (Sigma-Aldrich Company, St. Louis, USA). The resulting dark green coloured extract was filtered through double layered muslin cloth and the filtrate was evaporated to dryness. The yield was 18.5%. This product was stored in airtied bottles at 4°C until use.

2.3 Phytochemical analysis of *S.brachiata* methanolic extract

The methanolic extract of *S.brachiata* was subjected to qualitative analysis for alkaloids, saponins, flavonoids, tannins, phenols, steroids, glycosides and diterpenes [22].

2.4 *In vitro* evaluation of sun protection factor of methanolic extract of *S.brachiata*

The solid product of *S.brachiata* obtained was redissolved in methanol (ACS reagent, 99.8% purity from Sigma-Aldrich) to prepare a solution of 2.0 mgmL⁻¹. 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.brachiata* and Dermatone[®] was 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. SPF values were then determined using the Mansur equation [7,12,16] given below.

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

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

2.5 Statistical Analysis

The results are given as mean ± SEM. Statistical comparisons were made using Mann-Whitney U-test. Significance was set at P<0.05.

III. RESULTS

The results obtained and computed are depicted in Tables 1 and 2. As shown in Table 1, methanolic extract of *S.brachiata* displayed markedly high absorbance values (range: 3.0 to 3.2) as the reference agent, Dermatone[®] (range: 3.1 to 3.6). As shown in Table 2, computed SPF values of *S.brachiata* and Dermatone[®] are 30.89 and 34.23 respectively. These values did not differ significantly (P>0.05).

Phytochemical screening of *S.brachiata* methanolic extract revealed the presence of flavonoids, phenols, tannins and steroids. On the other hand, alkaloids, saponins, glycosides and diterpenes were absent.

IV. DISCUSSION

This study investigated the sun screen potential (in terms of SPF) of a Sri Lankan salt marshy plant, *S. brachiata in vitro*. The efficacy of a sun screen is usually expressed by SPF [23]. The higher the SPF, the more effective is the agent as a sun protective [12,23]. SPF value was determined using UV absorption spectroscopy (290-320 nm) technique and Mansur equation [7,12,16]. This *in vitro* technique is well recognized, simple, quick, reliable, validated, inexpensive one and is widely used in assessing sun screen potential of natural and synthetic products/formulations [2,4,,5,7,12,16]. Further, this technique bypasses the variability and ethical issues associated with using animals and human as subjects [23]. Since, SPF value is reported to vary with several factors such as type of solvent, concentration of the test material, time and temperature of equilibration and quality of the spectrophotometer [4,23], a methanolic extract of the plant having a concentration of 2.0 mgmL⁻¹, equilibration time of 1 h , an ambient temperature of 23°C and a high quality spectrophotometer was used as done by other workers [3, 4, 5,6,23]. Hence, the results obtained are valid and reliable, and meaningfully interpreted and compared with that of others.

The results clearly show that, the 2.0 mgmL⁻¹ methanolic extract of *S. brachiata* possesses a remarkable degree of sunscreen activity with a SPF value of 30.89. This is a novel finding indicating a huge cosmeceutical potential of *S. brachiata* for development as a topical sunscreen. The SPF value of the extract was almost similar to dermatone[®], an efficacious sun protective agent containing 3% ensulizole, 7.5 % octinoxate and 9.8% zinc oxide [24], which is frequently prescribed by dermatologists[24]; dermatologists strongly recommend to apply sun screens having SPF values 15 or more to minimize harmful effects of UV rays[11]; and several herbal extracts which are claimed to have a high potential of developing as natural sunscreens have SPF values lower than what is reported in this study [5,8,25]. Another desirable feature exhibited by the extract, as a sunscreen, is its wide range of absorbance, (between 290-320 nm): it is claimed that wider the range of absorbance of a photoprotective formulation higher would be its effectiveness in preventing sunburns [23]. Sun burns are primarily caused by over exposure to suns UVB radiation [10].

It is well established that UVB rays provoke the production of variety of aggressive free radicals/molecules such as 'O₂', 'OH', 'HOO' in the skin [1,2,7,15]. Importantly, free radicals are closely

linked with photodamage of skin, and sunprotection activity of many herbal sunscreens are, often, mainly attributed to their antioxidant activity [1,2,7,13,26]. Strong antioxidant activity has been shown in methanolic extract of *S. brachiata* using four *in vitro* assays, namely ABTS, DPPH, FTC, and TBA [19,21]. Accordingly, it is reasonable to presume that one of the mechanisms responsible for the observed sun protection activity, in this study, is its antioxidant activity. It is well known that in plants antioxidant activity is mediated via phenolics like flavonoids and tannins [6,10,14,15,21,26], and vitamin C [6,10,14,15,21,26]. In this study, we demonstrated the presence of phenols, tannins, and flavonoids in the methanolic extract of *S. brachiata* in agreement with others [19,20,21]. In addition, these phytoconstituents in high amounts are also reported in ethanol and ethylacetate extracts [21] and aqueous extracts of this plant [26]. Besides, a high content of vitamin C has also been reported in this plant [27]. Accordingly, the sun screening activity seen in this study is most likely to be mediated via these phytoconstituents.

S.brachiata is found in salty arid coastal habitats where the environmental temperatures are high and is exposed to relatively high levels of UVB radiation [17,18]. Interestingly, high content of UV absorbing compounds have been shown in plants growing

in these environmental conditions [27]. Further, in several field studies, an increase in UV-absorbing compounds has been demonstrated in leaves to artificially elevated UVB levels [28]. It is very likely these UV – absorbing phytoconstituents are present in *S.brachiata* as well. And these could also contribute, at least partly, to the sunprotective action seen with the methanolic extract in this study.

V. CONCLUSION

It is concluded that methanolic extract of Sri Lankan tropical Salt marshy plant, *S.brachiata* possesses remarkable sun protection activity (SPF value=30.89) and display a huge potential to be developed as a safe, cheap and effective topical sun screen.

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Table 1: Absorbance of 2.0 mgmL⁻¹ methanolic extract of *Salicornia brachiata* and Dermatone[®]

Wavelength (nm)	EE x I	Absorbance	
		Extract	Dermatone [®]
290	0.0150	3.18 ± 0.0371	3.18 ± 0.0170
295	0.0817	3.17 ± 0.0704	3.33 ± 0.0349
300	0.2874	3.05 ± 0.0156	3.13 ± 0.0172
305	0.3278	3.09 ± 0.0333	3.61 ± 0.0849
310	0.1864	3.06 ± 0.0219	3.56 ± 0.1009
315	0.0837	3.14 ± 0.0412	3.48 ± 0.0404
320	0.0180	3.26 ± 0.0368	3.64 ± 0.1251

EE - Erythema effect spectrum: I – Solar intensity spectrum

Table 2: Sun protection factor (SPF) of 2.0 mgmL⁻¹ methanolic extract of *Salicornia brachiata* and Dermatone[®]

Methanolic extract tested	Sun Protection Factor (SPF)
<i>Salicornia brachiata</i>	30.89
Dermatone [®]	34.23

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AUTHORS

First Author- W.D. Ratnasooriya, , Department of Basic Sciences, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahera, Sri Lanka.
Second Author- R.N.Pathirana, Department of Basic Sciences, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahera, Sri Lanka.
Third Author -A.S.Dissanayake, Department of Chemistry, Faculty of Science, University of Ruhuna, Matara, Sri Lanka.
Fourth Author -B.L.C.Samanmali, Department of Pharmacy, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahera, Sri Lanka.
Fifth Author -P.K.Desman, Department of Pharmacy, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahera, Sri Lanka.
Corresponding author- Snr Prof. W.D. Ratnasooriya, e-mail: rmedchem@gmail.com