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FP Merlin

School of Biomedical Science and Physiology, University of Wolverhampton, Wulfruna St, Wolverhampton WV1 1LY, United Kingdom

WD Ratnasooriya

Department of Basic Sciences, General Sir John Kotelawala Defence University, Kandawala Road, Ratmalana, Sri Lanka

RN Pathirana

Department of Basic Sciences, General Sir John Kotelawala Defence University, Kandawala Road, Ratmalana, Sri Lanka

In vitro investigation of sunscreen activity and evaluation of phytochemical profile of Methanolic leaf extract of *Rauvolfia tetraphylla*

FP Merlin, WD Ratnasooriya and RN Pathirana

Abstract

This study aimed at investigating the sun protective activity and phytochemical profile of *Rauvolfia tetraphylla*. Application of sun screen formulations is essential to protect the skin from harmful Ultra Violet radiation that causes inflammation, erythema (sunburn), hyperpigmentation (tanning), local immuno suppression, irritation, and skin cancers. Some commercially available sunscreen preparations exhibit side effects and they are relatively expensive. At present, there is a huge demand for efficacious, cheap, safe and herbal sunscreens having broad spectrum sun protective activity. As such, a natural plant extract of *Rauvolfia tetraphylla* was studied for its sun protective activity. Its *in vitro* sun protective activity was assessed by determining the sun protection factor (SPF) in Methanol Leaf Extract of *R. tetraphylla* by using UV spectroscopic technique and Mansur equation. The SPF values of *R. tetraphylla* and the reference drug Dermatone® were found to be 19.27 and 33.73 respectively at the concentration 0.4mg/mL. The phytochemical analysis performed for *R. tetraphylla* revealed the presence of phenols, carbohydrates, alkaloids, saponins, flavonoids, quinones, steroids and terpenoids in both aqueous and methanol. As artificial sunscreens have adverse effects, there is an increasing demand for novel findings that is safe and consumer friendly. It is concluded that *R. tetraphylla* leaves has the capability to be developed into a topical sunscreen.

Keywords: *R. tetraphylla*, sun protective factor (SPF), sunscreen, methanol leaf extract (MLE)

Introduction

Sunscreen are photoprotective agents that protect the skin from Ultraviolet rays. They are also known as sunblock which is opaque in texture. Sunscreen increases the tolerance to UV rays. The function of sunscreen is to block, reflect and scatter sunlight. Mixture of organic compounds are present in chemical sunscreen to protect the skin from UV radiation. Sunscreen delays the occurrence of sunburn and they are available in forms of gels, lotion and creams which contain synthetic ingredients such as avobenzone, zinc oxide and titanium dioxide. These are efficient fast acting sunscreens that provide a broad spectrum protection against UV, but these sunscreens are expensive and induce many side effects. There are natural sunscreens that contain herbal ingredients like flavonoids, tannins and phenols which are safe and consumer friendly (Autier *et al.*, 1999; Latha *et al.*, 2013; Ratnasooriya *et al.*, 2016; Banu *et al.*, 2017) [5, 14, 1].

Although sunscreens are considered beneficial, it has adverse side effects such as photoallergic reactions, like dermatitis, cutaneous melanoma, increased number of nevi and basal cell skin cancer. Some sunscreens are phototoxic where application of these types of sunscreens during pregnancy can give birth to under weight babies and applying sunscreen in thick layers can decrease vitamin D synthesis in the skin (Autier *et al.*, 1999; Latha *et al.*, 2013) [5].

SPF is a measure of sun protective activity. This factor must be considered to make sure that the sunscreen is effective. Higher the SPF greater the protection provided. SPF measures UVB protection. SPF has an influence on duration of sun exposure. SPF is measured using Mansur's equation (Latha *et al.*, 2013) [5]

Sunlight is important for life. It contains Ultraviolet rays. Sun is the main source of UV radiations. Exposure to short term UV is beneficial as it stimulates hormone production, synthesizes vitamin D, regenerate skin cells and for melanin pigment. Day by day UV radiations are increasing due to depletion of stratospheric ozone layer. Skin cancers and photodamaging effects are increasing due to over exposure to ultraviolet radiations. Therefore, dermatologist prescribe sunscreen which reduces the symptoms and show beneficial effects. Synthetic sunscreen products, have side effects which are not safe. (Banu *et al.*, 2017 [1]; Ratnasooriya *et al.*, 2016; Latha *et al.*, 2013) [1, 14, 5]

Corresponding Author:**FP Merlin**

School of Biomedical Science and Physiology, University of Wolverhampton, Wulfruna St, Wolverhampton WV1 1LY, United Kingdom

UV radiations are broad spectrum ranging from 40 – 400nm and they can be divided as vacuum UV, far UV, UVC, UVB and UVA. UVB (290-320nm) and UVA (320-400nm) are medically important. Exposure to UVA is constant where UVB mostly occurs in summer. UVB can cause acute and chronic changes. Example sunburn, pigmentation, immune suppression and photocarcinogenesis. Both these radiation causes erythema, inflammation, sun burn and photoaging reactions. Photoaging means. Photocarcinogenesis, wrinkling and sagging that damages the skin cells and deoxyribonucleic acid (DNA). Therefore, dermatologist strongly recommend to apply a sunscreen with sun protective factor 15 or higher to protect the skin especially from UVB rays (Ratnasooriya *et al.*, 2016; Latha *et al.*, 2013) [14, 5].

Exposure of sunlight within 200 – 400 nm causes inflammation. Acute exposure can lead to erythema, tanning, edema, epidermal thickening and pruritus. Chronic exposure can cause carcinogenesis and skin aging. These are due to photochemical reactions occurring after the absorption of UV rays. Once the skin is exposed to UV rays, formation of prostaglandins and release of histamines increase which causes the activity of phospholipase too to increase causing more prostaglandin formation. Oxygen free radicals also take part in sunburn causing it to increase and Langerhans cell decrease in numbers. Macromolecules such as lipids, proteins and nucleic acid undergo oxidative modifications due to reactive oxygen species (ROS) by UVB rays. This not only causes inflammation, it too induces immunosuppression and gene mutation. (Hruza and Pentland, 1993; Li *et al.*, 2016) [7]. *Rauvolfia tetraphylla* is found in Sri Lanka and other countries like India, Myanmar and Bangladesh. Its commonly called as “Devil pepper”. *R. tetraphylla* is a woody shrub that is branched with creamy white flowers which grows up to 1.5m in height (Shariff *et al.*, 2006; Rahman and Ahfuza, 2015; Vinay *et al.*, 2016) [19, 12, 21].

There are approximately 85 species in this particular genus called *Rauvolfia* and is mainly found in tropical regions. It is rich in phytochemicals such as Ajmalicine, Serpentine, Raunescine, Reserpine, Alkaloids and Canescine. The leaf extract possesses anti-oxidant activity through glycosides, flavonoids, steroids and alkaloids (Rao *et al.*, 2012; Jyothi, Brijesh and Venkatesh, 2012; Vinay *et al.*, 2016) [13, 3, 21].

Materials and Methods

Collection of leaves

Two hundred grams of fresh leaves of *R. tetraphylla* were collected from Deraniyagala, Sri Lanka (Geographical coordinates: 6.9349° N, 80.3380° E).

Identification of plant

Taxonomic authentication of plant was conducted at National Herbarium, Peradeniya, Sri Lanka. A voucher specimen (MF/01/RT) of *R. tetraphylla* is kept at the Biochemistry laboratory in British College of Applied Studies Campus, Sri Lanka.

Preparation of methanolic leaf extract (MLE)

Fresh 200 grams of *R. tetraphylla* leaves were thoroughly washed in running tap water and shade dried until constant weight was obtained. Twenty grams of dried leaves were powdered using a grinder and were soaked in 200 mL of methanol for 7 days in an airtight container for maceration.

After 7 days, the leaf extract was filtered using a double layer muslin cloth and the filtrate was placed inside the water bath (under 67 °C) to evaporate to dryness and the volume was reduced to 100 mL. The extract was freeze dried to obtain a dried root extract of yield 5.43g (5%) which was stored under -20 °C until use.

Investigation of *in vitro* sun protective activity

Triplets of concentrations 0.4 mg/mL, 0.2 mg/mL and 0.1 mg/mL were prepared by serial dilution. Similarly, triplets of three different concentrations (0.4 mg/mL, 0.2 mg/mL and 0.1mg/mL) of reference drug Dermatone was prepared. Once all solutions were prepared, UV spectrophotometer was used to study the absorbance at different wave lengths, such as 290nm, 295nm, 300nm, 305nm, 310nm, 315nm and 320nm. Triplets of each concentration of *R. tetraphylla* leaves and Dermatone were used to measure the absorbance at each wavelength. 1mL of each sample was pipetted out and added into 1cm quartz cell and placed inside the UV spectrophotometer to obtain the absorbance readings. Methanol was used as blank. Before different concentration were added into the quartz cell, the cell was washed with distilled water. Sun Protective Factor was determined using Mansur equation

Mansur Equation

$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$ (Malli and Killedar, 2018)

Where, CF – Correction Factor (=10); EE(λ) – Erythral Effect spectrum; I(λ) – solar intensity spectrum; Abs(λ) – Absorbance of sunscreen product at the wavelength λ (Malli and Killedar, 2018). The predetermined values of Erythral Effect spectrum and solar intensity spectrum (EE x I) were obtained from the table 1 shown below.

Table 1: The relationship between the Erythral Effect and solar intensity spectrum (Malli and Killedar, 2018)

Wavelength	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Phytochemical Analysis

For the qualitative analysis, stock solution was prepared by dissolving 50mg of freeze dried methanol extract of *R. tetraphylla* leaves in 7 mL of distilled water and the same procedure was carried out for roots by dissolving 50 mg of freeze dried roots in 7 mL of water. Same tests were carried out for both *R. tetraphylla* leaves and roots.

Results

Spectrophotometric results of MLE of *R. tetraphylla*

The below figure 1 shows the absorbance at each wavelength. Dermatone shows higher absorbance than MLE. Absorbance for different concentrations (0.1 mg/mL, 0.2mg/mL, 0.4mg/mL) of MLE and Dermatone were studied and the graph was drawn using MS Excel.

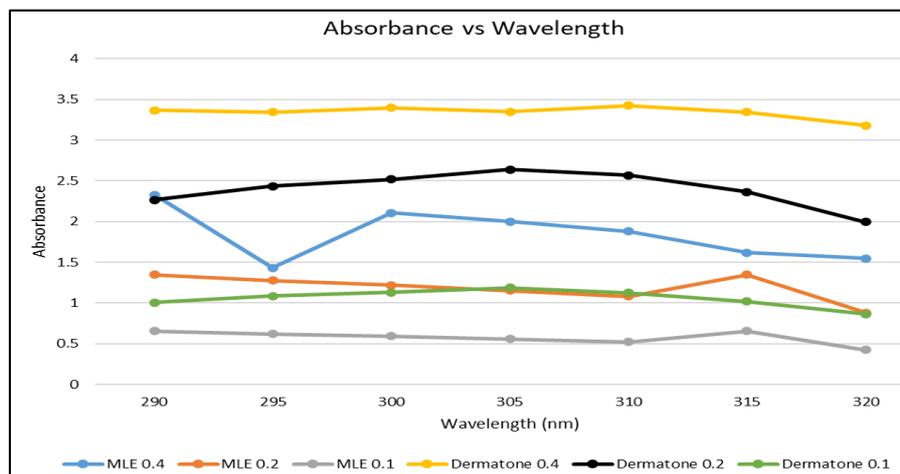


Fig 1: Absorbance vs Wavelength for MLE and Dermatone

Table 2 shows the mean values and SEM for absorbance at 290 nm, 295 nm, 300 nm, 305 nm, 310 nm, 315 nm, and 320 nm for concentrations 0.4 mg/mL, 0.2 mg/mL and 0.1 mg/mL. Dermatone and MLE shows highest absorption in concentration 0.4 mg/mL. The absorbance increases as the concentration increases.

Table 2: Absorbance of MLE

Absorbance	0.4mg/ml	0.2mg/ml	0.1mg/ml
290	2.329 ± 0.02	1.348 ± 0.24	0.654 ± 0.04
295	1.431 ± 0.02	1.275 ± 0.22	0.618 ± 0.04
300	2.105 ± 0.02	1.219 ± 0.21	0.591 ± 0.03
305	2.001 ± 0.02	1.152 ± 0.20	0.559 ± 0.03
310	1.879 ± 0.01	1.079 ± 0.20	0.523 ± 0.03
315	1.617 ± 0.001	1.348 ± 0.23	0.654 ± 0.04
320	1.548 ± 0.001	0.880 ± 0.17	0.426 ± 0.03

Mansur equation was used to calculate the Sun protective factor. Mean absorbance of each sample was multiplied using the values given in table 1 (EE x I). Table 2 shows the values after multiplying the absorbance with EE x I.

Table 2: Absorbance of MLE and EE x I

	0.4mg/ml EE x I	0.2mg/ml EE x I	0.1mg/ml EE x I
290	0.035	0.020	0.010
295	0.117	0.104	0.050
300	0.605	0.350	0.170
305	0.656	0.378	0.183
310	0.350	0.201	0.097
315	0.136	0.113	0.055
320	0.028	0.016	0.008
	3.373	2.537	1.129

Mean absorbance of Dermatone at concentrations 0.4 mg/mL, 0.2 mg/mL, 0.1 mg/mL was calculated multiplied by EE x I to calculate the SPF (table 3).

Table 3: Absorbance of Dermatone and EE x I

Absorbance	0.4	EE x I	0.2	EE x I	0.1	EE x I
290	3.366	0.050	2.264	0.034	1.005	0.015
295	3.343	0.273	2.436	0.200	1.084	0.089
300	3.399	0.977	2.521	0.725	1.132	0.325
305	3.351	1.098	2.640	0.865	1.186	0.389
310	3.425	0.638	2.568	0.479	1.127	0.210
315	3.343	0.280	2.362	0.198	1.018	0.085
320	3.181	0.057	1.995	0.036	0.862	0.016
		1.927		1.182		0.573

SPF of MLE of *R. tetraphylla* and Dermatone were determined by using the Mansur equation. MLE displayed absorbance values (range: 0.4 to 2.3). SPF of *R. tetraphylla* and Dermatone are 19.27 and 33.73 respectively at the concentration 0.4 mg/mL which is depicted in table 4.

Table 4: SPF of MLE of *R. tetraphylla* and Dermatone

mg/mL	MLE of <i>R. tetraphylla</i>	Dermatone
0.4	19.27	33.73
0.2	11.82	25.37
0.1	5.73	11.29

Phytochemical profile

The MLE of *R. tetraphylla* indicates the presence of carbohydrates, alkaloids, phenols, saponins, flavonoids, quinones, steroids, terpenoids and tanins as shown in table 5.

Table 5: Results obtained from the phytochemical analysis carried out

Tests	<i>R. tetraphylla</i> leaves
Carbohydrates	+
Alkaloids	+
Phenols	+
Saponins	+
Flavonoids	+
Quinones	+
Steroids	+
Terpenoids	+
Tannins	+
Coumarins	-

Discussion

The main objectives of this study are to determine the sun protection activity and phytochemical analysis of *R. tetraphylla*. UV spectrophotometric method was carried out to determine the Sun Protection Factor using Mansur equation (Leelaprakash and Dass, 2011; Sangeetha and Vidhya, 2016) [6, 16].

The qualitative phytochemical analysis of the MLE indicated the presence of carbohydrates, alkaloids, phenols, saponins, flavonoids, quinones, steroids, terpenoids and tanins and lack coumarins. The study was assessed *in vitro* because it is simple, validated, reliable and inexpensive compared to *in vivo*.

Moreover, *in vivo* techniques are expensive, involve ethical issues and time consuming (Banu *et al.*, 2017) [1].

This study investigates Sun Protective Factor (SPF) of MLE of *R. tetraphylla* which was found to be 19.27 at 0.4mg/mL. This is a novel finding which proves that it has a cosmeceutical potential to be developed into a natural sunscreen. In order to be recognized universally, sunscreen is determined by SPF (Schalka, Manoel and Dos Reis, 2011) [18]. Higher the SPF more effective the sunscreen (Saraf and Kaur, 2010) [17]. UV spectroscopic method is an easy and cost effective method (Kaur and Saraf, 2010) [4]. This technique bypasses ethical issues as no animals or humans were subjected (Mbanga *et al.*, 2014) [9] SPF was calculated by using absorption of UV spectroscopy and Mansur equation. Dermatologists recommend to use sunscreen with SPF 15 or more to prevent the skin from harmful UV rays (Banu *et al.*, 2017) [1]. Wide range of absorbance was studied from 290nm to 320nm which claims that wider the range of absorbance, higher the effectiveness of the sunscreen and prevent sunburns (Mbanga *et al.*, 2014) [9]. Sunburns are main cause of exposure to UVB radiation (Duraismi *et al.*, 2011) [2]. UVB rays provoke the production of free radicals like O₂, OH and HOO which are aggressive that cause photodamage to skin. Therefore, mainly a strong antioxidant sunscreen is prescribed. Anti-oxidant property is mainly present on phytochemicals such as polyphenols, flavonoids and tannins which are responsible for sun protective activity. Methanolic leaf extract of *R. tetraphylla* shows positivity to phenols, flavonoids and tannins. Therefore, the sun protective activity in this study, may be mediated through anti-oxidant property (Ratnasooriya *et al.*, 2016). Hence *R. tetraphylla* could be developed into a natural and safe sunscreen. Thus, greater the anti-oxidant activity higher the SPF and therefore, in future quantitative analysis needs to be carried out to study the quantity of phytochemicals present in *R. tetraphylla*.

Conclusion

In conclusion, the Sri Lankan MLE of *R. tetraphylla* leaves displays SPF of 19.27 which indicates that *R. tetraphylla* has a huge potential to be developed into a safe and effective topical sunscreen.

In conclusion the Srilankan MLE of *R. tetraphylla* leaves displays SPF of 19.27 which indicates that *R. tetraphylla* has a huge potential to be developed into a safe and effective topical sunscreen. In future, vivo techniques can be carried out to evaluate the photostability on sunscreen. In conclusion the Srilankan MLE of *R. tetraphylla* leaves displays SPF of 19.27 which indicates that *R. tetraphylla* has a huge potential to be developed into a safe and effective topical sunscreen. In future, vivo techniques can be carried out to evaluate the photostability on sunscreen

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