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Research Article

A Pilot Study of Sun Protective Factor of Selected Lichens from Himalayan Region

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Abstract

The Sun's ultraviolet light can causes early aging of the skin, leathery skin, Wrinkles, actinic keratosis and liver spots on our skin. Every plants contain many active constituents that can protect our skin form sun burn. There are many synthetic sunscreen are offered in market, but formulation of natural sunscreen is an important aspect in cosmetic industry. Thus the aim of the present study is to inspect the presence of UV light absorption ability of the selected lichens.

Keywords: Lichens, Sun Protection Factor, Sunscreen, UV light

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Introduction

Exposure to Sun rays may lead to skin damage and skin cancer also. Because skin is susceptible to the photo damage due to direct exposure to the solar radiation. However, UV also welfares human health by facilitating natural production of vitamin-D and endorphins in our skin, hence UV has complex and diverse effects on our health. UV radiation is divided into three regions, UV-A (400-320nm), UV-B (320-290nm), UV-C (290-200nm). UV-A can causes indirect damage to the microbe's DNA and causes darkening and tanning effect on human beings. UV-B mostly absorbed by the ozone layer and only a small amount reaches to the earth. But it is one thousand times more responsible to cause skin cancer and highly dangerous for human skin. UV-C rays are completely absorbed by ozone layer and not causing any more harmful effect on the skin (Nohynek et al. 2010). So, the protection from UV-A & UV-B is most important. Though there are many chemical products are available in the market but they pose many adverse effects. Therefore, evaluation of plant-based sunscreen is important. Lichen communities are familiar for their great diversity in high-altitude terrestrial ecosystems and they face the threats of UV radiation rising from the polar O_3 depletion (Marie et al. 2015). In presence of polar UV radiation, they increase the production and accumulation of different secondary biochemical in their body to decrease UV penetration (Bjorn 2006). In lichen UV protectant phytochemicals are produced by both the photosynthetic and fungal partner (Nguyen et al. 2013). To keep this view in mind, the goal of the present work is to find out the potency of seven selected lichen species as a skin protective agents and comparative SPF (Sun Protective Factor) values of them are also evaluated using Mansur equation.

Materials and Methods

Chemicals and Reagents

Methanol of analytical grades and procured from Merck, India Ltd.

Collection and Authentication of Plants

The Lichens were collected from different places of India. After the collection the specimens were dried properly and then studied morphologically (i.e. forms, size, structure etc.), anatomically (i.e. cellular structutre) and chemically (by color spot tests) to identify. Finally, we select seven lichen specimens to study (Table 1).

Extraction of Plant Material

After sample collection, the materials were washed properly and then shade dried. Then the dried lichens were grounded into powder. Then the dried lichens were grounded into powder. Then 10g powdered lichen added to 100ml methanol and kept on a rotary shaker for 24h. at 150 rpm at 25 ºC and then filtered through whatman No.1 filter paper. Next day extracts were concentrated under reduced pressure on a rotary evaporator at 40°C. Then the concentrated were kept in a desiccator until the traces of solvents was evaporated completely.

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Table 1. Details on lichens collection

SL No.	Lichen Name	Family	Habit (Thallus type)	Habitat (Based on substrate)	Place of collection	Date of collection
LS1	*Parmotrema austrosinense* (Zahlbr.) Hale	Parmeliaceae	Foliose	corticolous	Kalimpong Hills	March,2019
LS2	*Parmotrema sancti-angeli* (Lynge) Hale	,,	,,	,,	Nainital	Oct,2019
LS3	*P. tinctorum* (Nyl.) Hale	,,	,,	,,	Sikkim	Jun,2020
LS4	*Flavoparmelia caperata* (L.) Hale	,,	,,	,,	Nadia	July,2020
LS5	*Parmelia sulcata* Taylor	,,	,,	,,	Burdwan	,,
LS6	*Evernia prunastri* (L.) Ach.	,,	Fruticose	,,	Kalimpong Hills	Sept,2020
LS7	*Usnea florida* (L.) F. H. Wigg.	,,	,,	,,	,,	,,

Finally, the dried crude samples were kept in air tight vial and stored at 4 ºC for further studies.

Absorbance Capacity of Extracts in UV Region

The antisolar activity was executed by UV-visible spectrophotometry. Photoprotective elements that have specific absorbance at specific UV spectrum. To observe the UV absorbing property of the lichen extracts spectrophotometric readings were taken from 200 to 400 nm with 50 nm variation.

Sample preparation

1 mg of each extract dissolved in 1 ml of methanol dissolved in methanol to prepare stock solution (1mg/ml). Then made different concentrations such as 0.5mg/ml & 0.25mg/ml by serial dilutions.

Determination of SPF (Sun Protection Factor) of Lichen extract

The efficacy of sunscreen agent is usually expressed by the SPF, which is defined as the UV energy required to yield a MED (minimal erythema dose) on protected skin, divided by the amount of UV energy necessary to produce a MED on the undefended skin.

SPF = Minimal Erythemal Dose of Protected Skin / Minimal Erythemal Dose of Unprotected Skin.

To determine the SPF value of different concentration at 290-320 nm at 5 nm interval. The measurements were performed in triplicate for each concentration using 1 cm quartz cell and methanol was used as blank. Mansur equation (Kaur and Saraf 2010) was used to determine the SPF values of the formulations by using a UV spectrophotometer. The Equation is,

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

Where, CF = Correction Factor (10)
EE (λ) = Erythrogenic Effect of radiation
I (λ) = Solar Intensity spectrum
Abs (λ) = Spectrophotometric absorbance value.
The standards of EE x I are shown in Table 2.

Table 2. Normalized product function used in calculation of SPF

No	Wavelength (λ)	EE X I (normalized)
1	**290**	**0.0150**
2	**295**	**0.0817**
3	**300**	**0.2874**
4	**305**	**0.3278**
5	**310**	**0.1864**
6	**315**	**0.0839**
7	**320**	**0.0180**
Total		**1**

Results and Discussion

SPF value has become a global quantitative measurement of for determining the efficiency of sunscreen formulation. It provides a knowledge about how lengthy we can stay in the sunlight starved of getting hurt by the sun. The SPF number of methanol extracts of the lichen specimens was deliberate by applying mathematical equation. The absorbance (between 290-320 nm) nm and SPF values of the samples determined through the UV-Spectrophotometric method are revealed in Table 3-5. Table 3 represents the SPF Values of 1000µg/ml concentration of different lichen extracts. Table 4 represents the SPF Values of 500µg/ml concentration and Table 5 represents the SPF Values of 250µg/ml concentration of the lichen extracts. SPF value was calculated by absorption spectroscopy using the Mansur equation.

Table 3. Determination of *in vitro* SPF at 1000 µg/ml concentration of lichen extract

No	Wave length (λ)	EExI	L1		L2		L3		L4		L5		L6		L7	
			Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs
1	290	0.0150	2.438	0.0372	0.917	0.0138	2.341	0.0351	2.383	0.0357	2.394	0.0359	2.482	0.0372	2.429	0.0364
2	295	0.0817	2.468	0.2016	0.953	0.0779	2.372	0.1938	2.414	0.1972	2.423	0.1980	2.516	0.2056	2.461	0.2011
3	300	0.2874	2.455	0.7056	1.003	0.2883	2.358	0.6777	2.395	0.6883	2.409	0.6923	2.502	0.7191	2.442	0.7018
4	305	0.3278	2.357	0.7726	1.022	0.3350	2.257	0.7398	2.301	0.7543	2.318	0.7598	2.410	0.7899	2.351	0.7707
5	310	0.1864	2.377	0.4431	0.990	0.1845	2.268	0.4228	2.313	0.4311	2.329	0.4341	2.435	0.4539	2.373	0.4423
6	315	0.0839	2.314	0.1941	0.902	0.0757	2.118	0.1777	2.176	0.1826	2.228	0.1869	2.370	0.1988	2.308	0.1936
7	320	0.0180	2.475	0.0446	0.809	0.0146	1.654	0.0298	1.701	0.0306	2.154	0.0388	2.526	0.0455	2.456	0.0442
SPF				2.3988		0.9898		2.2767		2.3198		2.3458		2.4500		2.3901

Table 4. Determination of *in vitro* SPF at 500 µg/ml concentration of lichen extract

No	Wave length (λ)	EExI	L1		L2		L3		L4		L5		L6		L7	
			Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs
1	290	0.0150	2.396	0.0359	0.460	0.0069	2.292	0.0439	2.293	0.0344	2.034	0.0305	2.426	0.0364	2.212	0.0332
2	295	0.0817	2.422	0.1979	0.478	0.0391	2.329	0.1903	2.339	0.1911	2.038	0.1665	2.454	0.2005	2.127	0.1738
3	300	0.2874	2.404	0.6909	0.504	0.1448	2.310	0.6639	2.318	0.6662	2.026	0.5823	2.441	0.7015	2.055	0.5906
4	305	0.3278	2.307	0.7562	0.516	0.1691	2.188	0.7172	2.190	0.7179	1.932	0.6333	2.348	0.7697	1.989	0.6520
5	310	0.1864	2.322	0.4328	0.498	0.0928	2.036	0.3795	1.993	0.3715	1.788	0.3333	2.370	0.4418	1.977	0.3685
6	315	0.0839	2.251	0.1889	0.453	0.0380	1.518	0.1274	1.445	0.1212	1.506	0.1264	2.308	0.1936	1.895	0.1590
7	320	0.0180	2.365	0.0426	0.405	0.0073	0.954	0.0172	0.893	0.0161	1.188	0.0214	2.453	0.0442	1.819	0.0327
SPF				2.3452		0.4980		2.1394		2.1264		1.8937		2.3877		2.0098

Table 5. Determination of *in vitro* SPF at 250 µg/ml concentration of lichen extract

No	Wave length (λ)	EExI	L1		L2		L3		L4		L5		L6		L7	
			Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs
1	290	0.0150	2.187	0.0328	0.200	0.0030	1.398	0.0210	1.304	0.0196	0.876	0.0131	2.249	0.0337	0.979	0.0147
2	295	0.0817	2.116	0.1729	0.208	0.0170	1.468	0.1199	1.382	0.1129	0.869	0.0710	2.211	0.1806	0.885	0.0723
3	300	0.2874	2.064	0.5932	0.219	0.0629	1.495	0.4297	1.377	0.3957	0.863	0.2480	2.178	0.6259	0.838	0.2408
4	305	0.3278	1.996	0.6543	0.224	0.0734	1.310	0.4294	1.234	0.4045	0.821	0.2691	2.121	0.6953	0.818	0.2681
5	310	0.1864	1.972	0.3676	0.216	0.0403	1.035	0.1929	0.968	0.1804	0.733	0.1366	2.130	0.3970	0.803	0.1497
6	315	0.0839	1.870	0.1569	0.197	0.0165	0.691	0.0580	0.642	0.0539	0.598	0.0502	2.061	0.1729	0.770	0.0646
7	320	0.0180	1.792	0.0323	0.175	0.0032	0.414	0.0075	0.382	0.0069	0.458	0.0082	2.070	0.0373	0.711	0.0128
SPF				2.0100		0.2163		1.2584		1.1739		0.7962		2.1427		0.8230

SPF of the lichen extracts was determined by taking diverse concentrations of the methanolic extracts at 290–320 at 5 nm interval. It was detected that a rise in absorption is concentration reliant. The calculated SPF values were ranges between 0.2 to 2.4 (Fig 1). Among the seven lichens used in this study, the 1000µg/ml concentration of lichen extract of L6 offered highest SPF activity, i.e.,

2.4500 and 250µg/ml concentration of L2 lichen showed the lowest SPF activity when compared to the other remaining lichen extracts. The SPF activity of the different concentration of selected lichens with their graphical representation is displayed in Fig 2.

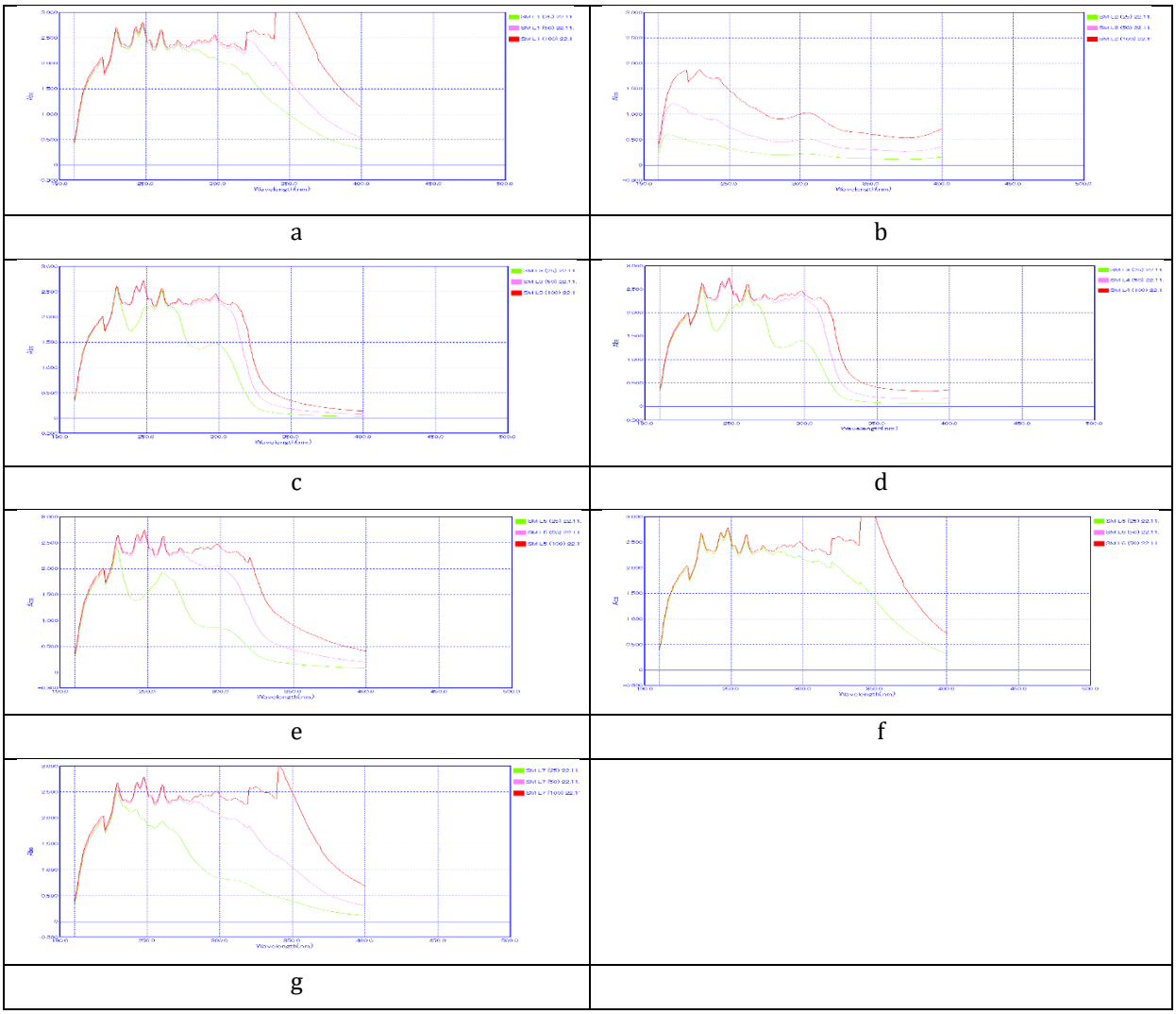

Figure 1. UV-VIS spectra of different Lichen extracts at various concentrations. a.UV-VIS spectra of L1, b. UV-VIS spectra of L2, c. UV-VIS spectra of L3, d. UV-VIS spectra of L4, e. UV-VIS spectra of L5, f. UV-VIS spectra of L6, g. UV-VIS spectra of L7.

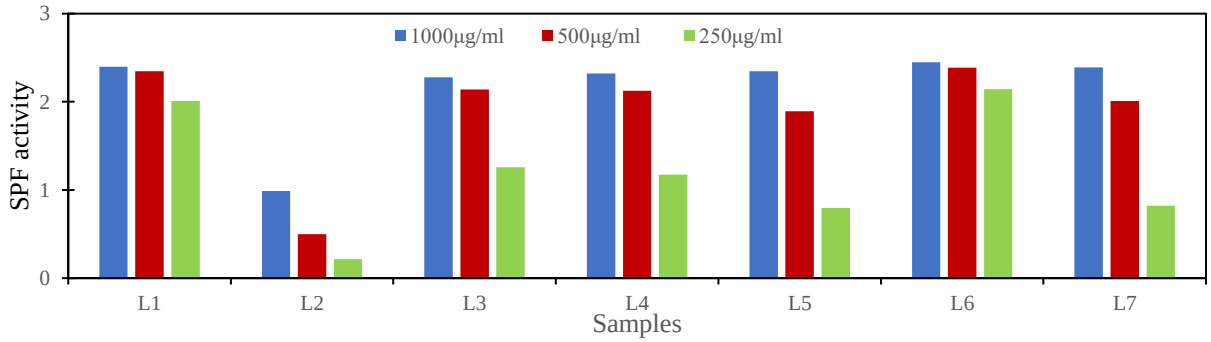

Figure 2. Graphical representation of SPF activity of different lichens at different concentrations

Although there is various synthetic sunscreen in the market, their application is inadequate because of their destructive effects on human skin (Mbanga et al. 2015). A sunscreen must have enough amounts of photoprotective agents to offer a high-level shield. Herbal products are known to be safe and have been universally accepted by clients. They can stimulate the immune response, detoxify carcinogens and block oxidative damage of DNA (Guyer et al. 2003). Thus, these natural products perform several roles in amending the route of carcinogenesis. Therefore, these natural or harbal products preparations at ideal concentrations could yield numerous valuable effects to our skin. The available literature explains that there is a positive correlation among the SPF and phenolic phytochemicals (Yasmeen and Gupta 2016) UV ray is extremely genotoxic and this causes the earliest phase of skin cancer. Sunscreen gives the protections to sunburn and numerous skin damage (Svobodova et al. 2003). Another study conducted by Mishra et al., has exhibited that flavonoid and phenolic compounds have excellent photoprotective properties (Mishra et al. 2012). T. Nguyen and his coworkers showed the SPF activity of extract of ***Parmotrema sancti-angeli*** and ***P. tinctorum*** and said that the SPF value to be 4.1 and 2.1 which is more or less similar to our result obtained from the present work (Nguyen et al. 2010). There are already many products processed from ***Evernia prunastri*** are available in market. Therefore, the present study displays that these lichen formulations at ideal concentrations could yield several valuable effects to our skin as an UV filter. In future laboratory studies could evaluate different quenching process in different lichens to estimate the degree of protection during threat by UV radiation, as has been done by Veerman et al. 2007 for *P. sulcate*.

Conclusion

The pilot findings of the present study reveal that the SPF values of the methanolic extracts of some lichen sources were calculated. It was established that all of the studied lichen has the UV protection abilities and can be used in sunscreen formulations. These lichens could become a decent, inexpensive and easily available components used in sunscreen products.

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