Assessment of *In Vitro* Sun Protection Factor of Plant Extracts by Ultraviolet Spectroscopy Method

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Abstract-Exposure to sunlight can trigger various biological responses ranging from sun-burn, erythema to skin cancer. Synthetic sunscreen formulations available in market pose variety of adverse effects. Therefore, formulation of the herbal sunscreen formulation and evaluation of its sun protection activity is an important aspect in the cosmetic industry. The purpose of present study was to evaluate the sun protection factor (SPF) of aqueous and methanolic extract of *Aloe barbadensis miller* and *Cocos nucifera* by ultraviolet (UV) spectroscopy method. Methanolic extract of Cocos nucifera showed the highest SPF value (3.2**)** amongst all extracts. The results indicated presence of active components responsible for ultraviolet absorption which may be extracted from these plant extracts and maybe used in sunscreens preparations for better protection against sun rays.

Key Words: UV radiations, SPF, *Aloe barbadensis miller* and *Cocos nucifera*

Introduction

The human body is constantly exposed to an array of chemical and physical exogenous pollutants (Perluigi et al. 2010). The harmful effects of solar radiations are caused predominantly by the ultraviolet (UV) region of the electromagnetic spectrum, which can be divided into three regions: UVA (400-320 nm) UVB (320-290nm) and UVC (290-200 nm) (Dutra et al. 2004). UVC radiations are filtered out by the atmosphere before reaching earth. UVB radiations are not completely filtered out by the ozone layer and are responsible for the damage due to sunburn and pyrimidine dimmers formation. UVA radiation reaches the deeper layers of the epidermis, dermis and provokes the premature ageing of the skin and is responsible for the generation of free radicals. UVB radiations are involved in 65% damage of all skin and are responsible for causing sunburn, cutaneous degeneration, photosensensitivity, phototoxicity, and ectinic elastisis (Hawk, 2001; Mbanga et al. 2014). The main destroying factors of UV radiations for skin are oxygenated molecules which are often call free radicals such as; superoxide anions (O2.-), hydroxyl radical (.OH), singlet oxygen, hydrogen peroxide (H_2O_2), ferric ion, nitric oxide (NO) etc. It is well documented that ultraviolet (UV) light indices immune suppression and oxidative stress, which play an important role in the induction of skin cancers (Mukhtar and Elmets, 1996). Previous reports have suggested that ultraviolet (UV) radiation is responsible for distinct mutations in keratinocytes that ultimately contribute to the development of the non-melanoma skin cancers, which include basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Thus it becomes necessary to protect the skin from such carcinogenic radiation. Sunscreens and sunblocks are chemicals that absorb or block UV rays and show a variety of immunosuppressive effects of sunlight. There are several agents available from both synthetic and natural sources with UV-filtering properties. Given their potential to produce considerable human local and systemic exposure, UV filters have to be safe (Nohynek *et al.,* 2010). Synthetic UV filters are known to have potential toxicity in humans and also showed ability to interfere only in selected pathways of multistage process of carcinogenesis (Chanchal and Swarnlata, 2009).To stimulate the skin, to repair and build itself naturally, we need find an arsenal of potent ingredients. In a quest to find effective topical photoprotective agents, plant-derived products have been researched because of their antioxidant activity mostly due to presence of phytochemical substances. Effective botanical antioxidant compounds are widely used in traditional medicine including tocopherols, flavonoids, phenolic acids, nitrogen containing compounds (indoles, alkaloids, amines, and amino acids), and monoterpenes. The sun protection factor of a sunscreen is a laboratory measure of the effectiveness of sunscreen, the higher the SPF, the more protection a sunscreen offers against UV-B (the ultraviolet radiation that causes sunburn) (Ramos-e-Silva and Carneiro, 2007). The SPF is the

amount of UV radiation required to cause sunburn on skin with the sunscreen on, relative to the amount required without the sunscreen (FDA, 2009). There is an immense need to explore the sunburn protective properties of herbal plants because of their proved medicinal properties due to rich source of phytoconstituents and oxidation inhibitors. Keeping the above factors in consideration, the present study was planned to evaluate SPF vaules and phytochemical properties of *Aloe barbadensis miller* and *Cocos nucifera*.

Material and Methods

Sample Preparation

The Coconut and *Aloe vera* samples were washed with distilled water twice and dried. The samples were then grinded separately in a mixer grinder and 20 gm from each were taken separately in a beaker. This powder was weighed and loaded to the Soxhlet apparatus and 200 ml of methanol/water was used as solvent for preparing the alcoholic and aqueous extract respectively. The extraction process was carried out overnight and the extract was then filtered .The filtered extract was suitably diluted with methanol/water or dried and kept at 4˚C for measuring SPF and phytochemical anaylsis.

Phytochemical Analysis Of Plant Extract

Flavonoid Test: 5ml of diluted ammonia solution was added to aqueous filtrate of the plant extract followed by the addition of concentrated H_2SO_4. Formation of yellow color indicates the presence of flavonoids.

Saponins Test: One ml of plant extract was diluted with 20 ml distilled water and the tube was shaken. Formation of foam indicates the presence of saponins.

Alkaloids Test: **To one** ml of plant extract, 3ml of ammonium solution was added and incubated at 37 ˚C for few minutes. The tubes were then placed in water bath and then mayer's reagent was added. Formation of cream color precipitation showed the presence of alkaloids.

Terpenoids Test: 2ml of chloroform was added to 5ml of plant extract. Conc. H_2SO_4 (3ml) was then carefully added to form a layer. Reddish brown coloration of the interface indicated the presence of terpenoids.

Carbohydrate Test: 1ml of Fehling's A and Fehling's B were heated in a boiling water bath for 5-10 min with the plant extract. Appearance of reddish orange precipitate shows the carbohydrate presence.

Tanins Test: About 2ml of the plant extract was stirred with 2ml of distilled water and few drops of $FeCl_3$ solution (5%w/v) were added. The formation of a green precipitate was an indication for the presence of tannins.

Phenol Test: **Plant e**xtracts were treated with 3-4 drops of $FeCl_3$ solution. Formation of bluish black colour indicates the presence of phenols.

Steroid Test: A red colour produced in the lower chloroform layer on addition of 2 ml plant extract to 2 ml of chloroform and 2 ml conc. H_2S0_4 indicates the presence of steroids.

Determination of SPF

100 mg of aqueous and methanolic extract were dissolved in 100ml of distilled water. From this 2 ml and 4ml of the extract was withdrawn and diluted to 10 ml with distilled water so as to prepare extract with the final concentration of 200µg/ml and 400µg/ml. Thereafter, the absorbance of these extracts was taken by spectrophotometer from wavelength ranging from 290 to 320 at 5nm. SPF for aqueous and methanolic extract was calculated by the formula given by Mansur equation and by utilizing values given by Sayre (Kaur and Saraf, 2010). SPF was calculated three times and then mean value was taken in consideration.

In vitro SPF can be calculated by following equation**:**

$$\mathbf{SPF = CF \times \sum EE \times I \times Abs}$$

Where;

(I) - the solar irradiance spectrum,

EE (I) - the erythemal action spectrum,

Abs- absorbance of sunscreen product,

CF-correction factor (=10)

The value of EE x I are constant and predetermine as shown in table-1

Table-1 Values of EE×I used in the calculation of SPF

Wavelength (nm)	EE*I (Normalised)
290nm	0.0150
295nm	0.0817
300 nm	0.2874
305nm	0.3278
310nm	1.864
315nm	0.0839
320nm	0.0180
TOTAL	1

Results and Discussion

3.1 Phytochemical Analysis Of Plant Extracts

Phytochemical examination revealed the presence of constituents such as carbohydrates, alkaloids, glycosides, saponins, tannins, flavinoids, phenol and terpenoids (Table -2).

Table-2 Phytochemical Analysis

Constituents	Aloe vera (*Aloe barbadensis Miller*)	Coconut (*Cocos nucifera)*
Carbohydrates	+	+
Saponins	+	+
Alkaloids		+
Tarpinoids	-	+
Flavonoids	+	+
Phenols	-	+
Tannin	+	+
Steroids	-	+

Raphael (2012) conducted a study on phytochemical analysis of Aloe vera. Their study revealed the presence of tannins, flavonoids, terpenoids, carbohydrates, and alkaloids in *A. vera* plant whereas saponins, glycosides phlobatannins, antiquinones carbohydrates, and steroids were absent. Odenigbo and Otisi (2011) conducted study on phytochemical analysis of Coconut oil and reported the presence of alkaloid, resins, glycosides, terpenoids and tannins.

Determination of SPF

The sun protection factor or SPF is a measure of the fraction of damage-producing UV rays that reach the skin. It also gives an idea of how much time you can stay in sun without any protection (sunscreen) and without any damage to the skin. In order to protect against UV radiations, the formulation should have good SPF number and also the formulation should have wide range of absorbance between 290-400 nm ranges. In the present research work aqueous and methanolic extract of *Aloe barbadensis miller* and *Cocos nucifera* were subjected for SPF evaluation by UV spectroscopic method. SPF value for sunscreen above 2 is considered as having good sunscreen activity. The calculated values of SPF of aqueous and methanoic extract of *Aloe barbadensis miller and Cocos nucifera* are presented in the table-3-6 *SPF value of* aqueous extract of *Cocos*

nucifera was 0.647 and 1.454 at concentration of 200µg/ml and 400µg/ml respectively. Methanolic extract of Coconut have SPF value 1.305 and 3.207 at a concentration of 200µg/ml and 400µg/ml respectively. It was found that aqueous extract of have *Aloe barbadensis miller* SPF value of 0.14802 and 0.082276 at concentration of 200µg/ml and 400µg/ml respectively. Methanolic extract of *Aloe barbadensis miller* have SPF value 0.60412 and 1.96168 at a concentration of 200µg/ml and 400µg/ml respectively.

Table-3 Absorbance and SPF value of methanolic extract of *Cocos nucifera*

S.No	λ	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	0.845	0.012675	2.022	0.03033
2	**295**	0.817	0.789	0.644613	1.923	1.571091
3	**300**	0.2874	0.748	0.2149752	1.845	0.530253
4	**305**	0.3278	0.718	0.2353604	1.783	0.5844674
5	**310**	0.1864	0.695	0.129548	1.726	0.3217264
6	**315**	0.0837	0.682	0.0570834	1.678	0.1404486
7	**320**	0.018	0.642	0.011556	1.63	0.02934
	Total			**1.305811**		**3.2076564**

Table-4 Absorbance and SPF value of aqueous extract of *Cocos nucifera*

S.No	λ	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	0.443	0.006645	0.991	0.014865
2	**295**	0.817	0.4	0.3268	0.894	0.730398
3	**300**	0.2874	0.37	0.106338	0.83	0.238542
4	**305**	0.3278	0.348	0.1140744	0.785	0.257323
5	**310**	0.1864	0.332	0.0618848	0.752	0.1401728
6	**315**	0.0837	0.319	0.0267003	0.724	0.0605988
7	**320**	0.018	0.306	0.005508	0.699	0.012582
	Total			**0.6479505**		**1.4544816**

Table-5 Absorbance and SPF value of aqueous extract of *Aloe barbadensis miller*

S.No.	λ	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	0.111	0.001665	0.059	0.000885
2	**295**	0.817	0.101	0.082517	0.053	0.043301
3	**300**	0.2874	0.091	0.0261534	0.047	0.0135078
4	**305**	0.3278	0.083	0.0272074	0.043	0.0140954
5	**310**	0.1864	0.038	0.0070832	0.038	0.0070832
6	**315**	0.0837	0.034	0.0028458	0.034	0.0028458
7	**320**	0.018	0.031	0.000558	0.031	0.000558
	Total			**0.1480298**		**0.0822762**

Table-6 Absorbance and SPF value of methanolic extract of *Aloe barbadensis miller*

S.No.	λ	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	0.152	0.00228	1.192	0.01788
2	**295**	0.817	0.140	0.11438	1.162	0.94935
3	**300**	0.2874	0.129	0.03672	1.146	0.32936
4	**305**	0.3278	0.101	0.03310	1.112	0.36451
5	**310**	0.1864	0.091	0.01696	1.106	0.21361
6	**315**	0.0837	0.083	0.06947	0.910	0.07616
7	**320**	0.018	0.041	0.00073	0.601	0.01081
	Total			**0.60412**		**1.96168**

The different SPF values of *Aloe barbadensis* miller and *Cocos nucifera* indicates that aqueous and methanolic extract of them found near the range of good sunscreen activity. Methanolic extract of *Cocos nucifera* showed the highest SPF value amongst all extracts. Thus it can be proposed that these plant extract can absorb the ultraviolet radiation since they possess good sun protection activity against ultraviolet radiations.

Malsawmtluagi et al. (2013) conducted a similar study on some aqueous extract of vegetables to determine the ultraviolet (UV) absorption properties determining the sun protection factor (SPF) number. It was observed that all of the tested herbals showed some UV protection capabilities with aqueous Coconut extract showing the highest SPF number of 7.38 while watermelon showed the lowest SPF number of 0.97. Suva (2014) conducted a study to evaluate the sun protection factor (SPF) of methanolic extract of flowers and leaves of *Azadirachta indica* by ultraviolet (UV) spectroscopy method. It was reported that methanolic extract of leaves of *Azadirachta indica* have SPF value about 1.47658 and methanolic extract of flowers of *Azadirachta indica* have SPF value about 1.3565.

Conclusion

The SPF values of the aqueous extracts of some *Aloe barbadensis miller and Cocos nucifera* were evaluated. It was found these extracts possess UV protection capabilities and active components responsible for ultraviolet absorption which maybe isolated from these plant extracts. Along with their many beneficial effects and safety, these botanicals could thus become good, cheap and easily available formulation ingredients in sunscreen products.

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