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Estimation and Comparison of Hydro-Alcoholic and Water Extract for Sun Protection Factor Activity from Naturally Available Resources

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Abstract

Expose to sunlight causes various biological responses ranging from sun-burn, erythema to skin cancer. Synthetic sunscreen formulations are readily available in market but are expensive and includes a wide range 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. There are various naturally available herbal agents which contain significant sunscreen properties which may be useful for formulating herbal sunscreen creams and lotions. So, evaluation of its sun protection activity is an important aspect in the cosmetic industry. The comparative values of the calculated SPF of eleven natural resources were evaluated using mansur equation. Hydroalcoholic and water herbal extraction was carried out and absorbances were recorded between 290-320nm using UV-VIS spectrophotometer. It was observed that all the tested herbal extracts showed some uv protection capabilities with water and hydroalcoholic extract. Hydroalcoholic extract of mulberry showed highest spf i.e. 203.03 while tomatoe extract showed lowest spf i.e. 6.43. Water extract of mulberry showed highest spf i.e. 183.19 while kiwi extract showed lowest spf i.e. 8.08. Thus, natural resources extract can be formulated in the form of cosmetics formulation because of their better activity, acceptability, less irritant nature and these are less expensive, easily available.

Keywords: Sun protection factor (SPF); Mansur equation; Hydro alcoholic extract; Water extract

Abbreviations: SPF: Sun Protection Factor

Introduction

Solar ultraviolet radiation (UVR) is divided into three categories: UV-C(200-280nm), UV-B(280-320nm), UV-A(320-400nm). UV-C radiation is filtered by the atmosphere before reaching the earth. UV-B radiation is not completely filtered by the ozone layer and is one of reason for sunburn. UV-A reaches to our deeper layer of epidermis and dermis and provokes the prematuration of skin. So, to protect dermis and epidermis from UV-A radiation different sunscreen are employed. Sunscreen preparation can be used for protection from both UV-A and UV-B radiations. Mainly two factors are responsible for protection of skin.

a) thickness of stratum corneum

b) pigmentation of the skin.

The thickening of the stratum corneum occurs due to effect of solar irradiation by increasing mitotic rate of epidermal cells and thus making it more permeable to passage of erythemogenic radiation. Increase in melanin content of epidermis also increases protection of skin. UV radiation causes excess formation of melanin which migrates upwards towards stratum corneum and skin surface increases and thus increases the resistance. The efficacy of a sunscreen is described by the sun protection factor (SPF). SPF is defined as the UV energy required to produce a minimal erythema dose (MED) in protected skin divided by the UV energy required to produce same med in unprotected skin. The med is defined as the lowest time interval or dosage of UV light radiation sufficient to produce a minimal, acceptable erythema on unprotected skin.

Sun screen preparations

The ideal sunscreen agent should have following characteristic:

a) Absorb light preferentially over the range of 280nm-320nm.

b) Be stable to heat, light and perspiration.

c) Be non-toxic and non-irritant.

d) Not be rapidly absorbed.

e) Be neutral.

f) Be readily soluble in suitable vehicles.

The sunscreen can work in different ways

a) A protective layer can be formed on skin that prevent the UV rays to reach the skin either by absorbing or by reflecting them. Zinc oxide and titanium dioxide both have such property. But this preparation has disadvantages of eliminating the beneficial rays along with the harmful ones.

b) To incorporate substances in preparations to filter the sun rays by absorbing medium range UV rays (280nm-320nm) but allowing rays of higher wavelength to pass. All modern suntan preparations are based on this principle and contains such substances.

c) Biologically effective substances can be used effectively to prevent symptoms of inflammation without reduction of tanning. Because the damage of cells by sunburn liberates histamine in tissue.

d) Substances that cause or accelerate tanning of skin can be applied. Dioxyacetone causes tanning by forming a brown complex with keration of corneal layer.

SPF (sun protection factor)

The higher is SPF, the more effective the product is in preventing sunburn. So, it is necessary to determine SPF value of the products used for preventing sunscreen. SPF on package can range from as low as 2 to as high as 100. These number refers to ability of product to screen or block out the sun burning rays. If the product refer as SPF15 means that $1/15^{th}$ of the burning radiation reach the skin through the recommended thickness of sunscreen.

SPF can be determined by *in vivo* or *in vitro* methods, but the *in-vivo* method is expensive and introduces the ethical testing on humans and animal. So, mostly the *in- vitro* method that is rapid, cost effective is used. *In-vitro* testing can be used as formulation tool to identify new filters, optimize combination of old one and new sunscreen formulas prior to *in-vivo* testing in humans.

Mechanism of used resources in protection against sunburn

a) Tomatoe: The red tomato helps protect the skin from sunburn and skin ageing caused by sunlight exposure. Lycopene also helps increase the collagen in the skin, keeping it more elastic and preventing sagging lycopene also helps increase the collagen in the skin, keeping it more elastic and preventing sagging. Protects skin antioxidant system ((glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase activities), prevention of UV-B radiation-induced liposome peroxidation SPF.

b) Kiwi: Kiwi are packed with more vitamin c which is thought to smooth out wrinkles caused from the sun by promoting the production of collagen than any other fruit. Reduces DNA damage and erythema formation due to protection of DNA repair enzymes from inactivation by ROS.

c) Orange: Orange contains highest amount of vitamin c which in turns help skin to protect from UV rays. Protects skin via transcriptional mechanisms of nf-κb and mapk signaling

d) Mulberry: Mulberry helps to prevent oxidative stress due to UV radiation which causes mutagenicity and wrinkling of skin. Mulberry are highest source of vitamin c.

e) Broccoli: Broccoli reduces the skin redness and inflammation caused by exposure to ultraviolet (UV) radiation. Protects skin's antioxidant systems (glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase activities).

f) Flax Seed: Flaxseed oil in particular, can protect your skin against UV rays and keep your skin moisturized so it won't dry out.

g) Mangoe: Yellow foods contain beta-carotene, an antioxidant that may help prevent sunburn. Inhibits solar radiation induced p53 powerful antioxidant enhancer.

h) Aloe Vera: Aloe Vera is touted as the "burn plant," the perfect natural remedy for a bad sunburn. A compound in aloe called aloin is found to be responsible for the plant's anti-inflammatory benefits. Aloe Vera can also help to moisturize the skin and prevent the peeling that sometimes happens with sunburns.

i) Pomegranate: Inhibits the adverse effects of UV-B exposure including translocation of transcription factors nf-kb and ap-1, over expression of the pro-inflammatory cytokine il-8, cleavage of procaspase-3 (a key step in apoptotic pathway), and DNA fragmentation

j) Honey: Honey can be a sweet relief to a sunburn due to its antibacterial properties.

k) Beetroot: Beetroot are greatest source of vitamin c which helps in prevention against sunburn.

l) Ginger: Ginger contains anti-inflammatory properties which will help in reducing redness and swelling. Thus, help in sunburn. Scavenge ROS, by interrupting the activation of protein kinase-c. Enhance glutathione content and gst activity. Inhibit lipid peroxidation and arachidonic acid. Inhibit ornithin decarboxylase (ODC) activity.

Methods used to determine SPF (sun protection factor)

There are different methods used to determine and evaluate the efficiency of sunscreen by its SPF (sun protection factor). We know higher is SPF, more efficient is sunscreen and more will be protection against UV-A and UV-B radiations.

The methods are:

Spectrophotometric evaluation: This is basically to evaluate the UV radiation absorption ability of the sunscreen compounds. Using a UV-Spectrophotometer and taking specific concentration

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of the substance on the preparation, molar extinction coefficient or absorbency can be determined and compared with any other standard substances.

Erythemal dosage: It is important to estimate the erythemally effective radiation or e-vitons/cm^2, transmitted by a suntan preparation. The erythemal energy is the product of the solar energy transmitted through the film of suntan preparation and the effectiveness factor at that wavelength.

Sunscreen index: This is evaluation of the relative screening activity of the sunscreen compounds. This is measurement of extinction coefficient at 308µm wavelength and comparison with other. 308µm is the peak wavelength for effective sunburn.

***In-vivo* skin testing:** This is a direct test on animal skin, particularly rabbit, the site normally used is either backside or abdomen as these sites have maximum sensitivity. Preparations are applied on a site for a specified period of time. The effects are observed at the end of the period.

***In-vitro* method:** There are two *in-vitro* methods:

a) method which involve the measurement of absorption or the transmission of UV radiation through sunscreen product film in quartz plates or biomembranes.

b) method in which absorption characteristics of the sunscreen agents are determine based on spectrophotometric analysis of dilute solutions which is known *as mansur equation method*.

In-vitro estimation of SPF (sun protection factor) by mansur equation method

Mansur developed a very simple mathematical equation which calculates and provide SPF value of wide range from 290-320nm. Here, the test substances are prepared and subjected for photo protective activity study by UV-Spectrophotometer in the range of 290-320nm.

$$SPF_{invitro} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

Where, CF= correction factor (10), EE(λ)= erythmogenic effect of radiation with wavelength λ, abs(λ)= spectrophotometric absorbance value at wavelength λ.

Table 1: Standard EE×I value used in mansur equation.

Wavelength(λ)	Ee×i
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.018

The value of EE×I (λ) are constants (Table 1).

Material and Methods

UV-Visible spectrophotometer: model: shimadzu-1800, software: UV probe, version: 2.42.

Weighing balance: Company- reptech, capacity- 0.01-300gram.

Centrifuge: Company- remi-electro technic limited.

Glasswares- made up of borosilicate glass.

Reagents- distilled water, methanol.

Sample used- honey, aloevera, mulberry, pomegranate, ginger, flax seed, tomatoe, mangoe, kiwi, orange, broccoli are obtained from local market.

Procedure for *in-vitro* estimation of SPF by Mansur Equation Method

To determine SPF by mansur method, first sample is extracted with its suitable solvent overnight, centrifuge and then filtered out. The filtrate is then diluted with required quantity of methanol and is measured at wavelength 280-320nm which is 5nm increments using 1cm quartz cell. The obtained absorbance is kept in mansur equation by multiplying with standard value of EE×I which is shown in Table 2.

$$SPF_{invitro} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

Table 2: Standard EE×I value used in mansur equation.

Wavelength(λ)	EE×I
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315	0.0837
320	0.018

The standard value of EE×I by mansur are shown below:

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Procedure for preparation of water extracts:

10 gram of sample is weighed in 150ml beaker followed by solvent addition of (100ml of distilled water) and is kept overnight for extraction. After extraction, it is filtered out using whatman filter paper. The filtrate is then centrifuge at 2000rpm for 20min. The obtained clear solution is diluted upto methanol and examined for its absorbance using UV-Spectrophotometer.

Procedure for preparation of hydro-alcoholic extracts

10 gram of sample is weighed in 150ml beaker followed by solvent mixture addition (60ml of distilled water + 40ml of methanol) and is kept overnight for extraction. After extraction,

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it is filtered out using whatman filter paper. The filtrate is then centrifuge at 2000rpm for 20min. The obtained clear solution is diluted upto methanol and examined for its absorbance using UV-Spectrophotometer.

Preparation of standard solution of extract

The clear solution(1ml) after centrifuge is taken and is diluted upto 10ml with water.

Preparation of dilution solution of extract

The above standard solution (1ml) is taken and is diluted up to 10ml with water and is measured at 290-320nm using 1cm quartz cell and water as blank by UV-Spectrophotometer.

Result

$$SPF_{invitro} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

(Table 3 & 4)

Table 3: Absorbance of hydro-alcoholic extract samples taken from 290-320nm (5 increments) using UV-Spectrophotometer.

		Absorbance											
wavelength (nm)	EE×I	Beetroot	Ginger	Tomatoe	Orange	Broccoli	Kiwi	Promegranate	Aloevera	Honey	Mulberry	Flax seed	Mangoe
290	0.015	0.4445	0.5198	0.1034	0.3249	0.7643	0.2518	0.8969	0.1647	1.2618	2.9735	1.7107	1.4264
295	0.0817	0.4239	0.3604	0.0876	0.2741	0.668	0.2158	0.8085	0.1383	1.125	2.5434	1.495	0.9435
300	0.2874	0.4018	0.2932	0.0774	0.2369	0.6002	0.1873	0.7173	0.1113	0.9656	2.2643	1.3734	0.8095
305	0.3278	0.3737	0.2616	0.0712	0.2176	0.5496	0.1662	0.6436	0.0857	0.8123	1.9908	1.3078	0.7652
310	0.1864	0.3457	0.2457	0.0665	0.2137	0.5154	0.1484	0.5881	0.0662	0.6829	1.7393	1.2623	0.7118
315	0.0837	0.3242	0.2343	0.0645	0.2165	0.4892	0.1331	0.5467	0.0527	0.5845	1.5302	1.2144	0.6231
320	0.018	0.305	0.225	0.0624	0.2187	0.4646	0.1217	0.5151	0.0445	0.5053	1.3895	1.1579	0.5445

Table 4: Absorbance of water extract samples taken from 290-320nm (5 increments) using UV-Spectrophotometer.

		Absorbance											
Wavelength (nm)	EE×I	Beetroot	Ginger	Tomatoe	Orange	Broccoli	Kiwi	Promegranate	Aloevera	Honey	Mulberry	Flax seed	Mangoe
290	0.015	0.2435	0.8367	0.2114	0.1478	0.5944	0.1165	0.3692	0.162	0.302	2.4498	0.8531	0.1543
295	0.0817	0.2275	0.706	0.1959	0.1335	0.5368	0.0986	0.3298	0.16	0.277	2.2129	0.7408	0.1352
300	0.2874	0.2165	0.6393	0.1839	0.1223	0.4913	0.0867	0.296	0.159	0.25	1.9925	0.6782	0.1149
305	0.3278	0.2083	0.6023	0.1743	0.1154	0.4567	0.0784	0.2668	0.14	0.222	1.791	0.6418	0.0943
310	0.1864	0.2015	0.5782	0.1677	0.1119	0.4315	0.0734	0.2413	0.106	0.196	1.6329	0.6157	0.0727
315	0.0837	0.192	0.5607	0.1615	0.1109	0.4139	0.0671	0.2218	0.064	0.172	1.4975	0.5931	0.0553
320	0.018	0.186	0.5459	0.1555	0.1088	0.3984	0.0623	0.2076	0.041	0.15	1.3876	0.5684	0.0415

Discussion

There is lack of awareness about chemical toxic agent used in sunscreen which causes skin cancer. This has lead to increase demand of effective protection against sunburn without any adverse effect. Thus, in above work some natural available resources (ginger, tomatoe, aloevera, promegranate, mangoe, kiwi, beet, broccoli, flax seed, orange, honey, mulberry) are checked and tested for SPF (activity of protection).

Among all samples extracted and tested it was found that there was effect of solvent on extraction. As we can see in Table 5 & 6, that the lowest SPF in water extract was found in kiwi but lowest SPF for hydroalcoholic extract was found in tomatoe.

Table 5: Calculated SPF of hydro alcoholic extract using mansur equation.

	Sample	SPF Calculated
1	Tomatoe	6.430369
2	Promegranate	6.612845
3	Beet	8.487719
4	Aloevera	9.140136
5	Kiwi	17.07111
6	Orange	22.85731
7	Ginger	27.67192
8	Broccoli	66.61312
9	Mangoe	77.68968
10	Honey	83.99355
11	Flax seed	132.8994
12	Mulberry	203.0366

Table 6: Calculated SPF of water extract using mansur equation.

	Sample	SPF Calculated
1	Kiwi	8.083965
2	Mangoe	9.622103
3	Orange	11.82
4	Aloevera	13.29438
5	Tomatoe	17.67403
6	Beet	20.99042

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7	Honey	22.54303
8	Promegranate	26.79235
9	Broccoli	46.59247
10	Ginger	61.59327
11	Flax seed	65.32567
12	Mulberry	183.1965

But for highest SPF for both extract it was mulberry. Because mulberry is highly rich in vitamin c, they also help in good blood circulation that makes skin more shine. Mulberry also helps to prevent oxidative stress due to UV radiation which causes mutagenicity and wrinkling of skin.

We can also see the effect of solvent extraction in the series of sample positioned as show in Figure 1 & 2. This is due to some constituents may be very soluble in water rather than hydro-alcoholic and some constituents may have very less solubility in water and vice versa [1-23].

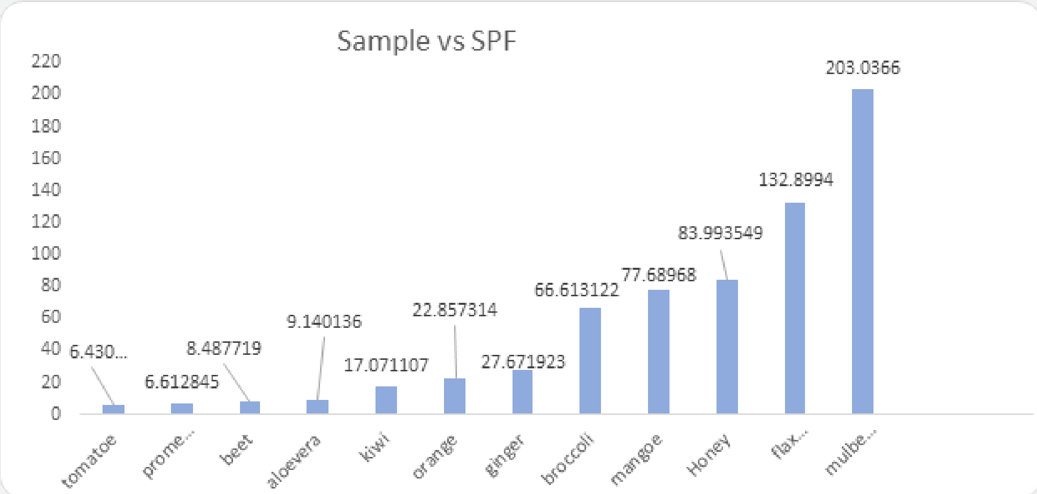

Figure 1: Graph of calculated SPF of hydroalcoholic extract showing lowest to highest activity: It was found from the spf calculated using mansur equation as shown in (figure 2) that hydroalcoholic extract of tomatoe have lowest SPF of value 6.43, while mulberry have highest SPF of value 203.03.

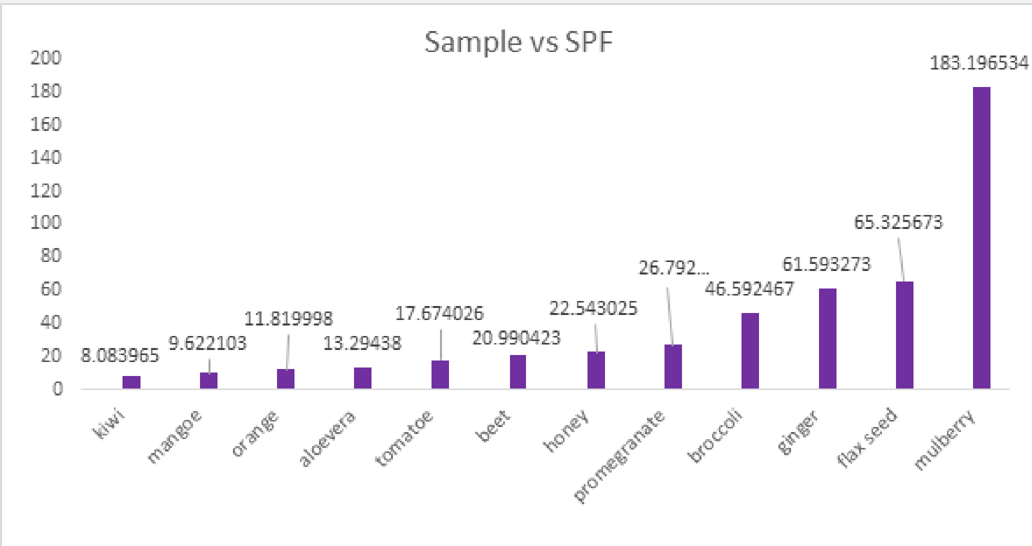

Figure 2: Graph of calculated SPF of water extract showing lowest to highest activity: It was found from the SPF calculated using mansur equation as shown in (figure 2) that water extract of kiwi has lowest SPF of value 8.08, while mulberry have highest SPF of value 183.19.

Conclusion

We can know there are some sunscreen commercially available in market with some natural sources but above all samples do not have any formulation supporting sunscreen preparations. So, it is a great concern for cosmetic industry to use natural source and invent new formulation with higher efficiency.

Our results showed that mulberry have highest SPF in both water and hydro alcoholic extract. So, a new formulation of

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mulberry for sun protection will show great activity, acceptability, safety and efficacy.

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