STUDY OF ANTIOXIDANT PROPERTY AND PHOTO PROTECTIVE ACTIVITY OF *HELIANTHUS ANNUS* AND *CORIANDRUM SATIVUM* UNDER DIFFERENT SOIL TREATMENTS.

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ABSTRACT

Now a days personal care products containing ingredients from the plant origin are getting increasing trend in pharmaceuticals, because various synthetic agents have been used as photoprotectives but they have limited use as they cause potential toxicity in humans and involve in photo carcinogenesis. So herbal antioxidants and sunscreen work on photo aging and other skin disorders. The effectiveness of sunscreens is determined by Sun Protection Factor(SPF), which is supposed to indicate the level of protection from UV radiation. The present work was designed to study the effect of different soil treatments on the antioxidant activity and UV absorption of Helianthus annus and Coriandrum sativum. The Photo protective activity (SPF) of these herbs showed remarkable increase in soil treatments compared to control. The sunscreen cream was prepared using the herbs which showed highest SPF. Helianthus showed SPF of 23.63 under soil treated with Pongamia leaves and Coriandrum showed SPF of 39.77 under soil treated with Spirulina. A survey of the prepared cream was also done.

Key words: Skin damage, Soil treatments, Antioxidants, UV absorption, SPF, Sunscreen cream, survey.

Introduction: Now a days cosmetics are considered to be one of the essential commodities of life (Sarvesh *et al* ., 2013). The world over there is a return towards the use of herbal products. People prefer natural food, herbal medicines and natural curing practices for healthy life. The

usage of herbal cosmetics has been increased many folds in personal care products. Herbal products are not only devoid of side effects but also equally effective in comparison to their modern counter parts. The use of bioactive ingredients in cosmetics influence biological functions of skin and provides nutrients necessary for the healthy skin. The use of phytoantioxidants in cosmetic products nourishes the skin and replenishes it with antioxidants.

Free radicals are of great importance to signaling processes in the human body. If their concentration exceeds a critical value, however, these highly reactive molecules can destroy cells or cell compartments. The reasons for enhanced radical formation in the human organism, specifically in the skin, are manifold. In addition to environmental factors, such as ultraviolet radiation of the sun and contact with environmental hazards, smoking and excessive alcohol consumption lead to the formation of free radicals in the skin. In addition, such formation can be stimulated by illness, insomnia, stress etc. ROS-induced damage on the skin and UV stress plays important role in photoaging (Wlaschek *et al*., 1995).

Overproduction of reactive oxygen species (ROS) results in oxidative stress thereby causing cytotoxicity and delayed wound healing. Therefore, elimination of ROS and estimation of antioxidants like SOD, catalase and glutathione in granulation tissues is also relevant because these antioxidants hasten the process of wound healing by destroying the free radicals. The bioactivity of flavonoids tightly correlated with their chemical structure and action mechanisms, mostly inhibitory on enzymatic systems involved in cellular activations.

Our body defends itself from these phenomena via endogenous and exogenous antioxidants. Antioxidants favors skin hydration, elasticity, and sebum production and stimulate the physiological properties of the skin and protect against UV-induced skin damage. The deleritious effect of UV radiation on human has increased the need for photoprotection. Sunscreens are widely used as photoprotective agents (Rai *et al*., 2007).

With the antioxidative network, the human body has developed a protective system against the harmful action of free radicals. The most important antioxidants in the human body, particularly in the skin, include vitamins A, C, E, and D; the carotenoids like β-carotene, lycopene, lutein and polyphenols.

Current thinking is that a combination of different phytoantioxidants would be the best defence strategy against ROS. The best approach is to measure antioxidants and their effects, which will lead to improved cosmetic formulation for the prevention of premature skin aging. With respect to topical application of phytoantioxidants, the stratum corneum is a prime target for cosmetic formulators, as it requires the contribution of antioxidants to protect itself from the environment.

Phytoantioxidants neutralize UV-induced oxidation of the stratum corneum, provide protection from the environment, and in cosmetic products may stimulate the stratum corneum to regenerate. Phytoantioxidants are therefore to be used both topically and orally and should be integrated into any antiaging strategy (Pouillot *et al*., 2011).

To avoid unwanted skin effects of the sun, the use of sunscreen preparations became absolutely necessary.The effectiveness of Sunscreens is determined by sun protection factors (SPF), which is supposed to indicate the level of protection from UV radiation (Kale et *al*.,2011).Efficacy of sunscreen is defined as the ability to protect the skin against ultraviolet-induced burning, with the level of performance indicated by the sun protection factor (SPF). The SPF is a quantitative measurement of the effectiveness of a sunscreen formulation to be effective in preventing sunburn and other skin damage, a sunscreen product should have a wide range of absorbance (Chanchal *et al*., 2010). Higher SPF sunscreens offer greater protection from sunburn.

This work was undertaken to study the antioxidant property of *Helianthus annus* and *Coriandrum sativum*.

Various treatments were chosen to study the variation in antioxidant property and UV absorption so that enhancement in antioxidant property and SPF could be analyzed.

Materials and Methods:

Selection of plants

The herbs *Helianthus annus* and *Coriandrum sativum* have previously been reported to have antioxidant property and sunscreen activity were chosen for the present study.

1. *Helianthus annus*

Common name- Sun flower

Family- Asteraceae

Part used- Leaves

The antioxidant capacity of *Helianthus annus* was also much stronger than other sprout samples in terms of free radical scavenging and reducing properties (Wang et al., 2011).

2. *Coriandrum sativum*

   Common name- Coriander

   Family- Apiaceae

   Part used- Leaves

Coriander is highly reputed ayurvedic medicinal herb growing throughout India. Various parts of this plant such as seed, leaves, flower and fruit, possess diuretic, antioxidant activity, anti-diabetic, anti-convulsant activity, sedative hypnotic activity, anti-microbial activity, anti-mutagenic and anthelmintic activity (Nimish *et al*., 2011).

Preparation of soil

1. Control- The garden soil was prepared with soil, sand and manure in the ratio of 1:1:1.

2. Cocopeat- The garden soil was mixed with coco peat($1/4^{th}$ of garden soil).

3. *Pongamia*- The garden soil was mixed with shade dried *Pongamia* leaf powder($1/5^{th}$ of garden soil).

4. Spirulina- Initially the seeds were made to germinate in garden soil, after which Spirulina was added to 10 days old plants every alternate day.

Preparation of plant extract

The plants grown under different treatments were harvested and shade dried separately in-between folds of paper for 6 to 8 days. Dried materials were made into fine powder and used for the extraction of bioactive phytochemicals using ethanol, methanol and water as solvents.

Determination of *in vitro* antioxidant property (Mensor *et al,.2001)*

Different concentrations of plant extract both aqueous and ethanol was prepared and plated in ELISA reader plates at different concentrations with 100 micro litre of DPPH solution added to each and the antioxidant activity was measured using an ELISA reader.

The results were calculated using the formula:

$$\textbf{DPPH inhibition(\%)} = \frac{\textbf{control value-test value}}{\textbf{Control value}} \times 100$$

Preparation of extracts for to study their UV absorption

The extract of different plants 100 mg of the powdered plant material was mixed with 10 ml ethanol to give concentration of 10000 micro gram/litre. Then the concentration was used to study the absorbance using UV-VISIBLE spectrophotometer at 290 nm to 320 nm with ethanol as blank (Kaur *et al.*, 2011).

Sun Protection Factor (SPF) determination

The *in vitro* determination of SPF was done by method described by Mansur *et al*., (1986). The aqueous extract was prepared. Then the absorbance of the extracts was determined from 290 nm to 320 nm at every 5nm interval, using distilled water as blank. The results were calculated using the formula:

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

Where CF (Correction factor) is 10, EE(λ) is Erythmogenic effect of radiation with wavelength lambda, Abs(λ) is spectrophotometric absorbance values at wavelength lambda. The values of EE(λ)×I(λ) are constant. The obtained absorbance values are multiplied with EE(λ)×I(λ) and then their summation is taken and multiplied with correction factor to obtain the SPF values.The standard EE(λ)×I(λ) values are given below:

Wavelength (nm)	EE(λ)×I(λ) employed
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180

Preparation of herbal skin cream

The dried herbs were allowed to steep in 80ml of olive oil for three days and filtered through the cloth. Fresh *Aloe vera* pulp was boiled in low flame until all the stickiness goes off and blended in the mixer to get a soft gel (100ml).15grams of bee wax was melted in 20ml of olive oil. All the ingredients were blended in the mixer for about 10 minutes to get a creamy consistency. Few drops of rose oil was added for fragrance. A survey of the prepared cream was done to assess the spreadability, texture, consistency and fragrance.

Results

The results of the investigations carried out are given below.

Table 1: Antioxidant activity of Helianthus annus by DPPH method.

Sl.no.	Plant treatment	Aqueous extract(SUNFLOWER)		
		DPPH Inhibition		
		5µl	10µl	15µl
1	Control	-3.672	0.816	-8.631
2	Spirulina	-5.589	3.918	9.104
3	Coco peat	1.503	2.577	-12.641
4	Pongamia	3.373	-1.658	-3.144

Figure 1 : Antioxidant activity of Helianthus annus by DPPH method.

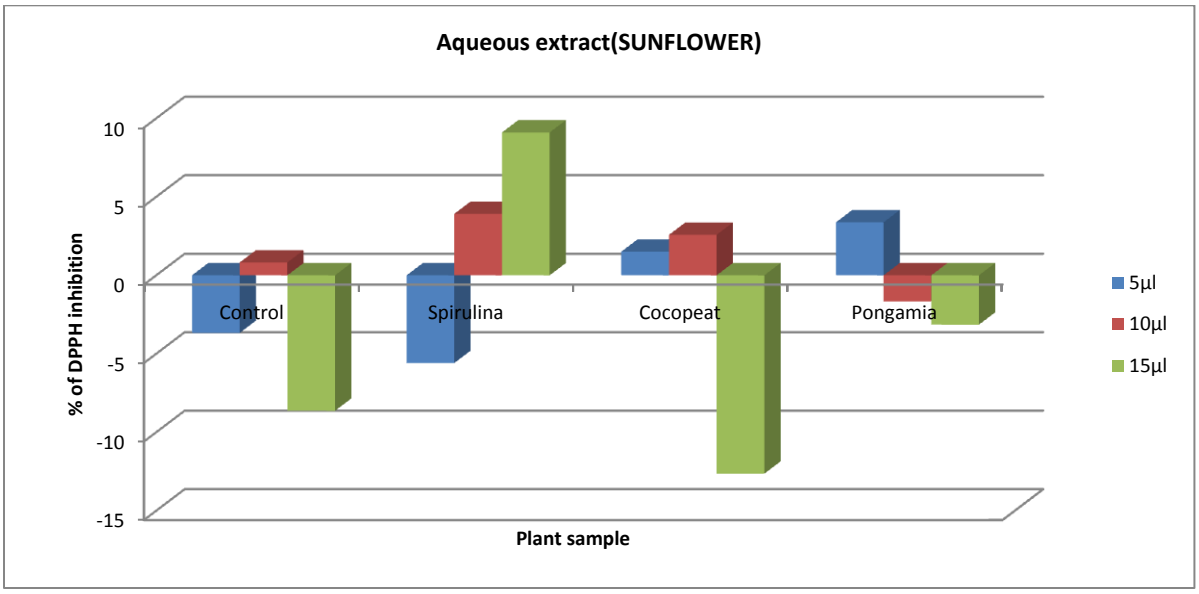


Table 2 : Antioxidant activity of Helianthus annus by DPPH method.

Sl.no.	**Plant treatment**	**Methanolic extract(SUNFLOWER)**		
		DPPH Inhibition		
		5µl	10µl	15µl
1	Control	-6.517	5.470	7.713
2	Spirulina	-2.480	1.881	-7.354
3	Coco peat	-3.340	3.3486	-0.937
4	*Pongamia*	-0.252	2.668	6.123

Figure 2 : Antioxidant activity of Helianthus annus by DPPH method.

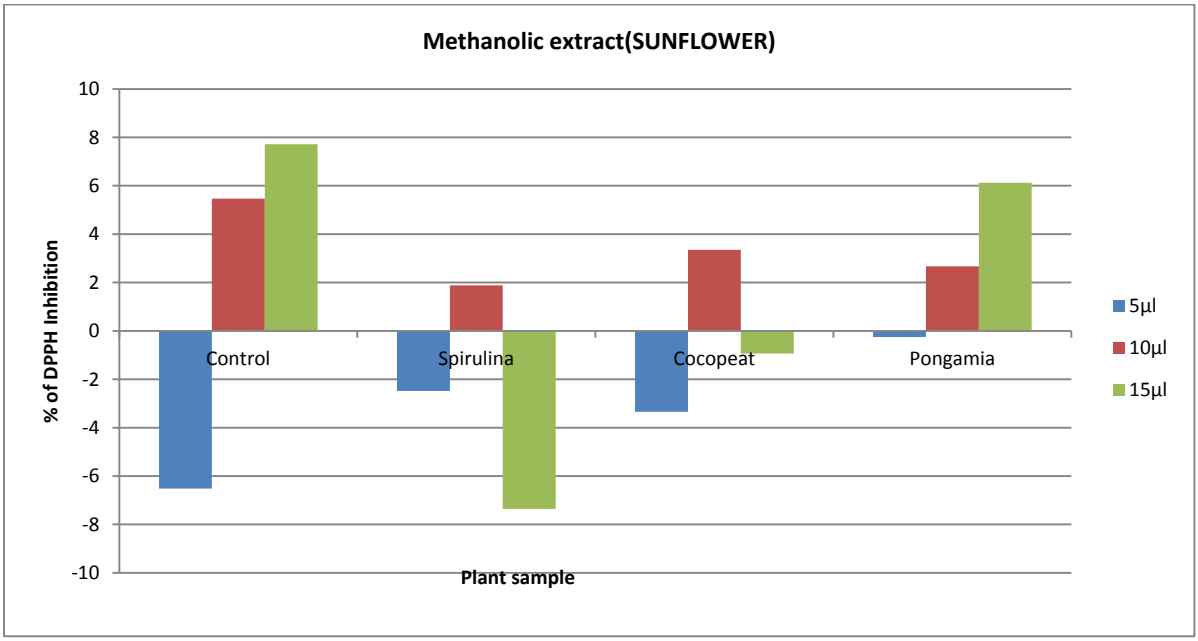


Table 3 : UV absorption of ethanolic extract of Helianthus annus.

Sl.no.	Plant sample	Wavelength in nm(SUNFLOWER)						
		290	295	300	305	310	315	320
1	Control	1.7422	1.7292	1.7510	1.7623	1.7987	1.8531	1.9262
2	Spirulina	1.9462	1.9287	1.9532	1.9702	2.0082	2.0781	2.1571
3	Coco peat	2.1072	2.0892	2.0941	2.1315	2.1650	2.2367	2.3091
4	*Pongamia*	1.9641	1.9580	1.9817	1.9946	2.0447	2.0957	2.1868

Figure 3 : UV absorption of ethanolic extract of Helianthus annus.

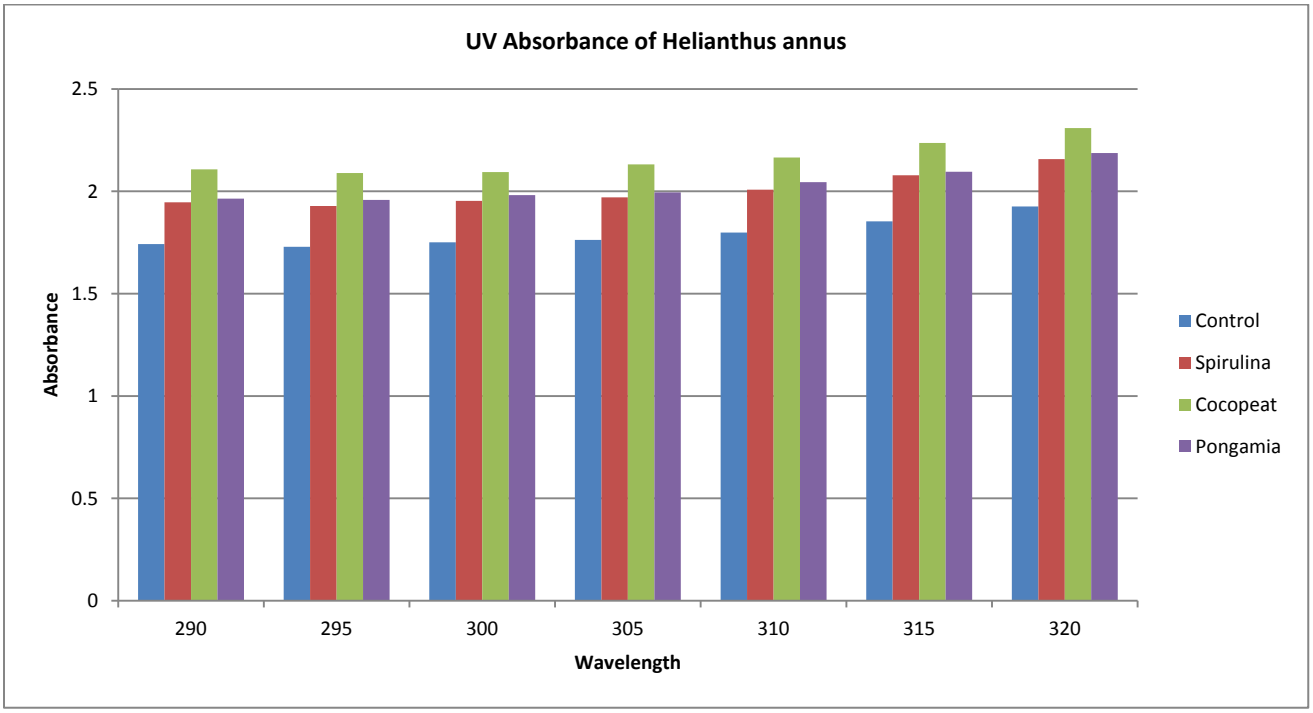


Table 4 : SPF Values Of Helianthus annus.

Sl.no.	**Plant sample** **(Helianthus annus)**	**SPF**
1	Control	17.73
2	Spirulina	19.807
3	Coco peat	21.3496
4	*Pongamia*	23.627

Figure 4 : SPF Values Of Helianthus annus.

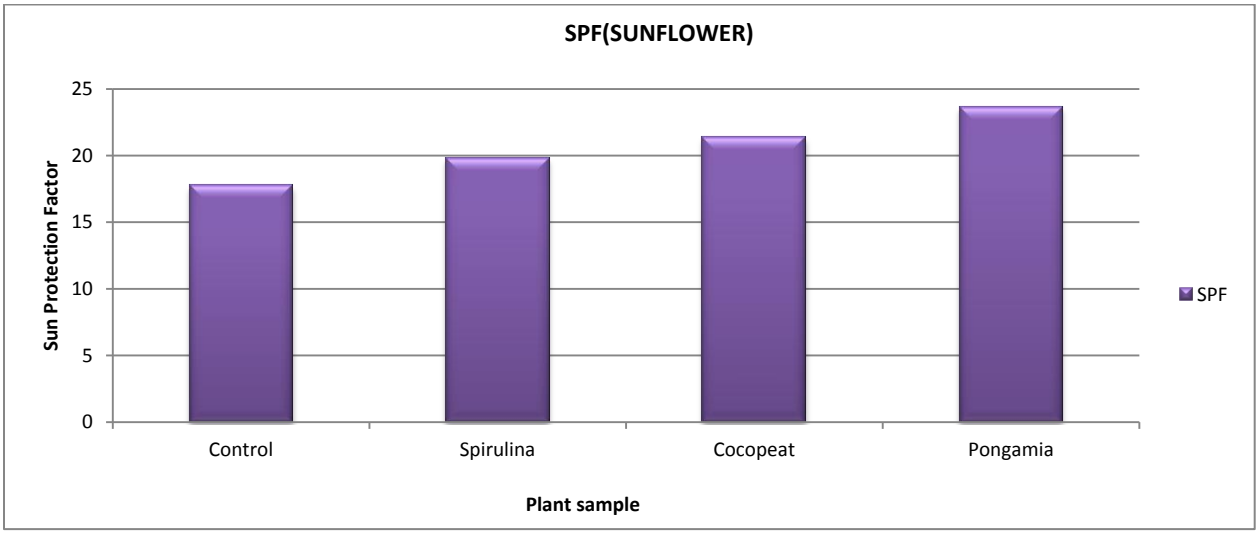


Table 5 : Antioxidant activity of Coriandrum sativum by DPPH method.

Sl.no.	**Plant treatment**	**Aqueous extract(CORIANDER)**		
		DPPH Inhibition		
		5µl	10µl	15µl
1	Control	-7.084	-3.216	-6.947
2	Spirulina	-1.117	2.45	-5.683
3	Coco peat	-7.538	-6.132	6.521
4	*Pongamia*	-5.254	-17.75	-11.784

Figure 5 : Antioxidant activity of Coriandrum sativum by DPPH method.

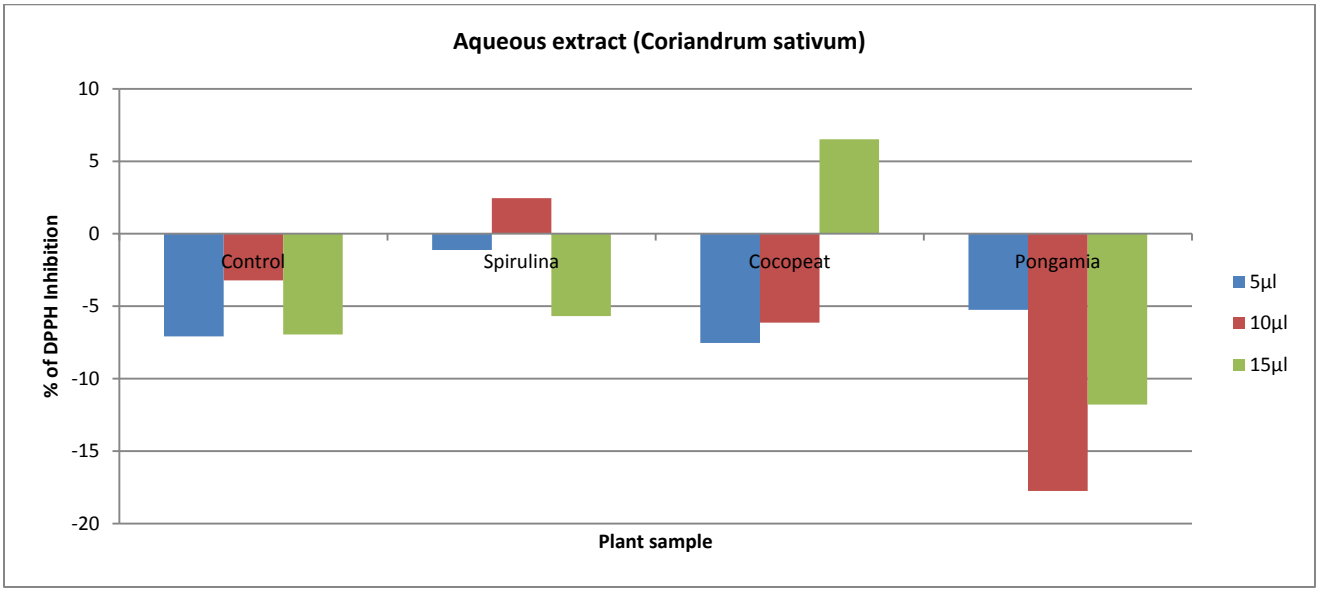


Table 6 : Antioxidant activity of Coriandrum sativum by DPPH method.

Sl.no.	Plant treatment	Methanolic extract(CORIANDER)		
		DPPH Inhibition		
		5µl	10µl	15µl
1	Control	-6.8	2.712	2.586
2	Spirulina	-0.153	-1.8711	-0.0708
3	Coco peat	-2.542	-2.678	-0.379
4	Pongamia	-14.056	-21.99	-0.22

Figure 6 : Antioxidant activity of Coriandrum sativum by DPPH method.

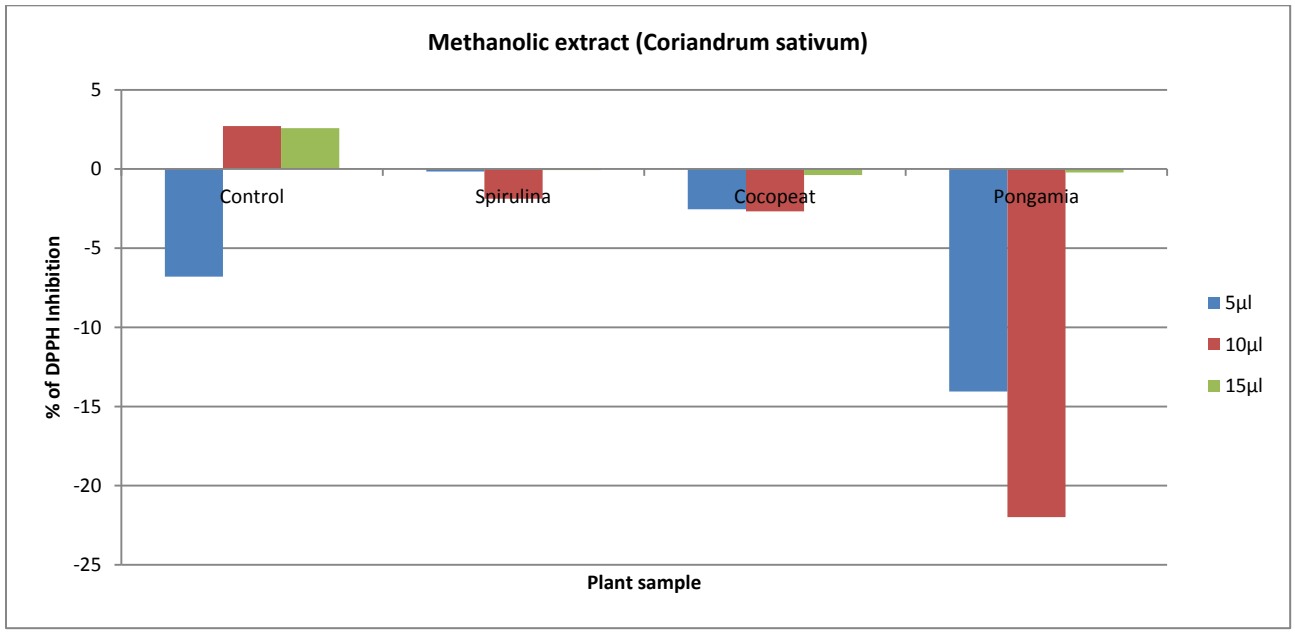


Table 7 : UV absorption of ethanolic extract of Coriandrum sativum.

Sl.no.	Plant sample	Wavelength in nm (CORIANDER)						
		290	295	300	305	310	315	320
1	Control	1.7153	1.7145	1.7201	1.6910	1.6976	1.6787	1.6877
2	Spirulina	3.9999	3.9999	3.9781	3.9999	3.9781	3.8339	3.9999
3	Coco peat	2.3761	2.9509	2.5156	2.5151	2.5713	2.4553	2.3234
4	*Pongamia*	3.3142	3.5519	3.9565	3.999	3.7620	3.9030	3.7180

Figure 7 : UV absorption of ethanolic extract of Coriandrum sativum.

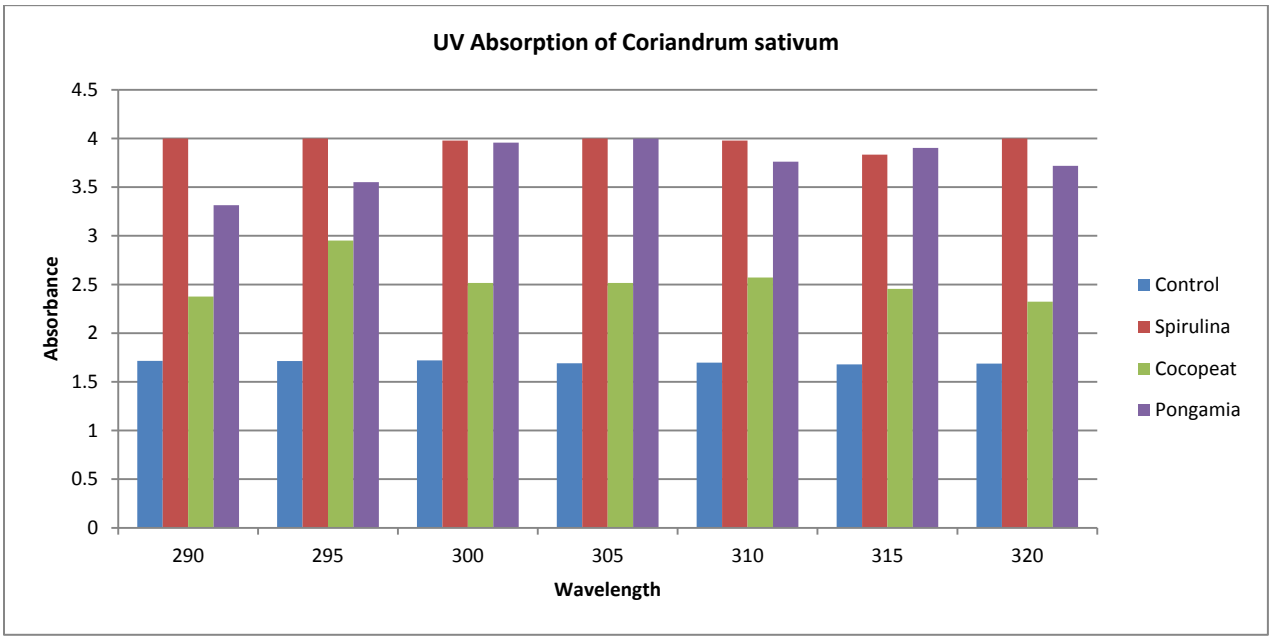


Table 8 : SPF Values of Coriandrum sativum.

Sl.no.	**Plant sample** ***(Coriandrum sativum)***	**SPF**
1	Control	17.004
2	Spirulina	39.763
3	Coco peat	25.096
4	*Pongamia*	38.826

Figure 8 : SPF Values of Coriandrum sativum.

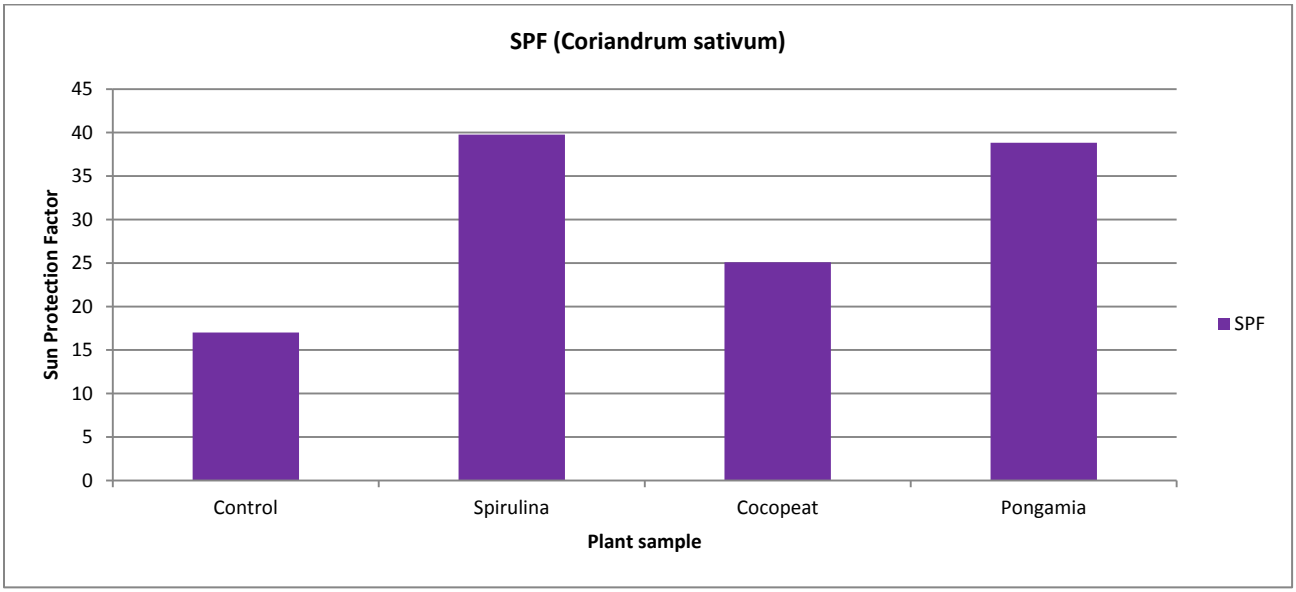
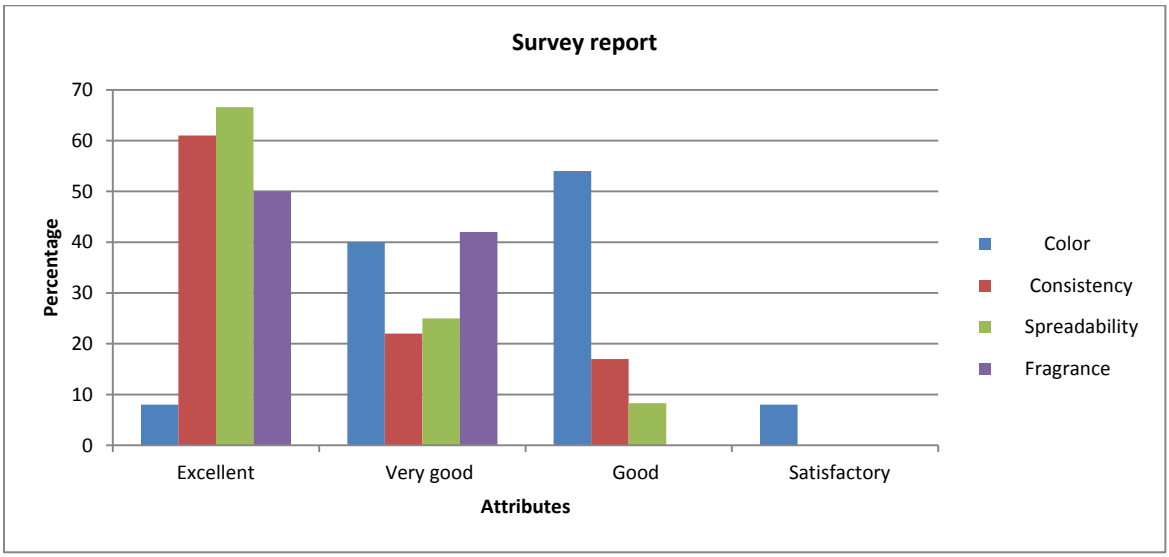


Table 9 : Survey of skin Cream.

Attributes	Percentage(%)			
	Excellent	Very good	Good	Satisfactory
Color	8	40	54	8
Consistency	61	22	17	0
Spreadability	66.6	25	8.3	0
Fragrance	50	42	0	0

Figure 9 : Survey of skin Cream



Discussion

The significance of the results are discussed below.

Antioxidant activity in aqueous extract

In *Helianthus*, Spirulina treatment showed an increased antioxidant activity in 15µL concentration followed by 10 µL (Table 1, Fig 1).

But *Coriandrum* did not show any significant results except in coco peat treatment 15 µL (Table 5, Fig 5)

Antioxidant activity in methanolic extract

Antioxidant activity of *Helianthus* was not significant in Spirulina and coco peat treatments, but *Pongamia* 15µL concentration showed comparatively more activity (Table 2, Fig 2). In *Coriandrum* there was no significant results seen.

UV absorption

The UV absorption in *Helianthus* was very much enhanced in all the three treatments when compared to control, the best being in coco peat followed by *Pongamia* and Spirulina (Table 3, Fig 3). In *Coriandrum* the maximum absorption was seen in Spirulina treatment followed by coco peat treatment (Table 7, Fig 7).

Sun Protection Factor(SPF)

There was an increase in the SPF values in *Helianthus* with all the treatments, the highest was in *Pongamia* treatment followed by coco peat and Spirulina (Table 4, Fig 4). In *Coriandrum*, Spirulina treatment showed maximum SPF value followed by *Pongamia* and coco peat treatments compared to control values (Table 8, Fig 8).

Survey of cream

The cream had very good rating with regard to all the parameters like consistency, spreadability and fragrance. The survey also showed there is a slight scope for improvement in colour (Table 9, Fig 9).

Conclusion

The present study revealed that *Coriandrum sativum* and *Helianthus annus* had good sunscreen activity, hence can be considered as sunscreen agents or can be incorporated into other sunscreen formulations and the SPF values were also increased with all the treatments when compared to control. The proposed UV spectrophotometric method is simple, rapid, employs low cost reagents and can be used in the *in vitro* determination of SPF values in plant extract and in many cosmetic formulations. These herbs showed good antioxidant activity, UV absorption and SPF. The sunscreen cream which was prepared incorporating these herbs had excellent feed back in terms of consistency, spreadability and fragrance.

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