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Spectrophotometric Determination of Sun Protection Factor and Antioxidant Potential of an Herbal Mixture

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Authors' contributions

This work was carried out in collaboration between both authors. Author VS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MS managed the analyses of the study. Author VS managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Objectives: Present research work deals with the determination of hydroxyl radical scavenging potential and Sun Protection Factor of herbal mixture which was prepared by the phytochemical composition of different herbal extracts.
Materials and Methods: The herbal mixture was prepared by the composition of important herbal plant extracts such as; 50% ethanolic extract of *Berberis aristata* root, 30% ethanolic extract of *Ficus benghalensis* bark, ethanolic extract of *Asparagus racemosus* root, aqueous extract of *Asparagus racemosus* root, 30% methanolic extract of *Butea monosperma* flowers, gel extract of *Aloe vera,* 80% ethanolic extract of *Terminalia arjuna* bark, 80% ethanolic extract of *Cyperus rotundus* root, 80% ethanolic extract of *Rubia cordifolia* root and 50% methanolic extract of *Hibiscus-rosa-sinensis* flowers, which was further proceed for hydroxyl radical scavenging activity and Sun Protection Factor determination at different concentrations *viz;*0.5%, 1%, 5% and 10%.
Results: IC_{50} values of Ascorbic acid was found to be 52.93±2.64% (Inhibition TBARS) at the concentration of 18.00 µg/ml and herbal mixture were 51.58±1.27% (Inhibition TBARS) observed at

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the concentration of 9.80 µg/ml respectively. The SPF values for different concentrations of herbal mixture were in between 2.14±0.15SPF to 12.97±0.07SPF. The results showed that 10% concentrated of herbal mixture has high SPF value of 12.97±0.07SPF which may be attributed due to the presence of active components.

Conclusion: Herbs and herbal preparations have high potential due to their antioxidant activity, primarily. The bioactive compounds such as flavonoids, phenolic acids, saponins etc. of this prepared herbal mixture may able to reduce skin damages which are caused due to long time exposure of skin in sun rays specially UVA and UVB rays. The proposed spectrophotometric method is simple and rapid for the *in vitro* determination of SPF values of sunscreens emulsions.

Keywords: Antioxidants; herbal mixture; skin; spectrophotometer; sun protection factor.

1. INTRODUCTION

The skin is the largest organ of the human body. It provides a major anatomical barrier between the internal and external environment. The body is constantly exposed to an array of chemical and physical exogenous pollutants [1]. 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) [2]. 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 dimers. 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 involved in 65% damage of all skin [3]. On the basis of research it seems that, the main destroying factors for skin are oxygenated molecules which are often call free radicals such as; superoxide anions (O_2.-), hydroxyl radical (.OH), singlet oxygen, hydrogen peroxide (H_2O_2), ferric ion, nitric oxide (NO) etc. The diseases associated with the ROS (Reactive oxygen species)/free radicals mainly depend on the balance of the pro-oxidant and the antioxidant concentration in the body. 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. In a research aromatherapy has proved that, the topical supplementation of antioxidants has been shown to affect the antioxidant network in the skin, applying aromatherapy formulations that are rich in antioxidants offers interesting avenues for future research [4]. 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) [5]. 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 [6]. 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. In this chain of research we have undertaken various parts of some important herbal plants such as; *Berberis aristata* (Family: *Berberidaceae*) commonly known as Daru-Haldi, *Ficus benghalensis* (Family: *Moraceae*) and commonly known as Banyan Tree, *Asparagus racemosus* (Family: *Asparagaceae*) commonly known as Satavari, *Butea monosperma* (Family: *Fabaceae*) commonly known as Palash or Flame of Forest, *Aloe vera* (Family: *Xanthorrhoeaceae*), *Terminalia arjuna* (Family: *Combretaceae*) commonly known as Arjun Tree, *Cyperus rotundus* (Family: *Cyperacea*) commonly known as Nagarmotha, *Rubia cordifolia* (Family: *Rubiaceae*) commonly known as Manjeeshtha and *Hibiscus-rosa-sinensis*(Family: Malvaceae) commonly known as China Rose. These plants are well known for their therapeutic potential and have proved their efficacy against various diseases especially skin related problems. In this research we have prepared an herbal mixture which was further evaluated for hydroxyl radical scavenging activity and Sun Protection Factor using *in vitro* tests.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Herbal Plants

Fresh plant parts were collected from Bhopal and forests of the Hoshangabad (M.P.) having rich diversity of medicinal plants by the permission of forest authorities. The Plant parts were identified by the Botanist at the department of Botany & Biotechnology, Sadhu Vaswani College, Bairagarh, Bhopal. Plant material was washed thoroughly and shade dried at room temperature. The material was crushed using mortar- pestle and grinding machine. Powders were stored at room temperature in airtight containers.

2.2 Reagents and Chemicals

One commercially available sunscreen lotion (SPF 20) was purchased from local market of Bhopal for the standard. Ethanol (Merck®) and other chemicals were analytical grade and purchased from CDH, Renchem, Hi-Media Ltd., India.

2.3 Extraction of Herbal Plants

The powder of *Berberis aristata* roots was subjected to 50% ethanolic extraction by soxhlet apparatus at 60℃ temperature and tagged as DH1. The bark powder of *Ficus benghalensis* was extracted with 30% ethanol at 80℃ by soxhlet apparatus and tagged as FB1. Coarsely powdered *Asparagus racemosus* root were extracted with ethanol and water. The part one extracted with ethanol by Maceration, tagged as ST1 and other part was extracted with water by percolation method, tagged as ST2. The flower powder of *Butea monosperma* was extracted with 30% methanol using a soxhlet unit at 70℃ temperature and tagged as PSE1. Fresh leaves of *Aloe vera* were collected, washed with tap water, peeled and gel was collected with the help of spatula in a beaker. Collected gel was dried at 40℃ in incubator. The dried sample was weighed and tagged as AV1. The bark powder of *Terminalia arjuna* was subjected for soxhlet extraction at 80℃ temperature using 80% ethanol as the solvent and labelled as AJ1. 80% ethanolic extract of *Cyperus rotundus* root powder was prepared by hot extraction process using soxhlet unit at 80℃ temperature and tagged as Ngm1. The 80% ethanolic extract of *Rubia cordifolia* root powder was prepared using soxhlet unit at 80℃ temperature and tagged as M2. The flower powder of *Hibiscus-rosa-sinensis* was extracted with 50% methanol by maceration process at room temperature and tagged as Cr2. Collected residues were kept at 45℃ in water bath to concentrate it and finally transfer into hot air oven to dry it. Dried extracts were collected, weighed and kept into air tight containers for further use.

2.4 Preparation of Herbal Mixture

Herbal mixture was prepared by mixing different plant extracts at different Concentrations. The herbal extracts and their quantities were selected on the basis of presence of phytochemicals and inhibitory concentration values. Each herbal extract was previously screened for phytochemical analysis and antioxidant potential using standard methods of Harborne [7] and Halliwell et al. [8]. The present phytochemicals and IC_{50} values of each extract were observed and only final standardized data has been summarized in Table 1. Total of 10% herbal mixture was prepared which was dissolved in 50% ethanol. It is reported that maximum of 50% ethanol could be used in cosmetic preparation [9]. Hence solubility of extracts was detected taking 10% to 50% of ethanol in DDW. The maximum solubility was observed in hydroalcoholic (50ethanol:50DDW) solution.

2.5 Antioxidant Assay

Direct antioxidant activity and free/hydroxyl radical scavenging potential indirectly through stimulation of cellular antioxidant was tested for the prepared herbal mixture. *In vitro* Antioxidant activity of this herbal mixture was determined according to the De-oxyribose method (Fenton reaction) of Halliwell et al. [8]. The Fenton reaction was used to generate hydroxyl radicals in a test system and the free radical scavenging activity was determined by the degradation of deoxyribose. The hydroxyl radical attached deoxyribose and initiated a series of reaction that eventually resulted in the formation of thiobarbituric acid reaction substance (TBARS). The measurement of TBARS thus gives an index of free radical scavenging activity. The absorbance was measured at 532 nm. Ascorbic acid was used as positive control for comparison. The percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = (\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}) / \text{Absorbance}_{\text{Control}} \times 100$$

Table 1. Composition of various plant extracts

S. no.	Plant extracts	Quantity (in mg)	Phytochemical analysis	Antioxidant potential (IC_{50} Value)
1.	Hydroalcoholic extract of *Berberis aristata* root (DH1)	5 mg	Carbohydrates, Alkaloids, Tannins, Terpenoids, Flavonoids	67.8 µg/ ml
2.	30% ethanolic extract of *Ficus benghalensis* bark (FB1)	4 mg	Carbohydrates, Phenol, Tannins, Terpenoids	48.00 µg/ ml
3.	Ethanolic extract of *Asparagus racemosus* root (ST1)	17 mg	Carbohydrates, Phenol, Tannins, Terpenoids, Glycosides	38.00 µg/ ml
4.	Aqueous extract of *Asparagus racemosus* root (ST2)	12 mg	Carbohydrates, Phenol, Tannins, Terpenoids	45.50 µg/ ml
5.	30% methanolic extract of *Butea monosperma* flowers (PSE1)	2 mg	Carbohydrates, Tannins, Glycosides	17.60 µg/ ml
6.	Gel extract of *Aloe vera* (AV1)	25 mg	Carbohydrates, Tannins, Phenol	34.50 µg/ ml
7.	80% alcoholic extract of *Terminalia arjuna* bark (Aj1)	20 mg	Alkaloids, Glycosides, Tannins, Phenol, Terpenoids, Saponins	37.80 µg/ ml
8.	80% ethanolic extract of *Cyperus rotundus* root (Ngm1)	5 mg	Carbohydrates, Alkaloids, Glycosides, Tannin, Phenol, Flavonoids, Saponin	87.00 µg/ ml
9.	80% ethanolic extract of *Rubia cordifolia* root (M2)	6 mg	Carbohydrates, Alkaloids, Tannin, Phenol, Flavonoids	50.00 µg/ ml
10.	Hydroalcoholic extract of *Hibiscus-rosa-sinensis* flowers (Cr2)	4 mg	Carbohydrates, Glycosides, Tannin, Phenol	58.00 µg/ ml

2.6 Sun Protection Factor (SPF) Determination

The Sun Protection Factor of herbal mixture was determined which was prepared by the phytochemical combination of different extracts of herbal plants. Efficacy of herbal formulation as sunscreens was determined by *in-vitro* method using UV-Visible spectrophotometer [10,11]. Standard sunscreen lotion (SPF 20) was taken at the concentration of 0.5mg/50ml in 50% ethanol which gives the dilution of 1% w/v for the evaluation. Initial stock is prepared by taking 10% w/v of herbal mixture in ethanol and distilled water (50:50) solution. Then, from this stock other concentrations such as 0.5%, 1%, 5% and 10% in v/v were prepared for *in vitro* SPF determination study. Thereafter, absorbance values of each aliquot prepared were determined from 290-320 nm, 5 nm intervals, and taking 50% ethanol as blank using spectrophotometer. SPF was calculated by using the equation derived by Mansaur et al*.* [10] and Santos et al*.* [11]. EE (λ) × I (λ) values determined by Sayre et al*.* [12] used in below Equation (1). Each sample observed in triplicate.

$$SPF_{spectrophotometric} = CF_{290}\Sigma^{320}EE(\lambda) \times I(\lambda) \times A(\lambda) \quad (1)$$

Where, correction factor, CF=10, EE (λ)= erythemogenic effect of radiation of wavelength λ, I(λ)= intensity of solar light of wavelength λ, A (λ)= spectrophotometric absorbance values at wavelength λ.

Normalized product function used in the calculation of SPF [12]

Wavelength (λnm)	EE*X*I normalized
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180

2.7 Statistical Analysis

The experimental results were expressed as Mean ± SEM and Mean ± SD.

3. RESULTS

3.1 Antioxidant Activity of Prepared Herbal Mixture

The ability of prepared herbal mixture to scavenge OH' radical was assessed using the

Fenton reaction assay. The Hydroxyl radical attacked to de-oxyribose and initiated a series of reaction that eventually resulted in the formation of Thiobarbeturic acid reaction substances (TBARs). The stock solution of herbal mixture was prepared at different concentrations. Extent of hydroxyl radical scavenged was determined by decreased intensity of pink colored chromospheres in the form of IC_{50} values. Lower IC_{50} values represent higher Antioxidant activity. The dose dependent inhibitions of TBARS formation at different concentration of herbal mixture were ranging from 10 µg/ml to100 µg/ml. These values were compared to Ascorbic acid, which was used as positive control.

The graphs, % of Inhibition versus different concentration of herbal mixture were plotted against ascorbic acid. In which IC_{50} values of Ascorbic acid was found to be 18.00 µg/ml and herbal mixture were observed at 9.80 µg/ml respectively. Results are summarized in Table 2.

3.2 Sun Protection Factor (SPF) Determination

In this research the herbal mixture (prepared by the phytochemical combination of different extract of herbal plants) was evaluated for the Sun Protection Factor determination at different concentrations *viz.,* 0.5% to 10% by UV-spectrophotometry with comparison of standard sunscreen (SPF 20). The absorbance values at various wavelengths (λ nm) from 290-320 nm of both prepared herbal mixture and marketed sunscreen lotion are given in the Table 3. The SPF values of herbal mixture and marketed sunscreen lotion were calculated and presented in Table 4. It can be observed from Table 4 that the SPF values for different concentrations of herbal mixture were in between 2.14±0.15SPF to 12.97±0.07SPF. The results showed that 10% Concentration of herbal mixture having high SPF value as12.97±0.07SPF which may be attributed due to the presence of active components. The 0.5%, 1% and 5% concentrations of herbal mixture showed minimum SPF values as 2.14±0.15SPF, 4.18SPF±0.16 and 10.61±0.07SPF respectively. Hence the results suggest that, the Sun Protection Factor values of herbal mixture were increased at dose dependent manner. The marketed sunscreen lotion (SPF20) has shown SPF value as 17.11±0.63SPF in present study. Therefore, it was observed that the 10% concentration of herbal mixture has the best SPF value and a finding that will be helpful in the selection of herbal preparation in the formulation of sunscreen lotion.

4. DISCUSSION

Chronic exposure of human skin to solar ultraviolet (UV) radiation may cause several skin damages. These damages include sunburn, skin cancer and oxidative stress as well as

Table 2. *In vitro* antioxidant activity of prepared herbal mixture in the comparison of standard antioxidant

S. no.	Concentration (In µg/ml)	% of inhibition TBARS (in mean± SEM)	
		Ascorbic acid	Prepared herbal mixture
1.	10	27.84±2.54	51.58±1.27
2.	20	52.93±2.64	86.02±3.06
3.	30	64.39±1.63	101.97±5.78
4.	40	88.60±0.56	140.05±5.82
5.	50	96.99±3.45	158.14±4.40
6.	60	121.69±5.97	182.38±1.99
7.	70	140.30±3.74	187.36±1.99
8.	80	157.57±2.32	192.89±1.67
9.	90	170.08±2.34	203.95±1.63
10.	100	184.23±1.92	208.11±1.39

IC_{50} Values:

S. no.	% Of inhibition (TBARS)	Inhibitory concentration (µg/ ml)	% of inhibition TBARS (in mean± SEM)
1.	Ascorbic acid	18.00	52.93±2.64
2.	Prepared herbal mixture	9.80	51.58±1.27

Table 3. Absorbance of prepared herbal mixture at different concentrations (.5%, 1%, 5%, 10%) given below in comparison of standard sunscreen

S. no.	Wavelength (λnm)	EE *X*I Normalized Employed	Marketed sunscreen (SPF20)	HM (0.5% conc.)	HM (1% conc.)	HM (5% conc.)	HM (10% conc.)
1.	290	0.0150	0.963±0.08	0.273±0.03	0.532±0.03	1.003±0.01	1.799±0.01
2.	295	0.0817	1.119±0.07	0.236±0.01	0.449±0.02	1.034±0.01	1.537±0.01
3.	300	0.2874	1.148±0.08	0.239±0.03	0.447±0.03	1.054±0.01	1.480±0.01
4.	305	0.3278	1.194±0.08	0.210±0.02	0.413±0.02	1.066±0.01	1.290±0.01
5.	310	0.1864	1.235±0.09	0.217±0.04	0.387±0.02	1.070±0.01	1.045±0.01
6.	315	0.0837	1.267±0.10	0.207±0.04	0.367±0.02	1.069±0.01	1.004±0.01
7.	320	0.0180	1.342±0.13	0.212±0.03	0.353±0.02	1.085±0.01	0.942±0.01

Table 4. Spectrophotometrically calculated sun protection factor values of prepared herbal mixture in comparison of marketed sunscreen lotion

S. no.	Experimental samples	SPF values (in Mean±SD)
1.	Marketed Sunscreen (SPF20)	17.11±0.63
2.	0.5% Concentration of HM	2.14±0.15
3.	1%Concentration of HM	4.18±0.16
4.	5%Concentration of HM	10.61±0.07
5.	10%Concentration of HM	12.97±0.07

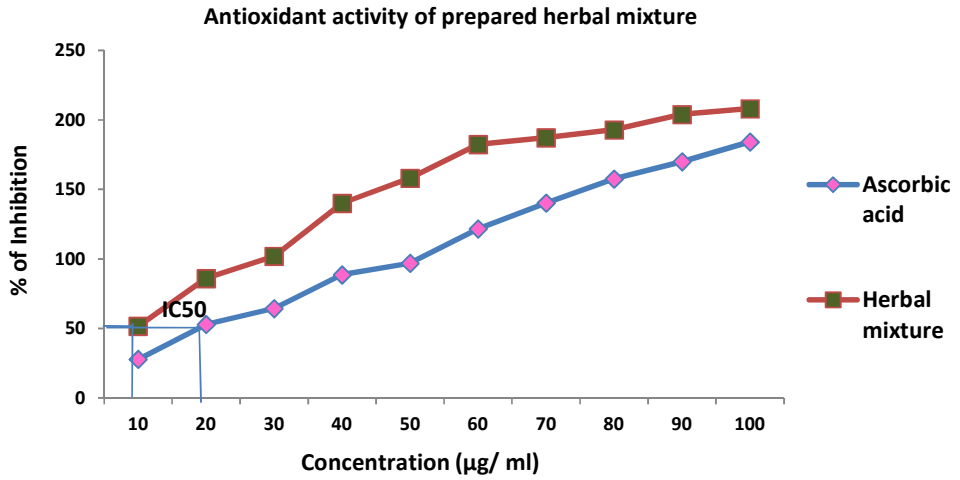

Fig. 1. Showing antioxidant activity of prepared herbal mixture in comparison of Ascorbic acid

photoaging depending on the amount and form of the UV radiation and on the type of the individual exposed [13]. Plant extracts, due to containing a wide range of natural compounds, usually cover full range of UV wavelengths. One approach to protecting the body from the harmful effects of UV irradiation is to use active photoprotectives. In recent years, naturally occurring compounds have gained considerable attention as protective agents [14]. Natural antioxidants present in herbs and spices are responsible for inhibiting the severe consequences of oxidative stress. Herbs and spices contain certain polyphenols, flavonoids and phenolics which possess radical scavenging activity [9]. The phenolics may be beneficial in preventing UV-induced oxygen free radical generation and lipid peroxidation, i.e. events involved in pathological states such as photo aging and skin cancer [15]. Antioxidant activity is important in UV protection [16]. 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, *viz.,* between 290-400 nm.

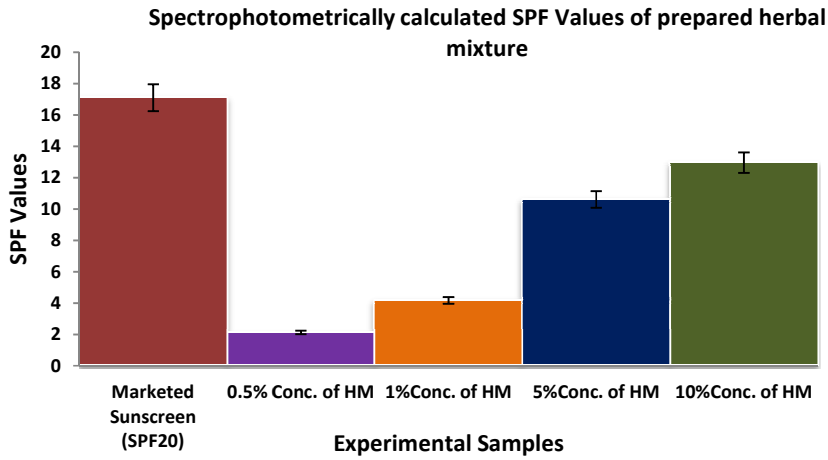

Fig. 2. Showing SPF values of prepared herbal mixture in comparison of marketed sunscreen

The efficacy of sunscreen is usually express by sun protection factor which is defined as "UV energy required producing minimal erythemal dose (MED) in protected skin divided by UV energy required to produce MED in unprotected skin". The *in vitro* screening methods may represent a fast and reasonable tool reducing the number of *in vivo* experiments and risks related to UV exposure of human subjects, when the technical test parameters are adjusted and optimized [17]. The *in vitro* methods are in general of two types: Methods that involve in the measurement of absorption or the transmission of UV radiation through sunscreen product films in quartz plates or bio-membranes, and methods in which the absorption characteristics of the sunscreen agents are determined based on spectrophotometric analysis of dilute solutions [18-21]. However, there are many factors affecting the determination of SPF values, like the use of different solvents in which the sunscreens are dissolved; the combination and concentration of the sunscreens; the type of emulsion; the effects and interactions of vehicle components, such as esters, emollients and emulsifiers used in the formulation; the interaction of the vehicle with the skin; the addition of other active ingredients; the pH system and the emulsion rheological properties, among other factors, which can increase or decrease UV absorption of each sunscreen [9]. However, the present research work revealed that combination prepared using different herbal extracts showed higher antioxidant activity at lower concentration as well as high SPF values in *in vitro* screening methods. First time this type of combination has been prepared for sunburn study and screened for preliminary investigation only. This research may give future scope for the formulation of herbal sunscreen products.

5. CONCLUSION

The proposed Spectrophotometric method is simple and rapid for the *in vitro* determination of SPF values of sunscreens emulsions. The study is helpful in selection of sunscreens formulations used in cosmetics with better safety and high SPF values. The *in vitro* SPF determination study is useful for screening test during product development. The plants studied here, those are used for the formulation of herbal remedy, may prove potential source of useful drugs which also justifies the traditional uses as medicines and the claims about their therapeutic values. These plant material contains various bioactive components specially Alkaloids, tannins, phenols, flavonoids etc. which have known ROS/hydroxyl radical scavenger and strong antioxidant potential. These antioxidants protect our skin from sunburn and skin cancer.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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