

Journal of Clinical and Biomedical Sciences

Original Article

Anti-solar, antioxidant and antihemolytic properties of a few selected medicinal plants: A preliminary study.

Priyanka Srinivasan¹, Mary Shobha Rani Inala², Nandhini Huthur Sriramaiah², Kiranmayee Pamidimukkala^{2*}

- 1. Department of Microbiology and Cell Biology, Indian Institute of Science, Bengaluru.
- 2. Department of Cell Biology and Molecular Genetics, Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar.

Abstract

Background: As the depletion of ozone layer is posing a serious problem on the existing environment, there is a need to develop agents for skin protection in order to avoid adverse conditions drawn by direct exposure to such harmful radiations.

Aim: The aim of the study is to identify the antisolar, antioxidant and anti-haemolytic properties from edible sources.

Methods and material: Using various solvents like water, methanol, ethanol, ethyl acetate, hexane and acetone, extracts were made and processed to study their sun protective capacity, antioxidant and antihemolytic property by spectrophotometric method. To evaluate Sun Protection Factor (SPF)/ antisolar activity, readings were taken at the corresponding nanometres. For total antioxidant activity, conducted phosphomolybdate assay and 2% Red Blood Cell (RBC) suspension in saline was used for antihemolytic activity.

Statistical analysis: ANOVA was performed to find out the significant difference between and among the groups and p value was calculated.

Results: Preliminary analysis established the highest photo-protectivity by onion leaves with significant antioxidant capacity and antihemolytic property followed by peppermint. The respective photo-protection was moderate to least with chilli and banana peel extracts respectively. All extracts showed significant antioxidant and antihemolytic activity.

Conclusion: The widely used edible plants have a well-defined photo-protective, antioxidant and antihmolytic properties.

Keywords: Antioxidant, anti-hemolytic, sun protection factor, Ultra Violet radiation.

Introduction

Sun Protection Factor (SPF), a scientific measure, is an important rationale in the sunscreen marketing. It represents in terms of number, for example 15, which means that, with sunscreen on, it gives 15 times longer protection to burn the skin than

*Corresponding Author Dr. Kiranmayee Pamidimukkala.

Department of Cell Biology and Molecular Genetics, Sri Devaraj Urs Medical College,

Sri Devaraj Urs Academy of Higher Education and Research. Tamaka. Kolar.

Mobile No: 7899346701

E-mail: kiranmayee@sduu.ac.in

Conflict of Interest: None

Financial Aid: Nil

without sunscreen application. The growth of commercially available sunscreen products is an indicative of peoples' awareness of solar radiation as one of the risk factors for skin cancer and other medical complications. The ultraviolet region of the electro-magnetic spectrum is the main source of harmful effects like photoaging and skin cancer. The three regions of an electromagnetic spectrum are: UltraViolet A (UVA), UltraViolet B (UVB) and UltraViolet C (UVC). UVA, the least powerful wavelength, starts between 320 and 400 nm of UltraViolet (UV) radiation. It is a long-wave, black light and not absorbed by the ozone layer. Primarily, in the skin, UVA acts to cause the melanin pigments to oxidize/darken. Though this creates the cosmetic tan, there is a limited influence to cause erythema.

Between 280 and 320 nm, starts the UVB region, primarily these wavelengths are associated with erythema (sunburn). For the production of vitamin D. skin needs UVB and stimulates the increased production of melanin. Specifically at 305 nm, UVB has about 1,000 times more erythema power when compared to UVA wavelengths. It is a medium wave, mostly absorbed by the ozone layer. Approximately between 200 and 280 nm, the germicidal UV in the electromagnetic region is the UVC. It is a short-wave, and totally captivated by the ozone layer in the atmosphere. Even a short exposure to UVC is very harmful, in particular, to the eyes and causes severe erythema (sunburn).^{1,2} Exposure to UV generates free radicals by oxidizing essential molecules in the skin inducing oxidative damage. Excess free radicles deteriorates the structure and function of cells as well as damages DNA inducing abnormal expression of cellular genes. Although skin holds antioxidant system to protect from harmful effects induced by UV radiations, the vast and uninterrupted exposure could over reach the antioxidant capacity of the skin.3 It would be impossible to lower the excessive exposure radiations: hence photochemoprevention/ photoprotection has been introduced to overcome the Photochemopreventive/photoprotective agents function by preventing the damage or modulating cellular responses caused by UV rays.4 To lessen the extent of UV radiation penetrating the skin, topical application of sunscreens with sun protective factor (SPF) value of 15 or greater is recommended to include in the cosmetics.5 Usage of chemical based products develops hypersensitivity, dermatitis, allergies, melanoma etc.6 Mixing of herbal agents in sunscreen products can better avoid these consequences. Extracts of plants, fruits and vegetables are capable to reduce oxidative damage, since they are rich sources of ascorbic acid, vitamin E and phenolics compounds.7

We have selected *Capsicum annum* L. (green chillies), belongs to Solanaceae, *Mentha piperita* L. (peppermint), belongs to Lamiaceae, *Allium cepa* L. (onion), belongs to Amaryllidaceae and *Musa acuminate* Colla (banana), belongs to Musaceae for our study. Though literature shows the antioxidant activity of these plants, the solvents used in this study for other activities, *viz.*, SPF and antihemolytic activity are the variants. The selected plant materials are rich with flavonoids and phenolics. We would like to understand whether these phytochemicals with potential antioxidant property have the capacity to absorb UV light as antisolar agents and at the same time do they have antihemolytic activity. The goal of the study is to assess:

- 1. The SPF to evaluate the antisolar activity of the extracts
- 2. Presence and absence of phytochemicals in the extracts
- 3. Antioxidant and antihemolytic activities
- 4. Correlation among the studied activities

Materials and methods

Reagents used were all analytical grade. Apparatus: PerkinElmer Lambda 35 UV-Visible spectrophotometer.

Extraction of plant materials

Plants for the study were acquired from the local market of Kolar area and validated by Horticulture College, Kolar. Running tap water was used for surface cleaning of the plant material and washed further with autoclaved double distilled water. Leaves of peppermint and onion; banana peel were finely chopped, seeds and skin of green chillies were separated, shade dried, powdered, sieved (12-20 mesh) and stored in air tight containers at room temperature until use. These materials were extracted by various solvents.

Methanol extract

About 1:10 ratios of the powdered *Capsicum annum* seeds and skin and *Mentha piperita* leaves were taken with methanol each in separate tubes and mixed, kept on shaker for one day at room temperature at 100 rpm. It was later clarified by centrifuging for half an hour at 5000 rpm, filtered the supernatant and left at room temperature for complete vaporization of the solvent.⁸

Hot water extract

About 1:16 ratio of *Capsicum annum* seeds and skin and *Mentha piperita* leaves were taken in separate tubes. During the extraction procedure, water was added to the powder, mixed and boiled to reduce the volume to one-fourth of its original volume. It was then cooled, strained, filtered through Whatman number 1 filter paper and then kept in hot air oven at 60°C in order to evaporate water content in it.8

Extracts of A. cepa with different solvents

About 10 g of dried powder of *Allium cepa* leaves were taken and mixed with 100 ml of solvents like ethanol, ethyl acetate and hexane in different tubes. The sample containing tubes were kept for maceration on orbital shaker for 48 h, filtered using Whatman number 1 filter paper and the filtrate was evaporated to dryness. Weight of the obtained dried extract was noted and used for analysis.⁹

Acetone extract of banana peel

Near about 20 g of banana peel powder was soaked in 100 ml of 70% acetone for 72 h followed by filtration. Filtrate was concentrated at 40° C in hot air oven until the evaporation of solvent.

Sample preparation to determine *in vitro* sun protection factor

From the dried extract, 1mg/mL stock solution, aliquot of $50~\mu L$, $100~and~150~\mu L$ were taken and by using respective solvents, the volume was made to one milliliter. Spectrophotometric (PerkinElmer Lambda 35~UV-Visible spectrophotometer) readings of the triplicate solutions were taken in wavelength ranging from 200~nm to 320~nm at 5~nm interval and the readings were noted and calculated by the following equation:

SPF = CF x
$$^{320}\Sigma_{290}$$
 EE (λ) x Abs (λ)

SPF was calculated by these values

EE (l) - erythemal effect spectrum; I (l) - solar intensity spectrum; Abs (l) - absorbance of sunscreen product; CF - correction factor (= 10). The values of EE x I are constants. They were determined by, and shown in Table 1.

Table 1: Normalized product function used in the calculation of Sun Protection Factor (SPF).

	` ,
Wavelength (λ nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

UV absorption capacity

Before proceeding with SPF values of the extracts, a rapid initial examination was carried out with all the water and methanol extracts. For this, 1mg/mL extract was made in respective solvent and Spectrophotometric (PerkinElmer Lambda 35 UV-Visible spectrophotometer) readings of all the triplicates were noted between 200 nm and 450 nm with 50 nm variation.

Phytochemical investigation

Phytochemical investigations of all the extracts were carried out by following the standard methods.^{11,12}

Total antioxidant capacity (Phosphomolybdate assay) (TCA)

The extracts were fractioned and dissolved (1mg/ml) in absolute respective solvents. Dilutions of each extract were made in the α -tocopherol 1 g/ mL stock solutions and checked for the total antioxidant capacity by phosphomolybdenum method. Diluted 0.1 mL extracts of each was shaken with 1 mL of phosphomolybdate reagent (0.6M sulphuric acid, 28 mM sodium phosphate, and 4mM ammonium molybdate) and incubated for one hour thirty minutes at 90°C, brought the samples to room temperature and measured absorbance at 695 nm with reagent alone as blank. The antioxidant capacity (total) was expressed in mM α -tocopherol acetate equivalent/g dry mass. 14

Antihemolytic activity

After obtaining ethics clearance from the institute (DMC/KLR/IEC/74/2017-'18), rejected blood samples were collected from Central Laboratory of R.L. Jalappa Hospital and Research Center, Kolar. The anti-hemolytic properties of extracts were measured by the spectrophotometry. 15 Collected blood samples in a heparin tube were centrifuged for 5 min at 1500 rpm and processed to obtain 2 % erythrocyte suspension. After discarding the supernatant, the obtained pellet (erythrocytes) was further washed twice with PBS by centrifuging after each wash. The washed pellet was re-suspended in 0.5% of the saline. The stock solution of 1mg/ml of plant extracts were made from which lowest concentrations were made. Extracts and 0.5% RBC were mixed carefully at 1:1 ratio and kept undisturbed for 30 min at 37°C. Then the incubated samples were centrifuged for 10 min at 1500 rpm and the absorbance of the supernatant was measured at 540 nm by spectrophotometer. The percentage of hemolysis was assessed with the hemolysis by H₂O₂ (Hydrogen peroxide) treated and PBS treated and were considered as negative and positive controls respectively. Inhibitory activity of different extracts were calculated and expressed in percentage.

The per cent hemolysis was calculated by using the following formula:

At: absorbance of the test sample
An: absorbance of the control (Positive control)
Ac: absorbance of the control (Negative control)
%Protection=100-(Hemolysis)

Statistical analysis

ANOVA was performed to know the significant difference between and among the groups and *p* value was calculated.

Results and Discussion Phytochemical investigation

The phytochemical tests provided the profiles of phytochemicals. Methanol and hot water extracts of Capsicum skin showed the presence of flavonoids, terpenoids, alkaloids, saponins, glycosides, phenolics, tannins, whereas, Capsicum seeds with methanol showed the presence of flavonoids, alkaloids, saponins, phenolics, tannins, and phytosterols; hot water extract contained alkaloids, saponins, phenolics and tannins.

Acetone extract of banana peel power contained flavonoids, terpenoids, alkaloids, saponins, glycosides, phenolics, tannins, reducing sugars, carbohydrates, proteins, steroids, anthraquinones and phytosterols.

Ethanol, ethyl acetate, hexane of onion leaf powder contained flavonoids, saponins, glycosides, phenolics, tannins and carbohydrates.

Phytochemicals of the plants are mainly involved in medicinal properties. The presence and absence of these phytochemicals depend on solvent system we used to isolate them. In this study, we used polar solvents (hexane, acetone, methanol, ethyl acetate, ethanol and water) to note the presence of

phytochemicals. The results are in agreement with the published data. $^{16,\,17}$

Though variety variation, phytochemicals were noted in all the extracts. Among them alkaloids, flavonoids and tannins have antioxidant property, whereas, saponins and terpenoids have antimicrobial activity.

UV absorption capacity

For wavelength determination and plant extract absorption, spectrophotometer scan with different wavelengths was performed. screening of UV absorption capacity of selected plant parts has been carried out from 200 nm to 450 nm with 50 nm interval. Table 2 shows the wide range of UV absorbance by various plant extracts of the mentioned nanometers. It is understood from the present study that UV absorbance exhibited by all the extracts were maximum at 200 nm and 250 nm except water extract of chilli skin coat i.e. 0.48 and 0.05 respectively and minimum at 450 nm with the least of 0.07 by water extract of chilli seeds. The highest absorption capacity (10) at 200 nm was found with chilli seed methanol, peppermint methanol and water, and onion ethanol extracts. With these finding, the study is further carried by calculating SPF of all the extracts of the study. In any analysis, where spectrophotometry technique is involved, both the transmission measures and path length through the sample are greatly rely on sample preparation.¹⁸

Table 2: Initial screening of UV absorption of selected plant parts from 200 nm to 450 nm with 50 nm interval.

UV nm	Chilli seeds		Chilli seeds Chilli skin coat		Peppermint		0	Banana Peel		
	Methanol	Water	Methanol	Water	Methanol	Water	Hexane	Ethanol	Ethyl Acetate	70% Acetone
200	10	5.1	5.21	0.48	10	10	4.18	10	7.8	8.1
250	5.4	5.1	4.09	0.05	10	10	3.19	3.3	7.5	7.7
300	1.1	1	1.33	0.82	1.6	2.08	1.26	1.9	2.3	5.2
350	0.65	0.23	0.72	0.6	1	2	1.56	1.65	2.1	0.6
400	0.55	0.16	0.33	0.44	0.18	0.76	2.35	0.81	2.4	0.26
450	0.42	0.07	0.21	0.17	0.11	1.05	1.18	0.46	2.5	0.14

ANOVA was performed for all the groups and the noted p value (0.2718) was not significant.

SPF

SPF of the extracts was checked by UV-Visible spectroscopy and calculated using Mansur equation method. Each sample's transmittance was measured to assess the SPF value. Spectroscopic transmittance

measurements of any extract/compound depend on the sample preparation, solvents used and the quartz cuvettes. Determination of SPF of the extracts was carried by taking varied concentrations of the extracts from 290 nm to 320 nm at 5 nm interval. Our findings

Kiranmayee et al. Anti-solar, antioxidant and antihemolytic properties of a few selected medicinal plants

indicated that absorption increased with respect to concentration. The values of extracts were in the range of 0.475 to 7.03. The highest SPF value is found in ethanol extract of onion leaves followed by methanol extract of peppermint with the values of 2.2 and 2 respectively at its lowest concentration. At its highest concentration peppermint methanol extract showed maximum SPF followed by ethyl acetate extract of onion. Onion and peppermint extracts showed higher activity with smallest rate by methanol extract of chilli seed and 70% acetone extract of banana peel. The graphical representation of the SPF activity was provided in tables and figure 1 (Tables 3-6 and Figure 1). As the plant's crude or

solvent extracts contain a variety of natural compounds, they generally cover complete range of UV wavelengths. All the extracts of the present study showed the presence of phenolics and flavonoids. phytochemicals These might be preventing UV-radiation induced oxygen free radical generation and lipid peroxidation. The conviction is, the phenolics and flavonoids inhibit DNA damage. Antioxidant activity is essential in UV protection. From the study, it is indicative that the presence of phenolics and flavonoids and SPF are related, though varied activity among the extracts. The result is in agreement with.18

Table 3: SPF values of the chilli seeds and chilli skin coat extracts at different concentrations at UV absorption between 290 nm and 320 nm with 5 nm difference based on Mansur equation, [EE (1) - Erythemal Effect spectrum].

		Chilli seeds						Chilli skin coat					
nm	EE*I (Norm	Methanol			Water		Methanol			Water			
	alized	50 μg/ml	100 μg/ml	150 μg/ml	50 μg/ml	100 μg/ml	150 μg/ml	50 μg/ml	100 μg/ml	150 μg/ml	50 μg/ml	100 μg/ml	150 μg/ml
290	0.015	0.0129	0.0417	0.0626	0.0181	0.031	0.0418	0.0202	0.035	0.0613	0.0123	0.0183	0.0252
295	0.0817	0.0539	0.1087	0.1617	0.0988	0.1446	0.2279	0.0948	0.18	0.29	0.049	0.142	0.1454
300	0.2874	0.1494	0.2443	0.3736	0.2902	0.5374	0.8104	0.3	0.5776	0.9541	0.1896	0.362	0.5029
305	0.3278	0.1475	0.223	0.3475	0.331	0.5900	0.885	0.3278	0.63	1.059	0.2097	0.399	0.5507
310	0.1864	0.0746	0.112	0.1734	0.1808	0.3243	0.4809	0.1864	0.35	0.5983	0.1155	0.2255	0.303
315	0.0839	0.0310	0.0461	0.0722	0.0788	0.1417	0.2097	0.0839	0.157	0.271	0.052	0.0998	0.1342
320	0.018	0.0059	0.0086	0.014	0.016	0.0286	0.0426	0.0167	0.0327	0.0574	0.0113	0.0203	0.027
SPF	1	0.47±0.05	0.78±0.05	1.20±0.31	1.01±0.09	1.797±0.16	2.69±0.54	1.03±0.11	1.96±0.78	3.29±0.18	0.64±0.14	1.27±0.07	1.68±0.51

Figure 1: Sun protection factor activity of the extracts of the selected plants.

Concentrations: Blue bars ($50 \,\mu\text{g/mL}$); red bars ($100 \,\mu\text{g/mL}$) and green bars ($150 \,\mu\text{g/mL}$) CSM: Chilli seed methanol; CSW: Chilli seed water extract; CSM: Chilli skin methanol extract; CSW: Chilli skin water extract; OH: onion hot water; OE: onion ethanol; OEA: onion ethyl acetate; PMM: pepper mint methanol; PMW: pepper mint water; BA: banana acetone.

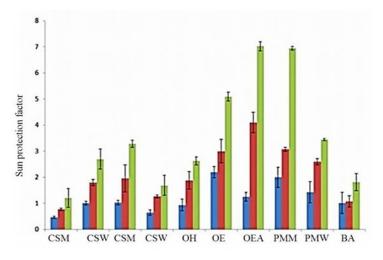


Table 4: Sun Protection Factor (SPF) values of the onion leaf extracts at different concentrations at UV absorption between 290 nm and 320 nm with 5 nm difference based on Mansur equation.

UV nm		Onion Leaves										
	EE*I (Norm alized)	Hexane			Ethanol			Ethyl Acetate				
		50 μg/ml	100 μg/ml	150 μg/ml	50 μg/ml	100 μg/ml	150 μg/ml	50 μg/ml	100 μg/ml	150 μg/ml		
290	0.015	0.0129	0.0279	0.0442	0.0316	0.0513	0.0786	0.01425	0.01005	0.0277		
295	0.0817	0.0801	0.1511	0.243	0.6634	0.267	0.4338	0.03268	0.2491	0.437		
300	0.2874	0.2758	0.523	0.848	0.4885	0.865	1.4657	0.2701	1.129	1.8278		
305	0.3278	0.3081	0.59	0.541	0.5474	0.97	1.6521	0.3933	1.3997	2.222		
310	0.1864	0.164	0.3112	0.503	0.3131	0.5536	0.948	0.2572	0.8332	1.2992		
315	0.0839	0.0738	0.14	0.227	0.1426	0.2525	0.4346	0.1384	0.4043	0.6141		
320	0.018	0.0158	0.137	0.224	0.031	0.0556	0.09486	0.148	0.0723	0.605		
SPF	1	0.94±0.28	1.88±0.45	2.63±0.20	2.2±0.30	3±0.62	5.1±0.21	1.25±0.21	4.1±0.55	7.03±0.24		

Table 5: Sun Protection Factor (SPF) values of the peppermint extracts at different concentrations at UV absorption between 290 nm and 320 nm with 5 nm difference based on Mansur equation.

UV nm		Peppermint								
	EE*I (Norm		Methano	l	Water					
	alized)	50 μg/ml	100 μg/ml	150 μg/ml	50 μg/ml	100 μg/ml	150 μg/ml			
290	0.015	0.0238	0.0568	0.0903	0.006	0.0375	0.0468			
295	0.0817	0.15114	0.3202	0.5065	0.629	0.2165	0.28			
300	0.2874	0.5805	0.865	1.819	0.2988	0.8047	0.8622			
305	0.3278	0.6687	0.9679	2.084	0.3605	0.9275	1.18			
310	0.1864	0.3951	0.5536	1.284	0.0212	0.356	0.6859			
315	0.0839	0.1879	0.2525	0.578	0.0998	0.2483	0.3196			
320	0.018	0.0423	0.0534	0.5913	0.02124	0.0054	0.0698			
SPF	1	2±0.54	3.07±0.11	6.95±0.093	1.43±0.56	2.6±0.16	3.44±0.056			

Table 6: Sun Protection Factor (SPF) values of banana peel extracts at different concentrations at UV absorption between 290 nm and 320 nm with 5 nm difference based on Mansur equation.

		Banana Peel							
UV nm	EE*I (Normalized)	70% Acetone							
		50 μg/ml	100 μg/ml	150 μg/ml					
290	0.015	0.0082	0.01425	0.0465					
295	0.0817	0.683	0.705	0.6862					
300	0.2874	0.1235	0.1235	0.5374					
305	0.3278	0.095	0.1147	0.25568					
310	0.1864	0.065	0.0738	0.1752					
315	0.0839	0.04	0.0394	0.0889					
320	0.018	0.003	0.01404	0.02376					
SPF	1	1.017±0.34	1.08±0.3	1.813±0.45					

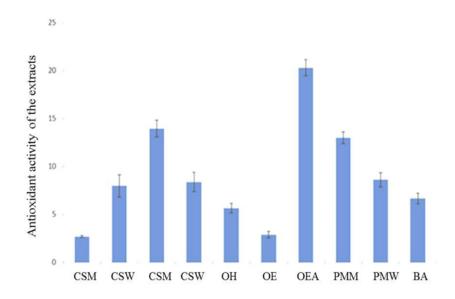
Antioxidant activity

Total antioxidant determination is a quantitative method based on the reduction of molybdenum (IV) to molybdenum (V) by the antioxidant action of extracts. From our findings, we noted that the ethyl acetate extract of onion revealed highest activity of 20.3 mg/mL followed by chilli coat methanol extract and peppermint with

the activity of 13.9 mg/mL and 13.03 mg/mL respectively. Antioxidant activity of water extracts lies approximately in the same range (8 mg/mL) with the least activity found in (methanol extract) chilli seed and (ethanol extract) onion. Remaining extract shows average activity ranging from 5-6 mg/mL (Figure 2).

Figure 2: Graphical presentation of antioxidant activity of plant extracts.

CSM: Chilli seed methanol; CSW: Chilli seed water extract; CSM: Chilli skin methanol extract; CSW: Chilli skin water extract; OH: onion hot water; OE: onion ethanol; OEA: onion ethyl acetate; PMM: pepper mint methanol; PMW: pepper mint water; BA: banana acetone.



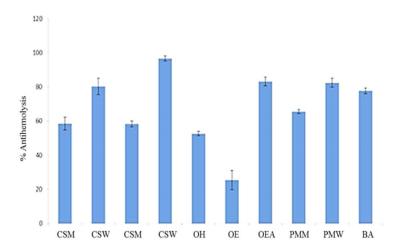
Antihemolytic activity

This experiment was aimed to determine anti-hemolytic effect of the selected plant extracts to prevent erythrocyte membrane damage by oxidation. Plant expressed hemolysis was represented as percentage hemolysis. The extracts of these plants displayed a differential pattern of anti-hemolytic activity. The observation was that the hemolysis induced in red blood cells were very minimal in most

of the extracts. Extracts prepared by polar, medium polar and non-polar solvents used in the present investigation were showing effective antihemolytic activity ranging between 75% and 96% followed by methanol extracts. Highest activity is observed in water extract of chilli coat and the lowest with ethanol extract of onion which are 96.6% and 25.4% respectively (Figure 3).

Figure 3: Per cent antihemolytic property of the extracts

CSM: Chilli seed methanol; CSW: Chilli seed water extract; CSM: Chilli skin methanol extract; CSW: Chilli skin water extract; OH: onion hot water; OE: onion ethanol; OEA: onion ethyl acetate; PMM: pepper mint methanol; PMW: pepper mint water; BA: banana acetone.



The powerful promoters of reactive oxygen species are the high concentrates of polyunsaturated fatty acids of the membranes and redox reactions of hemoglobin. Erythrocytes are the first targets for free radical attack and it is essential to protect them from these radicals by antioxidant agents. It is observed by²⁰, that flavonoids and phenolics of the plant extracts increase the half time of hemolysis by stabilizing the erythrocyte membrane. Searching for lipid peroxyl radicals play a role in antihemolytic activity. This will create a positive correlation between knowing antioxidant and antihemolytic activities of the extracts. Though, the extracts used in this study are non-toxic, harmless to the cells, further research is needed to prove that there is a strong correlation between antioxidant and antihemolytic activity of these extracts.

The Sun Protection Factor quantifies the protection that a product is capable to offer with respect to exposure time in relation to sunburn when compared to unprotected exposure. The effectiveness of any sunscreen formulations is a quantitative measurement. Any sunscreen product, which shows preventive action against sunburns or skin damages,

should have absorbance range of 290 nm - 400 nm. It has been observed that melanin equally contributes to filter all wavelengths of light. This reduces the UV radiation receiving capacity by five times. This protein provides photoprotection to a certain extent, minimizing phototoxicity and making the skin less prone to the acute and chronic phototoxic effects.²¹ on one side, UV radiation has a few benefits. We shall also be keen on its negative effect on human health. Nevertheless, skin shows the effects of photodamage in terms of pigmentation, wrinkling, sunburn and skin cancer is the serious result. UV is a strong physical mutagen. Its penetration into skin cells is likely to cause gene mutation and this is believed to be the first stage in skin cancer development.22 One of the approaches to shield the body from the harmful effects of UV irradiation is the use of active photo protectives. Plant extracts, due to containing a wide range of natural compounds, usually cover full range of UV wavelengths. 18 Ours and as an evident of these similar studies, common edible plants can also act as a photo protective agent. There is strong evidence that DNA-damaging UV light induces the accumulation of UV light-absorbing flavonoids and other phenolics in

dermal tissue of the plant body. The available and published literature in peer reviewed journals display that there is a strong correlation between SPF and phenolic compounds.²³ Recent research findings gave awareness and because of this, there has been an increasing interest in the use of antioxidants in sunscreens. This is to provide added photo protective action activity. Natural antioxidants from natural sources provide new possibilities for both treatment and prevention of UV-mediated diseases. Antioxidant activity is important in UV protection.24 From our studies it is shown that the extracts showing SPF and antisolar activity also show antioxidant activity. Apart from these findings, extracts also demonstrated the presence of antihemolytic properties by which these compounds might be used as pharmacologically important agents. Phenolic acids, flavonoids, lignins, anthocyanins, vitamin E and C are the natural plant antioxidants and there is relation between antioxidant, antihemolytic activities.25 Because of these, plants protect themselves from sun's damage).26 Equally, when we eat more and more of such compounds from plant origin, we get comparable action in our body. Antioxidants even at low concentrations delay or prevent oxidation of proteins, carbohydrates, lipids and DNA. Ayurveda rasayanas are non-toxic poly-herbal drugs with antioxidant properties. But in vitro investigations of a few phenolic compounds from plants have been mutagenic.

Plant based compounds are prospective sun protective resources as they have UV absorption qualities and antioxidant properties. In plants, there is an enormous amounts of phenolics and flavonoids in dermal tissue, which is epidermis including cuticle. Light when falls on the plant, the UV radiation induces the accumulation of these compounds. Peppers contain an antioxidant named as capsiate. When exposed to sun, capsiate decreases UVB-induced skin damage and also inhibits inflammation. Reports specify that onion has a high content of flavonoids like quercetin, thiosulphinates and it has been proven that they are antioxidants.²⁷ Banana peel has a wide array of phenolic and flavonoids (Ahmed).28 A wide distribution of flavonoids has been observed in peppermint leaves, seeds and flowers.²⁹ Therefore, it is the key concern of a formulator to develop sunscreens with high SPF and primarily, safety of the end-user. It is the prime responsibility not only to see the UV absorbance of the active compounds but also look into the vehicles like esters, emollients, emulsifiers and fragrances, which are used in the sun protection agent's formulations. The formulated sunscreens with any of these extracts might act as a good sunscreen agent. Sunscreens of natural origin

have a fewer side effects with additional beneficial effects of antiaging, antioxidant, antihemolytic and antimicrobial activity. This will be better and harmless substitute to harmful chemical/synthetic sunscreens that are in use now. To apply sunscreen topically is important, but to do that in conjunction with digesting particular foods that can boost the level of protection, or "eating your sunscreen," is the best known way to protect one's skin from the sun's rays overall.

Conclusions

From the study, we draw a conclusion that all the plant extracts showed promising results. Plant extracts have diversified characteristics to be an antioxidants, antisolar and antihemolytic. Peppermint showed better SPF and antisolar activity followed by onion and chilli extracts. Antioxidant activity and antihemolytic activity are considerably better in peppermint. Highest antihemolytic activity was observed in water extract of chilli coat and the lowest with ethanol extract of onion.

References

- 1. Dutra EA, Oliveira DAG da C, Kedor-Hackmann ERM, Santoro MIRM. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Rev Bra de Ciênc Farm 2004; 40(3):381–85.
- 2. Omar KA, Abdulrahman RS. Determination of Sun Protection Factor (SPF) of some sunscreens marketed in Kurdistan Region by UV-Visible spectrometery and study their Rheological properties. Inter J Pharm Chem 2015;5(2):40-44.
- 3. Katiyar SK, Elmets CA. Green tea polyphenolic antioxidants and skin photoprotection (Review). Int J Oncol 2001;18(6):1307–13.
- 4. Napagoda MT, Malkanthi BMAS, Abayawardana SAK, Qader MM, Jayasinghe L. Photoprotective potential in some medicinal plants used to treat skin diseases in Sri Lanka. BMC Complement Altern Med 2016;16:479-85.
- 5. Korać RR, Khambholja KM. Potential of herbs in skin protection from ultraviolet radiation. Pharmacogn Rev 2011; 5(10):164–73.
- 6. Chanchal D, Swarnlata S. Herbal Photoprotective Formulations and their Evaluation. The Open Natural Products J 2009; 2(1):71–76.
- Mahadik SU, Jagtap VN, Oswal H, Kumawat N. Kothari R. In vitro Antioxidant Activity of Ethanolic Extracts of *Celosia argentea* aerial parts, fresh fruits of *Fragaria vesca*, Tamarindus *indica, Psidium guajava, Zizyphus mauritiana*. Res J of Pharmacy and Technol 2011; 4(11):1782–84.
- 8. Azwanida NN. A Review on the Extraction

- Methods Use in Medicinal Plants, Principle, Strength and Limitation. Medicinal & Aromatic Plants 2015;4(3):1–6.
- 9. Niusha S, Shabnam M, Massoud A. Comparison of Different Methods in Quercetin Extraction from Leaves of *Raphanus sativus* L Pharma Sci 2016;23 (1):59–65.
- 10. Anokwuru CP, Anyasor GN, FakoyaO. Ajibaye O, Okebugwu. Effect of Extraction Solvents on Phenolic, Flavonoid and Antioxidant activities of Three Nigerian Medicinal plants. Nature and Science of Sleep 2011; 9(7):53-61.
- 11. Devi NN, Prabakaran JJ, Wahab F. Phytochemical analysis and enzyme analysis of endophytic fungi from *Centella asiatica*. Asian Pacific J Tropical Biomed 2012; 2(3), S1280-S84.
- 12. Harborne IB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.1998; 3rd ed. London, Chapman and Hall, 302.
- 13. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269(2):337–41.
- 14. Umamaheswari M, Chatterjee TK. In Vitro Antioxidant Activities of the Fractions of Coccinia *grandis* L. Leaf Extract. Afric J Tradit Complement Altern Med 2008; 5(1):61-73.
- 15. Kumar G, Karthik L, Rao KVB. Haemolytic activity of Indian medicinal plants toward human erythrocytes: an in vitro study. Applied Botany 2011; 40:5534-37.
- 16. Shivanand Bhat, Rajanna L. Preliminary phytochemical analysis and in vitro antioxidant potential of fruit stalk of *Capsicum annuum* var. Glabriusculum (Dunal) heiser & pickersgill. J Pharma Sci Res 2017; 9(8): 1283-87.
- 17. Kibria A A, Kamrunnessa M, Rahman M, Kar A. Extraction and evaluation of phytochemicals from banana peels (*Musa sapientum*) and banana plants (*Musa paradisiaca*). Malaysian J Halal Res J 2019; 2(1): 22-26.
- 18. Ebrahimzadeh MA, Enayatifard R, Khalili M, Ghaffarloo M, Saeedi M, Yazdani Charati J. Correlation between Sun Protection Factor and Antioxidant Activity, Phenol and Flavonoid Contents of some Medicinal Plants. Iran J Pharm Res 2014;13(3):1041-47.

- Nawaz H, Shad MA, Rehman N, Andaleeb H, Ullah N. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. Braz J Pharm Sci 2020; 56, e17129.
- Bouhlali ET, Bammou M, Sellam K, Benlyas M, Alem C, Filali-Zegzouti Y. Evaluation of antioxidant, antihemolytic and antibacterial potential of six Moroccan date fruit (*Phoenix dactylifera* L.) varieties. J King Saud Uni-Sci 2016; 28 (2), 136-142.
- 21. Kaidbey KH, Agin PP, Sayre RM, Kligman AM. Photoprotection by melanin--a comparison of black and Caucasian skin. J Am Acad Dermatol 1979; 1(3):249–60.
- 22. Matsumura Y, Ananthaswamy HN. Toxic effects of ultraviolet radiation on the skin. Toxicol Appl Pharmacol 2004; 195(3):298–08.
- 23. Yasmeen SH, Gupta PR. In vitro demonstration of *Dalbergia sissoo* (Indian rosewood) methanolic extracts as potential agents for sunscreening and DNA nick prevention. Int J Pharmacy Pharma Sci 2016; 8(6):175–81.
- 24. Ponnaree K, Panida B, Pimphaka W, Lakkhana P, Orathai P, Malyn C. Antimutagenic Potentials of Hydroalcoholic Herbal Extracts towards UV-Induced Mutation. Thai J Toxicol 2008; 23(1):27-34
- Skinner WA, Johnson HL, Ellis M. Parkhurst RM. Relation between Antioxidant and Antihemolytic Activities of Vitamin E Derivatives *In vitro*. J Pharmaceutical Sci 1971; 60 (4): 643-45.
- 26. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J et al. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. Int J Mol Sci 2017; 18(1): 96.
- 27. Griffiths G, Trueman L, Crowther T, Thomas B, Smith B. Onions a global benefit to health. Phytother Res 2002; 16(7):603–15.
- 28. Aboul-Enein AM, Salama ZA, Gaafar AA, Aly HF, Abou-Elella F, Ahmed HA. Identification of phenolic compounds from banana peel (*Musa paradaisica* L.) as antioxidant and antimicrobial agents. J Chem Pharma Res 2016; 8(4):46-55.
- 29. Naidu JR, Ismail RB, Yeng C, Sasidharan S, Kumar P. Chemical composition and antioxidant activity of the crude methanolic extracts of *Mentha spicata*. J Phytol 2012; 4(1): 13-18.