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Screening of *in vitro* sun protection factor of some medicinal plant extracts by ultraviolet spectroscopy method

Manisha Pralhad Sutar[1]*, Sanjay Ravindra Chaudhari[2]

[1]Department of Pharmacognosy, Amrutvahini Sheti and Shikshan Sansthas, Amrutvahini College of Pharmacy, Sangamner, Maharashtra, India, [2]Department of Pharmacognosy,Trinity College of Pharmacy, Pune, Maharashtra, India.

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ABSTRACT

The proposed work is aimed to investigate the ultraviolet (UV) light absorption capacity and sun protection factor (SPF) of selected plant extracts. Chemical agents are protecting against UV radiations but long exposure may cause allergies to the skin. The alternative is required to overcome such skin allergies, hyperpigmentation, sunburn, photoaging, and skin irritation. The plant products and their phytochemicals have recently been used for their antioxidant property, which can be used for UV absorbance ability and photoprotective property. The methanol extracts of flowers of *Butea monosperma*, leaves of *Neolamarckia cadamba*, peel of *Punica granatum* and leaves of *Cymbopogon citratus* were prepared by continuous Soxhlet extraction method and their UV absorbance was measured between 200 and 400 nm using UV spectrophotometer at 30µg/ml concentration. Further extracts were subjected to *in vitro* SPF determination at 20–40 µg/ml concentration by using the Mansur equation between 290 and 320 nm range using a spectrophotometer. All extracts showed UV absorbance capacity in 200–400 nm range and the *P. granatum* extracts having higher SPF. The SPF for *B. monosperma* flowers, *N. cadamba* leaves, *P. granatum*, and *C. citratus* leaves were found to be 2.1430 ± 0.0271, 2.2892 ± 0.0287, 4.1401 ± 0.0551, 0.8751 ± 0.0112 at 20 µg/ml, 2.3824 ± 0.0301, 2.3020 ± 0.0284, 4.3373 ± 0.0566, and 1.0940 ± 0.0140 at 30µg/ml and 2.5953 ± 0.0328, 2.7789 ± 0.0345, 6.0643 ± 0.0804, and 1.8765 ± 0.0239 at 40 µg/ml, respectively. The initial study proved the UV absorbing and Sun protecting capacity of selected plant extracts. This finding suggests that the extracts can be considered to develop photoprotective formulation in different combinations and proportions, which will provide an additive or synergetic effect. The proposed spectrophotometric method is simple, convenient and cost-effective for SPF determination.

1. INTRODUCTION

Solar ultraviolet (UV) radiation is divided into three regions, UV-C 290–200 nm, UV-B 320–290 nm, and UV-A 400–320 nm [1]. Scientific studies specified that exposure to solar radiation damages the skin in different ways. UV-C get filtered through stratospheric layer and not causing any more harmful effect on the skin. UV-B radiations are not completely filtered out by the ozone layer, are one thousand times more responsible to cause sunburn; DNA absorbs it and initiates carcinogenic processes. UV-A are long-wave UV-A or I (340–400 nm) and short-wave UV-A or II (320–340 nm). UV-A radiations produce immediate tanning effect and darkening of melanin in the epidermis. It causes premature photoaging, suppression of immunological functions and necrosis of endothelial cells. UV-A radiations reaches the deeper layers of the epidermis, dermis, and generates free radicals [2,3]. The oxygenated molecules such as superoxide anions, hydroxyl radical, singlet oxygen, hydrogen peroxide, ferric ion, and nitric oxide damage the skin. The diseases associated with the reactive oxygen species (ROSs) or free radicals mainly depend on the balance of the pro-oxidant and antioxidant concentration in the body [4]. Sunscreens are chemicals that protect against the adverse effects of solar radiation. Phytoconstituents extracted from plants have been recently considered as potential sunscreen resources because of their UV ray absorption capacity in the UV regions and their antioxidant property. Green tea polyphenols, aloe extract, aromatic compounds isolated from lichens, and glycosides are examples of natural substances evaluated for their sunscreen properties. There are reviews about the photoprotective effects of some naturally occurring herbal polyphenols and phenolic compound rich extracts in the skin damage induced by UV irradiation. Several studies have shown the flavonoids and phenolics act as free radical scavengers and enzyme inhibition causes oxidation [5]. Natural compounds act as catalysts in the light phase of photosynthesis and protect plant cells from scavenging ROS especially the antioxidants such as Vitamin C, Vitamin E, flavonoids, carotenoids, and phenolic acids [6].

Recent research focuses on the use of UV light absorbing property of plant extracts which are previously proved for their antioxidant activity

*Corresponding Author:
Manisha Pralhad Sutar,
Amrutvahini Sheti and Shikshan Sansthas, Amrutvahini College of Pharmacy, Sangamner, Maharashtra, India.
E-mail: manishasutar01@gmail.com

and the presence of polyphenolic compounds and their therapeutic values [7,8]. Butea flower known as Palash was studied for antioxidant activity by different *in vitro* assay [9] and also proven for the presence of flavonoid and phenolic content in ethanolic extract [10]. Ethanolic flower extract also proved for its sunscreen activity in a cream formulation [11]. *Neolamarckia* cadamba is also known as cadama plant having free radical scavenging property when studied for the methanolic extract of the leaf. It also shows the presence of phenolic compounds [12,13]. The plant bark with other plant extracts studied for its antioxidant assay [14]. The methanolic extract of the peel of *Punica granatum* possess better antioxidant activity, and phenolic content compared to aqueous and ether extract [15-17]. Ethanolic extract of *P. granatum* was investigated for its sunscreen activity [18]. The chloroform, methanol and water extract of *Cymbopogon citratus* leaf is shown potential *in vitro* antioxidant activity [19] and leaf shown the presence of flavonoids, tannins, and phenolic compounds [20].

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Methanol and petroleum ether (60–80°C) were of analytical grades and procured from Merck, India Ltd, Mumbai, India.

2.2. Collection and Authentication of Plants

The flowers of *B. monosperma* and fresh leaves of *N.* cadamba (Roxb.) were collected from Ahmedabad, Gujarat. The peels of *P. granatum* (Linn) and the fresh leaves of *C. citratus*, (Stapf) were collected from the medicinal plant garden of Alard College of Pharmacy, Pune. The plant specimens were identified at the Botanical Survey of India, Western Regional Centre, Pune. Specimen Voucher No. for *B. monosperma* (Lam.) family Fabaceae was BSI/WRC/IDEN. CER/2016/664, *N.* cadamba (Roxb.), family Rubiaceae was BSI / WRC/IDEN.CER. /2016/666, *P. granatum* (Linn), family Punicaceae was BSI/WRC/IDEN.CER/2016/665, and *C. citratus* (Stapf) family Gramineae was BSI/WRC/IDEN.CER. /2016/662.

2.3. Extraction of Plant Material

50 g of coarse powders were passed through a 40□mesh sieve, defatted with petroleum ether (60–80°C) and dried marc was extracted with 200 ml of methanol for 36 h using continuous hot extraction method in Soxhlet apparatus [21,22]. Extracts were concentrated under reduced pressure and at the temperature of 40°C using a rotary evaporator. The concentrated extracts were placed in the desiccator to remove the traces of the solvent. The percentage of yields were calculated and recorded. The extracts were named BM for methanolic extract of *B. monosperma,* NC for methanolic extract *N.* cadamba, PG for methanolic extract *P. granatum*, and CC for methanolic extract *C. citratus*.

Table 1: Normalized product function used in calculation of SPF

Sr. No	Wavelength (λ)	EE X I (normalized)
1	290	0.0150
2	295	0.0817
3	300	0.2874
4	305	0.3278
5	310	0.1864
6	315	0.0839
7	320	0.0180
Total		1

2.4. Spectra and Absorbance Measurement of Extracts in UV Region

Photoprotective are the chemicals that have specific absorbance in some parts of the UV spectrum and few have absorbance over the full range of UV. This property is important for any product to be considered as a sun protective agent. Plant extracts with a wide range of natural compounds usually cover UV range in 200–400 nm wavelength [23]. In recent years, naturally occurring compounds gaining attention as protective agents [24]. To examine the UV absorbing property of the plant extracts are required to expose to the UV region 200–400 nm using a spectrophotometer. The UV spectra were measured at 30 µg/ml concentration of each extract diluted with solvent methanol [25].

2.4.1. Sample preparation

10 mg of each extract dissolved in 100 ml of methanol to get 100 µg/ml of concentration, subjected to ultrasonication for 10 min and filtered through Whatman filter paper. 30 µg/ml concentration of each extract was scanned at over the wave length range from 200 nm to 400 nm using UV-visible a spectrophotometer (Make- JASCO and model V-630) in triplicate. The absorption spectra measured in 1 cm quartz cell using "Spectra Measurement" mode employing a reference cell containing methanol as pure solvent [26]. The absorption spectra and characteristic peaks of *B. monosperma* flower, *N.* cadamba leaves, *P. granatum peel,* and *C.* citratus leaves *extracts* were recorded.

2.5. Determination *In Vitro* Sun Protection Factor (SPF) of Extracts

There is an immense need to explore the sunburn protective properties of plants with proven medicinal value and a rich source of phytoconstituents. The efficacy of sunscreen agent is usually expressed by the SPF, which is defined as the UV energy required to produce a

Table 2: Absorption ability of extracts

Sr. No	Wavelength	Absorbance
BM extract		
1	371	1.037
2	314	0.681
3	269	1.012
4	228	1.339
NC extract		
1	326	0.315
2	309	0.294
3	284	0.435
4	264	0.253
PG extract		
1	367	0.206
2	340	0.173
3	258	1.389
4	217	2.056
CC extract		
1	386	0.119
2	279	0.620
3	265	0.576
4	212	1.663

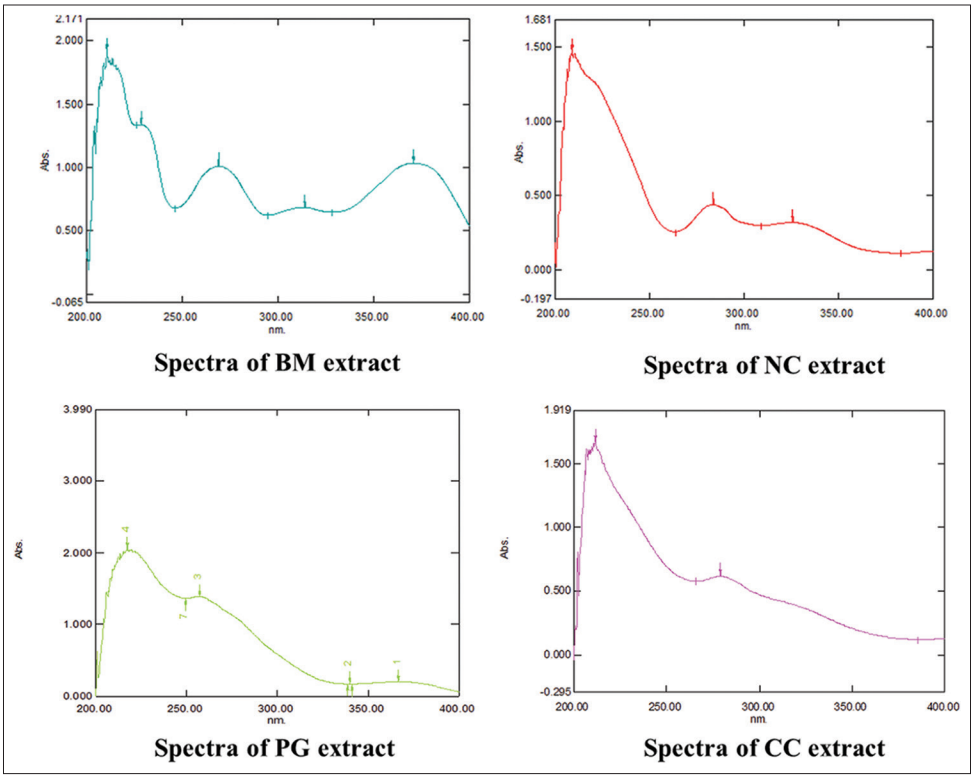

Figure 1: Spectra of selected plant extracts for ultraviolet absorbing capacity

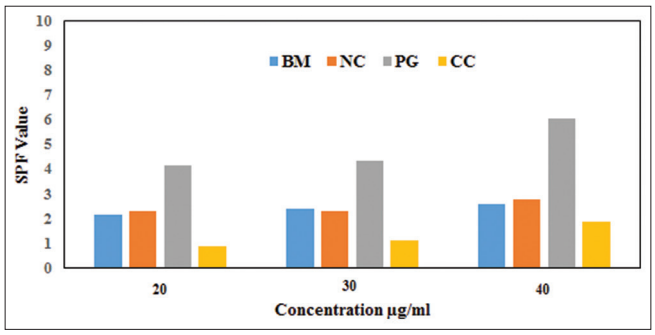

Figure 2: Graph for sun protection factor of plant extracts

minimal erythema dose (MED) on protected skin, divided by the UV energy required to produce a MED on unprotected skin [27,28].

$$SPF = \frac{\text{Minimal Erythemal Dose of Protected Skin}}{\text{Minimal Erythemal Dose of Unprotected Skin}} \quad (1)$$

It was reported that topical sunscreen formulations or extracts were exposed to UV radiation for their *in vivo* or *in vitro* protection determination. Human volunteers are used for *in vivo* determination but it is a time-consuming process and causing harm to skin [29,30]. *In vitro* analysis is the best solution for the SPF determination, using the measurement of absorption or the transmission of UV radiations. The extracts were further processed to determine the SPF [31].

The stock of 100 µg/ml of concentration was used to determine SPF of different concentrations (20 µg/ml, 30 µg/ml, and 40 µg/ml) and measured at 290–320 nm at 5 nm interval. The measurements were performed in triplicate for each concentration using 1 cm quartz cell and methanol was used as blank. A very simple mathematical equation was developed by Mansur *et al* which substitutes the in vitro method proposed by Sayre *et al* [32] by using a UV spectrophotometer [33]. Given formula was used to calculate the SPF,

The Equation is,

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

Where, CF = Correction Factor (10)

EE (λ) = Erythrogenic Effect of radiation

I (λ) = Solar Intensity spectrum

Abs (λ) = Spectrophotometric absorbance value.

The values of EE x I are constants. The results are shown in Table 1.

3. RESULTS AND DISCUSSION

3.1. Extraction of Plant Material

Percentage of yield for BM, NC, PG, and CC extract was found to be 2.2, 1.9, 5.7, and 1.7 % w/v, respectively. PG extract shown higher % of yield compared to other plant extracts.

3.2. Spectra and Absorbance Measurement of Extracts in UV Region

All extracts were subjected to spectra and absorbance measurement in the UV region which will be the indication of the UV absorbing or transmitting property of the extracts. The spectra and wavelength of 30 µg/ml of each solution were studied and maximum absorbance spectra with respective wavelengths were noted. All the extract exhibited significant absorption in the whole UV range (UV-A,

Table 3: Determination of *in vitro* SPF at 20 µg/ml concentration

Sr.No	Wave length (λ)	EExI	BM extract		NC extract		PG extract		CC extract	
			Conc. 20 µg/ml		Conc. 20 µg/ml		Conc. 20 µg/ml		Conc. 20 µg/ml	
			Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs
1	290	0.015	0.2228	0.0033	0.3065	0.0045	0.6439	0.0096	0.1024	0.0015
2	295	0.0817	0.2059	0.0168	0.2642	0.0218	0.5502	0.0449	0.0943	0.0077
3	300	0.2874	0.2090	0.0600	0.2385	0.0685	0.4737	0.1361	0.0907	0.0261
4	305	0.3278	0.2164	0.0709	0.2229	0.0730	0.4039	0.1324	0.0873	0.0286
5	310	0.1865	0.2200	0.0410	0.2140	0.0399	0.3383	0.0630	0.0838	0.0156
6	315	0.0839	0.2187	0.0183	0.2084	0.0174	0.2805	0.0235	0.0791	0.0066
7	320	0.018	0.2081	0.0037	0.2062	0.0037	0.2351	0.0042	0.0722	0.0013
SPF				2.1430±0.0271		2.2892±0.0287		4.1401±0.0551		0.8751±0.0112

*Values are calculated (*n*=3) as mean±SD

Table 4: Determination of *in vitro* SPF at 30 µg/ml concentration

Sr.No	Wave length (λ)	EExI	BM extract		NC extract		PG extract		CC extract	
			Conc. 30 µg/ml		Conc. 30 µg/ml		Conc. 30 µg/ml		Conc. 30 µg/ml	
			Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs	Abs	EExIx Abs
1	290	0.015	0.2616	0.0039	0.3342	0.0050	0.6022	0.0090	0.1292	0.0019
2	295	0.0817	0.2442	0.0199	0.2728	0.0222	0.5337	0.0436	0.1190	0.0097
3	300	0.2874	0.2397	0.0688	0.2397	0.0688	0.4768	0.1370	0.1130	0.0325
4	305	0.3278	0.2380	0.0780	0.2213	0.0725	0.4261	0.1397	0.1091	0.0358
5	310	0.1865	0.2361	0.0440	0.2090	0.0389	0.3788	0.0707	0.1048	0.0195
6	315	0.0839	0.2317	0.0194	0.2237	0.0187	0.3366	0.0282	0.0992	0.0083
7	320	0.018	0.2225	0.0040	0.2428	0.2285	0.3036	0.0055	0.0907	0.0016
SPF				2.3824±0.0301		2.3020±0.0284		4.3373±0.0566		1.0940±0.0140

*Values are calculated (*n*=3) as mean±SD

Table 5: Determination of *in vitro* SPF at 40 µG/ML concentration

Sr.No	Wave length (λ)	EExI	BM extract		NC extract		PG extract		CC extract	
			Conc. 40 µg/ml		Conc. 40 µg/ml		Conc. 40 µg/ml		Conc. 40 µg/ml	
			Abs	EExIx Abs	Abs	EEx I x Abs	Abs	EEx I x Abs	Abs	EExIx Abs
1	290	0.015	0.2637	0.0039	0.4102	0.0061	0.9267	0.0139	0.2212	0.0033
2	295	0.0817	0.2452	0.0200	0.3317	0.0271	0.7991	0.0653	0.2066	0.0169
3	300	0.2874	0.2511	0.0721	0.2902	0.0834	0.6917	0.1988	0.1953	0.0561
4	305	0.3278	0.2628	0.0861	0.2685	0.0880	0.5931	0.1944	0.1857	0.0609
5	310	0.1865	0.2689	0.0501	0.2550	0.0475	0.4986	0.0930	0.1782	0.0332
6	315	0.0839	0.2679	0.0224	0.2516	0.0211	0.4145	0.0348	0.1706	0.0143
7	320	0.018	0.2552	0.0045	0.2523	0.0045	0.3471	0.0062	0.1616	0.0029
SPF				2.5953±0.0328		2.7789±0.0345		6.0643±0.0804		1.8765±0.0239

*Values are calculated (*n*=3) as mean±SD

UV-B, and UV-C). Natural substances investigated in plants have recently studied for their potential as sunscreen candidates because of their UV-ray absorbing property. Literature showed the flavonoids and other phenolic compounds having an antioxidant property and previously studied for the given extracts. This data make a platform to exploit extracts in combination as potential ingredients in sunscreen formulations. The wavelength and their respective absorbance for all the subjected extracts were reported in Table 2 and shown in Figure 1.

3.3. Determination of *In Vitro* SPF of Extracts

SPF numbers have become a worldwide standard for measuring the effectiveness of photoprotective products. The *in vitro* SPF determination is one of the useful tools for screening tests during product development, as a supplement base for *in vivo* studies. Whereas the *in vivo* test is time consuming and includes various degrees of variability. The SPF numbers of extracts were calculated by applying Mansur mathematical equation in the UV-B region, which is considered to be the region of highest incidence during the day and

people are exposed for a longer time [34]. Whereas plant literature shows that natural substances extracted from plants have potential sunscreen activity as it gets absorbed in the UV region due to the presence of antioxidants [35].

The absorbance spectra indicated that the compound present in the extracts having the ability to absorb the UV radiation from the range of 200–400 nm and the values of SPF values determined with three different concentrations in the UV range from 290 to 320 nm. The absorbance values obtained were used to calculate the SPF and it was observed that there is an increase in SPF value with an increase in concentration. The current study was carried out at lower concentrations and shown appreciable SPF values. Hence, the higher concentration will definitely provide better sun protection values. The SFP values for 20, 30 and 40 µg/ml are shown in Tables 3-5, respectively. The highest SPF was observed in PG extract and lowest in CC extract as shown in Figure 2. The range of SPF values obtained was PG>NC>BM>CC.

CONCLUSION

This primary study revealed the UV absorbing capacity and sun protection property of the plant extracts. Along with their many therapeutic benefits, these botanicals could become beneficial, cost effective, and easily available ingredients to prove sun protection property. These plants were studied for various bioactive components such as alkaloids, tannins, phenols, and flavonoids which have known as a free-radical scavenger and strong antioxidants and useful to protect the skin from sun radiation induced damage. In past years, the plant of *Boerhavia diffusa* shown SPF values as 3.539–7.174 [36], fresh *Aloe vera* gel shown SPF of 0.0995 [37], the aqueous and methanolic extracts of *Zingiber officinale* at the lower concentration shown SPF 1.44–1.82 and 1.48–1.99, respectively [38]. Some studies were conducted on the mixture of herbs to get good amount of SPF value and considered for the development of topical formulations [39]. The undertaken study could be a useful tool for in-process quality control of formulation, product analysis and helpful to provide information before proceeding to *in vivo* studies. In future, these plant extracts in alone or in combinations with other additives can used to develop a photoprotective formulation such as creams and lotions with higher SPF.

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CONFLICT OF INTEREST

Authors declared that they do not have any conflicts of interest.

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