



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2020; 9(1): 40-44
Received: 22-11-2019
Accepted: 26-12-2019

Ishani Patel

Faculty of Pharmacy,
Kalabhavan, The Maharaja
Sayajirao University of Baroda,
Vadodara, Gujarat, India

Rajashree Mashru

Faculty of Pharmacy,
Kalabhavan, The Maharaja
Sayajirao University of Baroda,
Vadodara, Gujarat, India

Corresponding Author:**Rajashree Mashru**

Faculty of Pharmacy,
Kalabhavan, The Maharaja
Sayajirao University of Baroda,
Vadodara, Gujarat, India

Development of herbal SPF formulation

Ishani Patel and Rajashree Mashru

DOI: <https://doi.org/10.22271/phyto.2020.v9.i1a.10469>

Abstract

The study of Phytoconstituent is done by finding the uses of plants and by applying the acquired information to produce novel therapeutics drugs and their formulations. It has a number of benefits like anti-microbial, anti-inflammatory, anti-diabetic, as well as anti-hypertensive activities. To remove the oxidative stress induced diseases in plants, these plants will produce secondary metabolites which ultimately lead to therapeutic potential. According to the recent studies it is seen that there is a lot of damaging effect of sunlight such as sunburn, cracks, melanoma and cancer which make the cosmetic industries more prone towards sun protection formulations. The purpose of this research paper is to develop a herbal sunscreen product containing Phytoconstituent (flavonoids, phenolic) carrying the capacity to absorb radiation in the UV-A and UV-B region. The SPF value of the developed formulation was found to be 35. When Billi, Rose, and Mango were used together in the formulation.

Keywords: SPF sun protection factor, Phytoconstituent, anti-oxidant, phytochemical-screening

Introduction

In cosmetic industry, uses of herbal extract and natural molecules represents a new trend ^[1]. In some of the countries the sun protection cream is used as OTC product/cosmetic products ^[2] Sunlight contains approximately 40% of visible light, 10% of UV light, 50% of IR rays. We are only concerned for UV-light as it has deleterious effects on the biological living body. Some of the damaging effect like erythema, carcinogenic effect, premature skin aging, cell-mutation etc. UV radiation is divided into UV-C (200-280nm), UV-B (280-315nm), UV-A (315-400nm) ^[3].

Sunlight although having a lot of benefits for the human body such as stimulating the production of vitamin D3 and also helping out in the treatment of skin diseases like psoriasis and vitiligo in which case exposure of UV radiation is done, it also has some damaging effects as well ^[4, 5, 6]. Such as when UV radiation is exposed to the patient suffering from skin disease it is fine up until a certain limit after which excessive exposure may lead to skin cancer. UV-A and UV-B radiation is generally producing oxygen reactive species which include singlet oxygen, hydroxyl, peroxide and free iron ^[7, 8]. These reactive species may damage the nucleic acids, proteins and lipid membrane of our body which would in turn lead to mutation and necrosis of cells in the human body ^[9, 10].

Sunscreens are topical products which are also used to protect our skin against harmful UV rays. It will also protect against ageing, wrinkle formation, collagen loss and skin discoloration by damaging skin pigments. Phytocosmetic is used to refer to the plant extract and oils which possess cosmetic effects ^[11]. Due to ethical reason ISO introduced *in-vitro* methods to check the sun protection and sun irritation criteria for these Phytocosmetic products. For the calculation of UV-A sun protection factor we generally follow the ISO-2444:2012 guidelines as a standard to calculate SPF values by simple UV Spectrophotometry ^[12].

According to some guidelines there are certain standards of SPF values such as according to ANVISA (Agencia Nacional de Vigilancia Sanitaria) a SPF value greater than or equal to 6 is beneficial, where as in USFDA and FDA a SPF value greater than 2 and 15 respectively is allowed ^[13]. As herbal products have lesser side effects and more benefits people are more inclined towards using GREEN SUNSCREEN products ^[11]. Nowadays, in mostly all the sunscreen formulations there is one or more compound with anti-oxidant property which is added to the formulation ^[9] The human body generates compounds and enzymes such as superoxide dismutase, glutathione, ascorbic acid and tocopherol to protect us against free radicals providing antioxidant activity ^[14]. Anti-oxidant activity of certain plant extracts may be measured by DPPH (1, 1 Diphenyl 2-picryl hydrazyl) assay, Hydroxyl assay, Nitroxide assay, Chelating power assay.

Materials and Methods

Reagents

Methanol, Distilled water, Plant powder, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Ninhydrin, DPPH (1,1 Diphenyl 2-picryl hydrazyl), Copper acetate, Sulfanilamide, Naphthalenediamine, Sodium-nitroprusside, Saline phosphate, Ascorbic acid, Ferric chloride dihydrates, Ferrozine, EDTA (Ethylene di-amine tetra-acetic acid), Benedict, Fehling's reagent, Lead acetate.

Instruments and apparatus

UV-spectrophotometer (Shimadzu-1800), Mixer grinder, Magnetic stirrer, Micro-pipette, Filter-paper.

Plants materials

The plants which were used in the present study are enlisted in Table 1 as well as the plant parts which are used and their scientific names.

Table 1: List of plants and plant parts used respectively

Sr. No.	Common Name	Plant part	Scientific Name
1	Billi	Leaves	<i>Aegle marmelos</i>
2	Neem	Leaves	<i>Azadirachta indica</i>
3	Vad	Leaves	<i>Ficus benghalensis</i>
4	Asopalav	Leaves	<i>Saraca indica</i>
5	Mango	Leaves	<i>Mangifera indica</i>
6	Gado	Leaves	<i>Tinospora cordifolia</i>
7	Gulab	Flower petal	<i>Rosa</i>
8	Mogro	Flower petal	<i>Jasminum sambac</i>
9	Ked	Leaves	<i>Melophagus ovinus</i>
10	Garmalo	Flower petal	<i>Cassia fistula</i>

Extraction and *In-vitro* determination of SPF by UV-Spectrophotometer

The above presented plants were collected, washed and shade dried, after which they were grinded individually using a mixer grinder. Extraction was carried out by taking 0.5g of these dried plant powders separately in 50ml of methanol and placing them on a magnetic stirrer for 30 minutes after which this solution is filtered. From this the menstrum is taken and a further dilution of 10 times is done by taking 1ml of this menstrum and making the volume up to 10ml using methanol. The absorbance of this solution is recorded at different wavelengths in the range of 290nm to 320nm at every 5nm intervals.

Formulation

In case of the In-Situ formulation prepared in lab the dried methanolic plant extract is imparted into Aloe-Vera gel base and then the above mention process is repeated using 50ml methanol.¹⁵ The absorbance of the gel-based formulations is also taken between the wavelength range of 290-350nm in the increasing gap of 5nm.¹⁶ Then these absorbance values may be used to calculate the SPF value of the respective plants and their formulations using Equation 1 given below.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad \dots 1$$

Here CF = Correction factor (10), $EE(\lambda)$ = Erythrogenic effect of radiation with wavelength, $Abs(\lambda)$ = Spectrophotometric absorbance values at specific wavelengths. The value of $EE(\lambda) \times I(\lambda)$ also known as the normalized product functions is a constant at specific wavelengths and is given in Table 2^[17, 18].

Table 2: Normalized product functions used in the calculation of SPF

Wavelength (nm)	$EE(\lambda) \times I(\lambda)$
290	0.015
295	0.081
300	0.287
305	0.327
310	0.186
315	0.083
320	0.018

Phytochemical screening^[19, 20, 21]

Phytochemical screening for herbal extract is carried out according to the standard methods.

- Detection of alkaloids:** Extracts were dissolved individually in dilute Hydrochloric acid and then filtered.
 - Mayer's test:** Filtrates were treated with the Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.
 - Wagner's test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
 - Dragendorff's test:** Filtrates were treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
 - Hager's test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.
- Detection of carbohydrates:** Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
 - Molisch's test:** Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.
 - Benedict's test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
 - Fehling's test:** Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.
- Detection of glycosides:** Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.
 - Modified Borntrager's test:** Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.
 - Legal's test:** Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

- a. **Froth test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
- b. **Foam test:** 0.5 g of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phytosterols

- a. **Salkowski's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
- b. **Libermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

6. Detection of phenols

- a. **Ferric chloride test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

7. Detection of tannins

- a. **Gelatin test:** To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

8. Detection of flavonoids

- a. **Alkaline reagent test:** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
- b. **Lead acetate test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

9. Detection of proteins and amino acids

- a. **Xanthoproteic test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.
- b. **Ninhydrin test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

10. Detection of Diterpenes

- a. **Copper acetate Test:** Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of Diterpenes.

11. Detection of gums/mucilage

- a. **In methanolic extract:** add 2-3 drops of Ruthenium red it will not produce pink color. So mucilage is not present.

Estimation of antioxidant assay

1. DPPH (1, 1-diphenyl-2-picryl-hydrazyl) Radical Scavenging Activity [22]: The stock solution was prepared by dissolving 3.6mg of DPPH in 1.5ml of methanol and it was kept at 20 °C until further use. The stock solution was further diluted with methanol to optimize its absorbance (0.908 ± 0.01) at 517 nm. Now 100 µl of plant samples was mixed with 900 µl of DPPH aliquot and incubated for 15 min at room temperature in the dark. Absorbance was checked at wavelength of 517 nm by running Ascorbic acid as standard.

$$\text{DPPH scavenging activity(\%)} = \frac{(\text{control absorbance} - \text{sample absorbance})}{(\text{control absorbance})} \times 100 \dots (2)$$

2. Nitric oxide scavenging assay [23, 24]: Equimolar quantity of Naphthalenediamine (0.1%) in distilled water and sulphanilamide (1%) in phosphoric acid (5%) was added to prepare griess reagent. 100 µl of 10 mm sodium nitroprusside being prepared in saline phosphate buffer was added to 100 µl of each plant sample. Then 1 ml of griess reagent was added to, reaction mixture, incubated for 3 h and analyzed spectrophotometrically at 546 nm by using ascorbate as a positive control.

Result and Discussion

The simple and reliable method is used for determination of *in-vitro* Sun Protection Factor (SPF) with the help of their hydro-alcoholic extracts. The absorbance of the samples was taken between 290-320 nm at every 5 nm increment of wavelength. SPF values can be calculated using Mansur equation (Equation 1) which are represented in Table 3.

Table 3: SPF values of different plants

Name	SPF
Billi (<i>Aegle marmelos</i>)	10.947
Neem (<i>Azadirachta indica</i>)	4.296
Vad (<i>Ficus benghalensis</i>)	3.166
Asopalav (<i>Saraca indica</i>)	10.639
Mango (<i>Mangifera indica</i>)	19.986
Gado (<i>Tinospora cordifolia</i>)	5.063
Gulab (<i>Rosa</i>)	35.054
Mogro (<i>Jasminum sambac</i>)	3.072
Ked (<i>Melophagus ovinus</i>)	2.807
Garmado (<i>Cassia fistula</i>)	5.307

From the above listed plants the following three were shortlisted based on their higher individual SPF values and taking 0.5g of each plant.

1. Billi (*Aegle marmelos*)
2. Mango (*Mangifera indica*)
3. Rose (*Rosa*)

The reason behind choosing these three plants in combination is that Rose and Mango have the highest SPF values, but their combination alone did not show a good amount of stability, whereas with the addition of a third plant Billi which has an innate antimicrobial activity. Asopalav plant also could have been used as it also has around the same SPF value as Billi plant, but due to a lower cumulative SPF value of Asopalav with Rose and Mango (26.735) it was not chosen. The

cumulative SPF value of Billi, Mango and Rose on the other hand was found to be 35.537 by taking 0.5g of each plant and

applying the above mentioned method and taking its UV spectra as depicted in Figure 1.

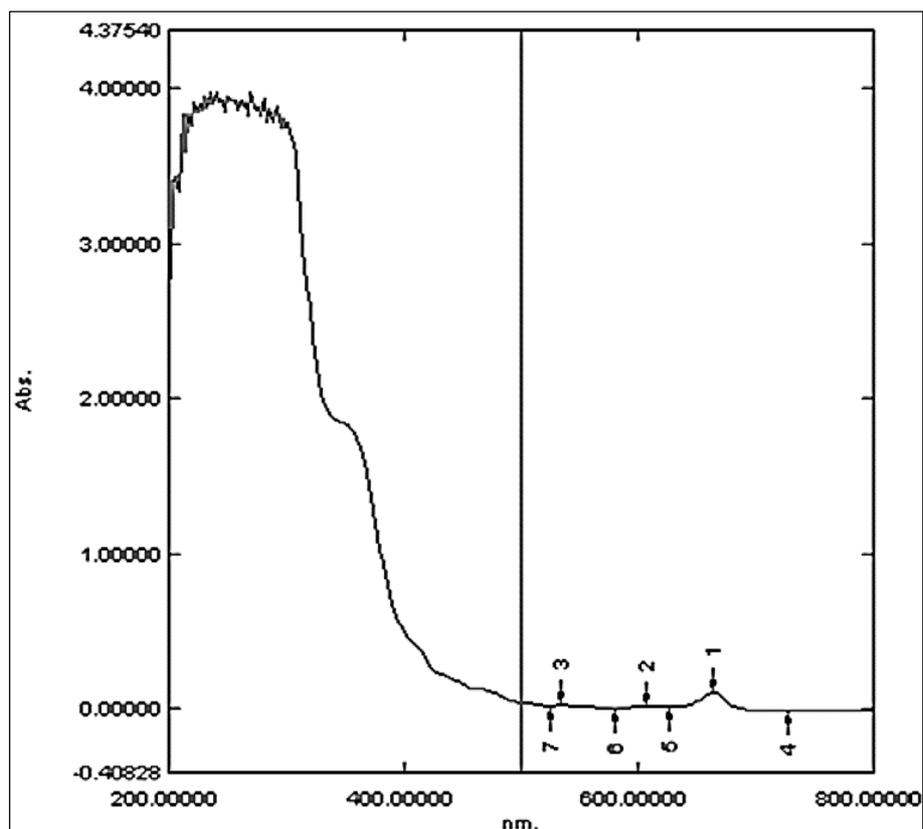


Fig 1: UV spectra of the combination selected

Phytochemical screening

Phytochemical screening of above plant mixture in the form

of methanolic extract was performed, the results of which are shown in Table 4.

Table 4: Results of phytochemical screening study

Sr. No.	Class	Test	Results of plant mixtures chosen
1	Alkaloid	Mayer's	+
2		Wagner's	+
3		Hager's	-
4		Dragendorff	+
5	Carbohydrate	Molish	+
6		Benedict	+
7		Fehling	+
8	Glycoside	Bronthager	+
9		Legal	-
10	Saponin	Foam	+
11		Froth	+
12	Phytosterol	Salkowski	+
13		Liebermann burchard	+
14	Phenol	Ferric chloride	+
15	Tannin	Gelatin	+
16	Flavonoids	Alkaline reagent	+
17		Lead acetate	+
18	Protein	Xanthoproteic	+
19		Ninhydrin	-
20	Diterpenes	Copper acetate	+
21	Gums/mucilage	Ruthenium red	-

Table 5: Results of antioxidant activity

Name	DPPH Assay	Nitric oxide Assay	% scavenging activity of sample	
			DPPH	Nitric oxide
Sample	0.278	0.39312	84.73%	55.72%
control	1.82	0.8836		

Estimation of anti-oxidant activity

From equation 2 we can calculate the percentage inhibition of free radical species which was found to be 84.73% and 55.72% in DPPH and Nitric oxide respectively as shown in Table 5.

Conclusion

SPF of the sunscreen is important as it has a direct effect on the human skin. In the present study a formulation mixture was prepared using Billi (*Aegle Marmelos*), Mango (*Mangifera Indica*) and Rose (*Rosa*) having a cumulative SPF value of 35.537 which is found to be higher than most of the other marketed formulations? As well as it is possible for one to formulate this mixture by themselves at home. It is having a high anti-oxidant property, totally herbal in nature and no skin irritations which makes it highly beneficial.

References

- Balogh TS, Velasco MV, Pedriali CA, Kaneko TM, Baby AR. Ultraviolet radiation protection: current available resources in photo protection. *A Bras Dermatol.* 2011; 86(4):732-42.
- Baker LA, Marchetti B, Karsili TNV, Stavros VG, Ashfold MNR. Photoprotection: Extending lessons learned from studying natural sunscreens to the design of artificial sunscreen constituents. *Chemical Society Reviews.* 2017; 46:3770-3791.
- Radice M, Manfredini S, Ziosi P, Dissette V, Buso P, Fallacara A *et al.* Herbal extracts, lichens and biomolecules as natural photo-protection alternatives to synthetic UV filters. A systematic review. *Fitoterapia.* 2016; 114:144-162.
- Gonzalez S, Fernandez-Lorente M, Gilaberte-Calzada Y. The latest on skin Photoprotection. *Clin. Dermatol.* 2008; 26:614-626.
- Palm MD, Donoghue MN. Update on Photoprotection. *Dermatol. Ther.* 2007; 20:360376.
- Pathak MA. Photoprotection against harmful effects of solar UVB and UVA radiation: an update. In: *Sunscreens: Development, Evaluation and Regulatory Aspects* (Lowe NJ, Shaath NA, Pathak MA Eds). Marcel Dekker, New York, NY, 1997, 59-82.
- Pourzand C, Tyrrell RM. Apoptosis, the role of oxidative stress and the example of solar UV radiation. *Photochem. Photobiol.* 1999; 70:380-390.
- Zanatta CF, Mitjans M, Urgatondo V, Rocha-Filho PA, Vinardell MP. Photo protective potential of emulsions formulated with Buriti oil (*Mauritia flexuosa*) against UV irradiation on keratinocytes and fibroblasts cell lines. *Food Chem. Toxicol.* 2010; 48:70-75.
- Giacomoni PU. Understanding reactive oxygen species. *Cosm. & Toil.* 2008; 122:36-40.
- Morita A, Krutmann J. Ultraviolet A radiation-induced apoptosis. *Methods Enzymol.* 2000; 319:302-309.
- Cefali LC, Ataide JA, Moriel P, Foglio MA, Mazzola PG. Plant compounds as active photo protectants in sunscreens. *International Journal of Cosmetic Science.* 2016; 38:346-353.
- Piergiacomo Buso, Matteo Radice, Anna Baldisserotto, Stefano Manfredini, Silvia Vertuani. *Guidelines for the Development of Herbal-Based Sunscreen*; chapter-4, 2017, 43-61.
- FDA. Food and Drug Administration. Sunscreen drug products for over-the-counter human use. Code of Federal Regulations. Title 21, 2013, 5.
- Niki E, Shimaski H, Mino M. *Antioxidantism-Free Radical and Biological Defense*, Gakkai Syuppin Center, Tokyo, Japan, 1994.
- Fonseca AP, Rafaela N. Determination of Sun Protection Factor by UV-Vis Spectrophotometry. *Health Care Current Reviews.* 2013; 1:108.
- Mansur JS, Breder MN, Mansur MC, Azulay RD. Determination of Sun Protection Factor by Spectrophotometry. *A Bras Dermatol.* 1986; 61:121-124.
- Malsawmtluangi C, Nath DK, Jamatia I, Lianhingthngi, Zarzoliana E, Pachuau L. Determination of Sun Protection Factor (SPF) number of some aqueous herbal extracts; *Journal of Applied Pharmaceutical Science.* 2013; 3(9):150-151.
- Mbanga L, Mpiana PT, Mbala M, Ilinga L, Ngoy B, Mvingu K *et al.* Comparative *in vitro* Sun Protection Factor (SPF) values of some herbal extracts found in Kinshasa by Ultraviolet Spectrophotometry *J of Physical and Chemical Sciences.* 2015; 2(4):1-6.
- Roopashree TS, Dang R, Rani SRH, Narendra C. Antibacterial activity of anti-psoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. *International Journal of Applied Research in Natural Products.* 2008; 1(3):20-28.
- Obasi NL, Egbuonu ACC, Ukoha PO, Ejikeme PM. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. *African journal of pure and applied chemistry.* 2010; 4(9):206-212.
- Audu SA, Mohammed I, Kaita HA. Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). *Life Science Journal.* 2007; 4(4):7579
- Brand-Williams W, Cuvelier M, Berset C. Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol.* 1995; 28:25-30.
- Bhaskar H, Balakrishnan N. *In vitro* antioxidant property of laticiferous plant species from Western Ghats Tamil Nadu, India. *Int. J Health Res.* 2009. <https://doi.org/10.4314/ijhr.v2i2.55413>
- Garrat DC. *The Quantitative analysis of Drugs.* Chapman and Hall Ltd., Japan. 1964; 3:456-458.