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Sunscreen and Anti-Oxidant Activities of Herbal Gel Formulations

Mahajan U.N.[1], Dr. Wasule D.D.[2]

[1]*Department of Pharmaceutical Sciences, Nagpur, Maharashtra, India.*
[2]*Department of Cosmetic Technology, L.A.D. & S.R.P. College for Women, Nagpur, Maharashtra, India.*
**Author for Correspondence:drdeepakdhanraj@yahoo.com ;unmahajan@yahoo.co.in*

ABSTRACT

The present study was designed to study sunscreen and anti-oxidant activity of herbal gel formulations. *Ocimum sanctum* (*O. tenuiflorum*; Family – *Lamiaceae* [Labiatae]) is one of the well-known drugs for its therapeutic values in traditional medicine. In the present study the drug was screened for its cosmetic use. All the three formulations containing aqueous, hydro-alcoholic as well as flavonoid extract showed sunscreen as well as anti-oxidant activity. The sunscreen activities of gel formulations could be related to the free radical scavenging properties of polyphenol compounds in the extracts incorporated in it.

KEY WORDS: *Ocimum sanctum*, sunscreen, anti-oxidant, gel formulation.

INTRODUCTION

Ocimum sanctum is a herbaceous plant found throughout India. The leaves are used as stimulant, aromatic, anti-catarrhal, spasmolytic and diaphoretic. The volatile oil from leaves is anti-bacterial and insecticidal [1].

The purpose of sunscreen product is to prevent the skin from tanning and burning by screening out the UV-A and UV-B radiations in sunlight [2].

The present study deals with the cosmetic use of *Ocimum sanctum*. The gel formulation containing aqueous, hydro-alcoholic and flavonoid extracts of *Ocimum sanctum* were screened for sunscreen and anti-oxidant activity.

MATERIALS AND METHODS

Plant Material

The fresh leaves were collected from Shri Sail Medi Farms, cultivators and dealers of medicinal herbs, Nagpur [Maharashtra] and authenticated by Dr. Prabha Bhugaokar, Head, Department of Botany, Vidarbha Mahavidyalaya Amravati. The leaves were shade dried.

Preparation of extracts

1. ***Aqueous Extract:***The shade dried leaves were coarsely powdered and extracted by cold maceration with distilled water. After filtration, the filtrate was concentrated under vacuum in a rotary evaporator.
2. ***Hydro-alcoholic Extract:*** After filtration, the plant material left was dried at room temperature and again extracted by cold maceration using distilled water and ethanol in 50:50 proportion. The filtrate obtained after filtration was concentrated under vacuum in a rotary evaporator.
3. ***Flavonoid Extract [3]:*** After weighing out, the coarsely powdered plant material, extraction was carried out in two steps'

(i) With Methanol: Water [9:1] and
(ii) With Methanol: Water [1:1]

After filtration, two extracts were combined and evaporated to about 1/3rd the original volume. The resultant aqueous extract was taken in a separating funnel and extracted with hexane or chloroform to remove low polarity contaminants such as terpenes, chlorophylls, xanthophylls etc. Then all the portions of extract were combined.

The solvent extracted aqueous layer containing the bulk of the flavonoids was then evaporated to dryness under vacuum in a rotary evaporator.

Preparation of gel formulation

Gel formulations of all the three extracts were prepared using formulae given in table 1.

Method / Procedure [4]

To prepare plain gel carbopol (ultrez 21, Noveon Inc.) was soaked in water. After some time, other ingredients were added and triturated well. The extract was then added slowly with triturating to plain gel to get a homogeneous dispersion of the extract in the gel.

Screening of Gel Formulations for Sunscreen Activity (Spectrophotometer Measurement) [5]

For the measurement, 50 mg of the finished preparation was dissolved in Isopropanol: Water

(50:50) and diluted to 100 ml. The solution was placed in 1 cm wide quartz cuvettes of spectrophotometer and its spectrum was recorded from 280 to 400 nm (Shimadzu UV 2401 PC).

The concentration prepared corresponds directly to the layer thickness at which the preparation is normally applied on the skin. 50 mg in 100 ml of solvent, measured at a layer thickness of 1 cm, corresponds to the optical behaviour of the undiluted preparations on the skin with a layer thickness of 0.005 mm.

Now days leading Indian manufacturers are marketing the product having SPF 15 so as a model for comparison we have selected the product having SPF 15. Further we tried to compare only the effect irrespective of composition knowing there is no single product available in the market, which is 100% natural. So finally we decided to compare our product, which is in the form of gel to be compared with the marketed gel product.

Screening of gel formulation for anti-oxidant activity

Anti-oxidant / anti-radical activity was measured by a decrease in absorbance at 517 nm of coloured DPPH [1, 1-diphenyl-2-picrylhydrazyl] brought about by the sample [6].

A stock solution of DPPH (Sigma Aldrich) 0.1 mM was prepared by dissolving 3.94 mg in 100 ml of methanol: water (50:50).

The stock solutions of the gel formulation 1 mg/ml were prepared using methanol: water (50:50) as a solvent. 0.1 ml of stock solution of each was pipetted out and diluted to 100 ml to get 10 μg/ml. Subsequent dilutions of stock solutions of gel were made to get the suitable concentration of the solutions.

1 ml of DPPH solution was added to 3 ml of hydro-alcoholic solution (Methanol: water [50:50]) and initial absorbance was recorded at 517 nm after 30 min. Decrease in absorbance in the presence of sample solutions at different concentrations was also noted after 30 min. The percent inhibition / percentage DPPH scavenging activity was calculated from the following formula;

$$\%\ DPPH\ Scavenging = \left[\frac{(Absorbance of\ Control\ -\ Absorbance of\ Test\ Sample)}{Absorbance of\ Control}\right]\times 100$$

IC_{50} was calculated from % DPPH scavenging activity. Ascorbic acid was used as standard.

RESULTS

In the present study, an attempt was made to evaluate sunscreen and anti-oxidant activity of gel formulations containing *Ocimum* sanctum extracts.

Sun screening Activity: All the three formulations showed sunscreen activity. The formulation containing total flavonoid extract seems to be the most potent sunscreen formulation [Figure 1 and Figure 2].

Anti-oxidant Activity: All the three gel formulation showed concentration dependent anti-radical activity by inhibiting DPPH radical. Table 2 exhibits % DPPH scavenging activity and IC_{50} values (concentration in μg/ml of gel formulations that inhibits the formation of DPPH radical by 50%) of three formulations.

DISCUSSION

It is evident from the result that gel formulations containing hydro-alcoholic extract and total flavonoid extract are potent sunscreen and anti-oxidant preparations as compared to the formulations containing aqueous extract. Free radical scavenging appears to be a major mechanism of sun protection by these formulations.

Methods

Table 1 : Formulation Chart

Ingredients	A (%)	B (%)	C (%)
Carbopol (Ultrez 21)	1.00	1.00	3.00
Methyl Paraben	0.50	0.50	0.50
Triethanolamine	q.s. (to pH 6-7)	q.s. (to pH 6-7)	q.s. (to pH 6-7)
Glycerin	5.00	5.00	5.00
Extract	45.00	40.00	45.00
Water	Up to 100.00	Up to 100.00	Up to 100.00

A: Gel containing aqueous extract

B: Gel containing hydro-alcoholic extract.

C: Gel containing flavonoid extract.

Table 2 : Anti-Oxidant / Anti-Radical Activity of Gel Formulations

S. No.	Sample	Concentration (µg/ml)	% Inhibition / % DPPH Scavenging Activity	IC_{50} (µg/ml)
1.	Gel Containing *Ocimum sanctum* Aqueous Extract	20	26.31	
		40	44.43	
		60	53.20	50
		80	56.38	
		100	59.95	
2.	Gel Containing *Ocimum sanctum* Hydroalcoholic Extract	5	24.00	
		10	43.22	
		20	48.64	
		40	54.65	24
		60	61.28	
		80	67.57	
		100	74.61	
3.	Gel Containing *Ocimum sanctum* Flavonoid Extract	1	34.44	
		2	36.30	5.80
		4	43.40	
		6	50.72	
4.	Ascorbic Acid	5	24.39	
		10	42.9	
		20	51.35	17.00
		40	56.76	
		60	62.09	

Six phenolic compounds isolated from *Ocimum sanctum* including eugenol, rosmarinic acid, apigenin and other flavonoids showed good to excellent anti-oxidant activity in vitro (7). Also two flavonoids orientin and vicenin isolated from leaves of *Ocimum sanctum* showed radio-protective effects in mice (8).

Thus, from the above study free radical scavenging activity of chemical constituents, particularly polyphenols of *Ocimum sanctum* appears to be one of the mechanism of sun protection.

Hence, it was concluded that *Ocimum sanctum* extract and especially, the isolated polyphenols / flavonoids can be effectively incorporated in cosmetic preparations as a potent sunscreen agents.

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