Research Article

STUDY OF SUNSCREEN ACTIVITY OF HERBAL CREAM CONTAINING FLOWER EXTRACT OF *NYCTANTHES ARBORTRISTIS L.* AND *TAGETES ERECTA L.*

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ABSTRACT

The aim of the present study was to evaluate the sunscreen activity of Herbal cream containing flower extract of *Nyctanthes arbortristis L.* (Oleaceae) and *Tagetes erecta L.* (Compositae). The shade dried flowers were extracted successively with petroleum ether and ethanol in soxhlet apparatus. Two different sunscreen creams were formulated using ethanolic extract and tested for the pysicochemical parameters such as colour, odour, spreadability, pH, specific gravity, limit test for lead and viscosity. Stability study and sensitivity study was done by centrifugation method and patch test for irritancy respectively. Sun protection factor is a laboratory measure of the effectiveness of sunscreen; the higher the SPF, the more protection a sunscreen offers against the ultraviolet radiations causing sunburn. The *in vitro* SPF of the formulations was determined according to the UV Spectrophotometric method of Mansur *et al*. The sunscreen cream containing ethanolic extract of *N. arbortristis* under study produced high absorbance at 290-320 nm wavelength range and SPF obtained was 10.21±2.18. The sunscreen cream containing ethanolic extract *T. erecta* obtained the SPF 8.67±1.35. From the result obtained in the study, we can positively conclude that N. *arbortristis* sunscreens will enhance and significantly contribute to the UV absorbing properties of conventional sunscreen formulation. It will also help in broadening the UV protection ability of the sunscreen along with the greatest advantage of avoiding the adverse and undesired effects of synthetic sunscreen compounds.

Keywords: *Nyctanthes arbortristis*, *Tagetes erecta,* sun protection factor (SPF).

INTRODUCTION

Exposure to solar radiation is recognized to have negative effects on human skin. It has been known for decades that sunscreens are capable of protecting man from harmful effects of solar radiation such as premature ageing or cutaneous cancer, basal cell carcinoma, sunburns and malignant melanomas.[1, 2] The harmful effects of solar radiation are caused predominantly by the ultraviolet (UV) region of electromagnetic spectrum, which can be divided into three regions: UVA, from 320 nm to 400nm, UVB, from 290 nm to 320 nm and UVC, from 200 nm to 290 nm. UVC radiation is filtered out by the ozone layer and is responsible for the damage due to sunburn. UVA radiation reaches the deeper layer of epidermis and dermis and provokes the premature ageing of the skin.[3] Ultraviolet radiations have been implicated as a causative factor of skin cancer. Due to these facts sunscreen substances are now incorporated into every day products such as moisturizers, creams, lotions, shampoos and other hair and skin preparations. The efficacy of sunscreen is usually expressed by the sun protection factor (SPF), which is defined as the UV energy required for producing a minimal erythema dose (MED) on protected skin, divided by the UV energy required for producing a MED on unprotected skin.

$$SPF = \frac{\text{Minimal erythema dose in sunscreen protected skin}}{\text{Minimal erythema dose in non-sunscreen protected skin}}$$

The minimal erythema dose (MED) is defined as the lowest time interval or dosage of UV light radiation sufficient to produce a minimal, perceptible erythema on unprotected skin.[4, 5] Nowadays because of the benefits of products containing natural compounds, acceptance of these products by the users, also the probability of the systemic absorption, using natural products that can absorb the ultraviolet radiation is of great interest. Natural substances extracted from plants have recently been considered as potential sunscreen resources because of their ultraviolet absorption in the UV region and their antioxidant activity.

The photoprotection afforded by topical sunscreen against solar ultraviolet radiation exposure can be determined *in vivo* or *in vitro* and it is ideally determined by photo-testing in human volunteers. This type of determination has been used for many years and although useful and precise, is a time consuming process, complex and expensive, particularly when information concerning to the protection against long wavelength is required.[6] As a consequence, much effort has been devoted to the development of *in vitro* techniques for assessing the photoprotection of sunscreen compounds. The methods *in vitro* are in general of two types. Methods which involve the measurement of absorption or the transmission of UV radiation through sunscreen product film in Quartz plates or Biomembrane and methods in which the absorption characteristics of the sunscreen agents are determine based on spectrophotometric analysis of dilute solution.[7]

Nyctanthes arbortristis Linn. (Fam. Oleaceae) commonly known as Harsingar or Night jasmine is common hardy large shrub. It is native of India, distributed wild in sub-Himalayan region and also found in Indian garden as

ornamental plant. Whole plant is used for the treatment of cancer. Roots for fever, sciatica, anorexia; bark as an expectorant, leaf for controlling fever, diabetes and as a chalogogue and diaphoric.[8] The decoction is use to treat arthritis, malaria, intestinal worms, tonic and laxative.[9] Antitrypanosomal, anti-inflammatory and antioxidant activity has also been exhibited by the various extracts of the plant. The antispasmodic and anthelmintic activities of flower, barks, seeds and leaves have been reported.[10,11]

Tagetes erecta Linn. (Fam. Compositae) locally known as Gendaphul is a stout, branching herb.[12] Different parts of this plant including flower are used in folk medicine to cure various diseases. Leaves are used as an antiseptic and in kidney troubles, muscular pain, piles and applied to boils. The flower is useful in fever, epileptic fits (Ayurveda), astringent, carminative, stomachic and scabies and in liver complaints and also said to purify blood and flower juice is given as remedy for bleeding piles and also in rheumatism, cold and bronchitis.[13]

The present work was planned to study the sunscreen activity of herbal sunscreen containing ethanolic extract of flower of *N. arbortristis* and *T. erecta*. It has become very important to study the sunscreen activity of herbal drugs, so as to avoid the various effects of synthetic chemical sunscreens like aminobenzoic acid derivatives, anthranilates, benzophenones, cinnamates, salicylates, inorganic sunscreens like titanium dioxide and zinc oxide. The therapeutic properties of *N. arbortristis* and *T. erecta* are very well recorded in the text of traditional Indian medicines Siddha and Ayurveda. However the sunscreens activity of flower of both the plants has not been reported till date. This forms the basis for selection of these plants for its sunscreens activity.

MATERIALS AND METHODS

Plant material

N. arbortristis and *T. erecta* flower were collected from medicinal garden of MIBP, Gondia. The plant material was identified and authenticated at Department of Botany, Rashtrasant Tukadoji Maharaj, Nagpur University, Nagpur (9229, 9230). A voucher specimen was deposited in the institute.

Extraction of plant material

The flowers were cleaned, dried under the shade and pulverized. The coarse powder extracted successively with petroleum ether and ethanol using Soxhlet apparatus. The extracts were dried using rotary vacuum evaporator and stored in desiccators until further use.

Reagents

Ethanol (Merck) analytical grade was used.

Instruments

UV Spectrophotometer: UV 1700 Shimadzu, Japan

Brookfield Viscometer: LVDV-I prime, Brookfield Engineering Laboratories Inc. U.S.A,

PH meter: ELICO LI 610

Micro centrifuge: REMI RM-12 C

Formulation of Sunscreen

Step I: Water phase was prepared by collecting deionized water (73%) and then (5%) water was remove aside from this for final volume makeup. Water soluble components disodium EDTA, sodium methyl paraben and triethanolamine were dissolved in deionized water; meanwhile carbopol was allowed to swell using a homogenizer and heated up to 80°C.

Step II: Oil phase was prepared by heating sodium propyl paraben, stearic acid, cetyl alcohol, cetomacrogal-1000, Cetosteryl alcohol and ethanolic extract at 80°C.

Step III: Oil phase was added in water phase at 80°c with continuous stirring for 20-25 min. and then it was homogenized till uniform emulsion is formed. The finished product has yellow color and gel like consistency. It was then poured into the wide mouth container and stored at temperature not exceeding 37°C (Table 1).

Table 1: Composition of ethanolic extract of flower of *N. arbortristis* and *T. erecta* Sunscreen cream

Sr. No.	Ingredients	Uses	Components (% w/w)
1.	Cetosteryl alcohol	Emulsifier	5
2.	Na Methyl Paraben	Preservative	0.3
3.	Na Propyl Paraben	Preservative	0.06
4.	Stearic acid	Emollient, Coemulsifier	2
5.	Cetomacrogal-1000	Emulsifier	2
6.	Carbopol	Gelling agent	0.5
7.	Triethanolamine	Surface active agent	0.5
8.	Disodium EDTA	Chelating agent	0.02
9.	Cetyl alcohol	Emollient, Coemulsifier	1
10.	*N. arbortristis*/ *T. erecta* ethanolic extract	Active Ingredients	1
11.	Purified water	Vehicle	q. s. (up to 100)

Physicochemical Analysis

Physical parameters of cream formulation such as color, odor, spreadability, PH, specific gravity (25°C), limit test for lead of the herbal sunscreens were determined by the standard technique and methods.[14] Viscosity of the sunscreens was measured using a Brookfield viscometer at 10-100 rpm, measurements were made at 25°C. Stability of each sunscreen was determined by centrifugation. During centrifugation studies both sunscreens were centrifuged at 3500-13500 rpm at the interval of 10 minutes and further observe for phase separation. To ensure sunscreens are free from adverse effects a sensitivity study using path test for irritancy was done.

Sample preparation

1.0 g of both the samples was weighed, transferred to 100 ml volumetric flask, diluted to volume with ethanol followed by ultrasonication for 5 min and then filtered through cotton, rejecting the first 10 ml. A 5.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol. Then 5.0 ml of aliquot was transferred to 25 ml volumetric flask and the volume completed with ethanol. Thereafter, absorbance values of each aliquot prepared were determined from 290-320 nm at 5 nm interval, taking ethanol as a blank. The measurements were taken thrice and the determinations were made at each point, followed by application of Mansur equation.

Mansur *et al.* (1986) developed a very simple mathematical equation which substitutes the *in vitro* method proposed by Sayre *et al.* (1979), utilizing UV Spectrophotometry and the following equation.[7, 15]

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

Where CF=Correction factor (10), EE (λ) = Erythmogenic effect of radiation with wavelength λ, Abs (λ) = Spectrophotometric absorbance values at wavelength λ. The values of EE×I are constant, they were determined by Sayre *et al.*

Sun Protection Factor Determination

The aliquots prepared were scanned between 290-320 nm and the obtained absorbance values were multiplied with the respective EE (λ) values. Then, their summation were taken and multiplied with the correction factor (10). Data was expressed as mean ± S.E.M.

RESULTS

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 between 290-400nm. Evaluation of the efficacy of sunscreen formulation for a long time has been assessed through *in vivo* test, which was performed with human volunteers. *In vivo* test is a time consuming, is subject to certain degree of variability, not mentioning the ethical problems of testing with humans. The *in vitro* SPF is useful for screening test during product development, a supplement of the *in vivo* SPF measures.

In this research herbal sunscreen creams containing ethanolic extract of flower of *N. arbortristis* and *T. erecta* were evaluated by UV spectrophotometry applying Mansur mathematical equation. The results of the physicochemical analysis of tested sunscreens showed in Table 2.

During the storage and handling of cosmetic formulation spreadability and viscosity are the prime parameters which affect the formulation acceptability. As the speed of rotation has increased viscosity of tested samples decreased, this behavior of both the formulation revealed the pseudoplastic behavior of products. Formulation with pseudoplastic flow produce a coherent protective film covering the skin surface and this activity is important for adherence on the skin.[16] Stability results tested by centrifugation method revealed that the phase separation was not observed. This showed the stability of these formulations at high stress condition. SPF values of samples obtained using the UV spectrophotometric methods are shown in Table 3.

We can clearly see that high absorbance values were obtained at 290-320 nm wavelength range. The value of SPF obtained for *N. arbortristis* sunscreen cream was 10.21±2.18. Whereas the value of SPF obtained for T. *erecta* sunscreen cream was SPF 8.67±1.35. The N. *arbortristis* sunscreen cream showed significant sunscreen activity as compared with T. *erecta* sunscreen cream.

Table 2: Physical parameters of sunscreens formulation

Sr. No.	Parameters	Observations	
		***N. arbortristis* cream**	***T. erecta* cream**
1	Color	Light yellow	Dark yellow
2	Odour	Characteristics	Characteristics
3.	Spreadability	Good and uniform	Good and uniform
4.	PH	6.5	6.4
5.	Specific gravity	0.93	0.89
6.	Limit test for lead	passes	Passes
7.	Viscosity (cps)	814	827
8.	Patch test for irritancy	No irritation reaction persists	No irritation reaction persists

Table 3: Determination of SPF values of *N. arbortristis* and *T. erecta* Sunscreen Cream

Sr. No.	Wave length	EE value	*N. arbortristis*	*T. erecta*
1	290	0.015	0.0248±0.30	1.2195±0.19
2	295	0.0817	0.1037±0.18	0.9421±0.05
3	300	0.2874	0.2957±0.95	0.8906±0.06
4	305	0.3278	0.3255±0.19	0.8683±0.03
5	310	0.1864	0.1812±0.06	0.8132±0.10
6	315	0.0837	0.0759±0.09	0.7972±0.15
7	320	0.018	0.0151±0.05	0.7557±0.12
Sun Protection Factor			**10.21±2.18**	**8.67±1.35**

However there are many factors affecting the determination of SPF values as for example, the use of different solvents in which the sunscreens are dissolved; the combination and concentration of the sunscreen; the type of emulsion, the effect and interactions of vehicle components, such as esters, emollients and emulsifiers used in the formulations; the interaction of vehicle with the skin, the addition of other active ingredients; the PH system and emulsion rheological properties, among other factors, which can increase or decrease the UV absorption of each sunscreen. The effect of different solvents and emollients have upon the wavelength of maximum absorbance and upon the UV absorbance of several sunscreens chemical, alone or in combination is well known and documented. Excipients and other active ingredients can also produce UV absorption bands, thus interfering with those of UVA and UVB sunscreens. This effect is reflected in a finished formulation especially for lotions with an SPF greater than 15.[17,18]

DISCUSSION

The proposed UV spectrophotometric method is simple, rapid, uses low cost reagents and can be used for *in vitro* determination of SPF values in many cosmetic formulations. It can be performed both during production process, on final product. In recent years, natural compounds or bioactive products have gained considerable attention as UV protective agents due to the presumable safe utilization, ecological issues and minimum side effects besides their antioxidant activity. Plant extracts, due to containing wide range of phenolic acids, flavonoids and high molecular weight polyphenols usually cover the full range of UV wavelengths. Green tea polyphenols, *Rosa damascene* flower extracts, *Aloe barbadensis* extract, aromatic compounds isolated from lichens and flowering tops of *Dracocephalum moldavica and Viola tricolor* are examples of natural substances evaluated for their sunscreens properties.[19, 20, 21, 22] Topical application of *Culcitium reflexum* extract in the form of gel proved to be a significant in vivo protection against the UV induced skin erythema in healthy human volunteers.[22] The flavonolic fraction of *Sedum telephium l*eaf extract also appears to possess potent protective effects against UV-induced skin erythema in human volunteers. Several studies have shown the flavonoids to act as scavengers of superoxide anions, singlet oxygen, hydroxyl radicals, and lipid peroxyl radicals. There are also reports of many enzymes, including lipo-oxygenase, cyclo-oxygenase, mono-oxygenase, mitochondrial succinate dehydrogenase and NADP-oxidase, phospholipase A2, protein kinases and nuclear transcription factor. Phenolics are believed to be capable of acting in redoxsensitive signalling cascades to inhibit DNA damage. Many flavonoids such as quercetin, luteolin and catechins are better antioxidants than the nutrients vitamin C, vitamin E and β-carotene. Therefore, the phenolics may be beneficial in preventing UV-induced oxygen free radical generation and lipid peroxidation, i.e. events involved in pathological status such as photoageing and skin cancer.[23]

The phytochemical investigation of *N. arbortristis* revealed the presence of flavonoids, tannins, phenolics, phytosterols, glycosides saponins etc.[24, 25] However the phytochemical studies of *T. erecta* revealed the presence of flavonoids, triterpenoids, carotenoids, thiophenes, phenolics, quercetagetin, a glucoside of quercetagetin etc.[26, 27] Absorption of UV radiation is the main characteristics for identification of flavonoids in natural sources. The result showed strong-to-moderate absorption of UV radiation along the whole range and this ability may be due to the presence of flavonoids. The result obtained were showed the ability of *N. arbortristis* and *T. erecta sunscreens* creams to absorb UV radiation and hence proved its UV protection ability. The *N. arbortristis* sunscreen cream showed prominent sunscreens activity as compared with *T. erecta* sunscreen cream.

From the result obtained in the study we can positively conclude that *N. arbortristis* sunscreens have significant UV absorbing property. It will also help in broadening the UV protection ability of the conventional sunscreen formulation. Considering the distress of the patient suffering from the skin cancers along with the adverse effects and associated deficits of the synthetic sunscreen compounds currently used, it is the need of the time to seek out various herbal plants which would exhibit prophylactic utility when formulated into efficacious sunscreen formulations.

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