

FORMULATION AND *IN-VITRO* EVALUATION OF ANTIOXIDANT ACTIVITY OF O/W SUNSCREEN CREAM CONTAINING HERBAL OIL AS DISPERSED PHASE

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ABSTRACT

Free radical scavenging activity of O/W sunscreen cream using calendula oil was measured using DPPH method, Nitric oxide method and H₂O₂ assay method. For this, Flowers of calendula were collected for oil extraction. Calendula oil in concentration ranging 1-5% was used as dispersed phase in O/W sunscreen cream. The free radical scavenging activity was evaluated for equally diluted samples and the activity was found in concentration dependent manner by all three adopted methods. The sunscreen activities of cream formulations may be related to the free radical scavenging properties of flavonoid compounds in the oil phase incorporated in it. Calendula oil is one of the well-known drugs for its therapeutic potential in traditional system of medicine. In the present study, the O/W cream was screened for its free radical scavenging activity. All the formulations especially F5 showed good anti-oxidant activity. The comparisons were made with marketed formulation FS. The IC₅₀ value by DPPH methods for diluted cream samples was 0.24±0.02 whereas by NO method, it was 0.38±0.014. When measured by H₂O₂ method, it was found to be 0.45 ± 0.03. All the formulations were stable and it was concluded that calendula oil sunscreen cream diluted formulation F55 is very pronounced antioxidant activity.

KEY WORDS: *Calendula officinalis*, antioxidant, Sunscreen cream, dispersed phase.

INTRODUCTION

Calendula officinalis (family Asteraceae) is an important plant of genus *Calendula* which is having several therapeutic applications in India and world wide⁽¹⁾. It grows widely in North America, Eastern Europe and Germany regions⁽²⁾. *Calendula* is fast growing annual herb that

is easy to germinate and simple to care⁽³⁾. Due to having unique properties and assisting nature in the cell rejuvenation, wound healing, reducing inflammation, soothing and softening the skin, its applicability is increasing in cosmetics. The extract of flowers, essential oil from

flowers is used in treatments of several ailments, mainly in dermatological diseases ⁽⁴⁾. In India, the oil extracted from flower, is consumed as edible oil ⁽⁵⁾. In Ayurvedic books, calendula oil is claimed for having very good potential to reduce the wound, scar and its emollient property has been proved recently ⁽⁶⁾. The tribal persons are using still calendula herb in form of decoction and in form of aqueous extract for treatment of pain ⁽⁷⁾. The calendula oil is having number of beneficial activities so far as it is used in cosmetics products. Keeping in mind these perspective and presence of polyphenolic flavanoids content in calendula oil, attempts were made to formulate O/W sunscreen cream and the calendula oil was used as dispersed phase in all the formulations with concentration ranging 1-5%. Topical antioxidants provide greater protection against environmental damage to the skin and may be somewhat effective in slowing down the skin aging ⁽⁸⁾. Antioxidants are chemicals that protect cells by neutralizing external forces (such as damage from the sun, pollution, wind and temperature) and internal factors (for example, emotions, metabolism and the presence of excess oxygen) ⁽⁹⁾. Common antioxidants are Vitamins A, C, E and beta carotene ^(10, 11). These special chemicals assist in skin repair and the strengthening of blood vessels. Antioxidants are necessary because they combat free radicals which cause skin damage ⁽¹²⁾. Free radicals attempt to gain an electron from natural proteins in the skin to acquire stability. Therefore, the structure of our skin is damaged and its cellular structure becomes weak. Free radicals also alter our DNA, which results in aging and illness ⁽¹³⁾. Scientist of dermatology are claiming that natural antioxidant in certain proportion, vitamins

and minerals can assist in our overall well being by combating the free radicals⁽¹⁴⁾.

Among body tissues particularly for aging, skin constitutes of the major targets, since it is exposed to external oxidant and free radical generators as UV light, chemical pollutants, oxygen, etc. Biological activity assessment of sunscreen cream formulation as antioxidant activity was performed. Sunscreen formula was adopted in present study because sunburn like conditions is originated from UV-B radiation by free radical mechanism ⁽¹⁵⁾. So for an effective sunscreen cream, the essential fact is that it must be having great potential to quench free radical.

The antioxidant activity of each formulation was evaluated with the assessment of concentration suitable for a good biological activity.

MATERIAL AND METHODS

Plant material

The fresh flowers of *Calendula officinalis* has been collected in January 2009 from botanical garden and park area of IFTM, Moradabad. The flowers were identified, authenticated and voucher specimen (No. IFTM/CO/1/2009) is kept in herbarium section of Phytochemistry laboratory, IFTM-Pharmacy College, Moradabad, India for future reference. The collected flowers were properly washed with tap water; petals were disaggregated (total weight 500 gm) and packed in distillation flask of Clavenger's apparatus with sufficient quantity of water and few pieces of porcelain chips to avoid bumping during distillation. The extraction was continued for six to eight hours. The

calendula oil was collected from graduated receiver and purified by anhydrous sodium sulphate in order to remove the water traces.

Reagents

1,1-diphenyl-2-picryl-hydrazyl (DPPH), Curcumin, EDTA, Ascorbic acid, perchloric acid were purchased from Sigma, St. Louis, USA. All other chemicals and reagents as sulphanilamide, hydrogen peroxide naphthylethylene diamine dihydrochloride, sodium nitroprusside, methanol and phosphoric acid used were of analytical grade. Other chemicals needed for formulations were of the highest grade commercially available.

Formulation of the Sunscreen as Cosmetic Products using calendula oil as dispersed phase

Five O/W sunscreen creams were preliminary prepared with the ingredients listed in Table 1. Physicochemical characters were determined by pH meter (Ri, India) and Viscometer Pro (Brookfield, USA) and found within the range. All of the creams were preliminary tested on physical stability by centrifugation assays at 10,000 ppm for 15 min under ambient temperature and found stable. All five creams which passed the stability test were further evaluated for skin irritation test which were carried out on 14 rabbits (white strains) and the creams were scored for erythema and edema. The result of skin irritation test showed no irritancy on skin surface.

All the five formulations with calendula oil (1-5%) were chosen for further

experiment. Further 0.5gm quantity of all the formulations were diluted with 10ml of dispersion phase (double distilled water) and mixed well in a way to get consistency. These diluted formulations were used as test samples in antioxidant activity evaluation and named F₁₁, F₂₂, F₃₃, F₄₄ and F₅₅ respectively. The standard O/W sunscreen cream for the experiment was a commercially available product purchased from local market of Moradabad distt and the diluted sample was named F_{SS}.

Free radical scavenging activity assay

DPPH method ⁽¹⁷⁾

The antioxidant activities were measured using the free stable radical, 1, 1-diphenyl-2- picrylhydrazyl (DPPH) of five equally diluted samples of O/W sunscreen cream formulations. One ml of 100 µM DPPH in methanol was mixed with equal volume of the diluted sample solution in phosphate buffer (pH 7.4), mixed well and the test tubes were kept in a dark room for 30 min. After incubation at 37°C for 30 minutes the absorbance of each solution was determined at 517 nm using double beam UV spectrophotometer. The corresponding blank reading were also taken and the remaining free radical of DPPH was calculated by using the following formula,

$$\text{DPPH radical scavenging activity (\%)} = \frac{[\text{Abs (control)} - \text{Abs (test)}] / \text{Abs (control)}}{\times 100}.$$

Where Abs is the absorbance and IC₅₀ value is the concentration of the sample required to scavenge 50% DPPH free radical. The assays were done in triplicate. An IC₅₀ values was obtained by plotting

means of % inhibitions (with SD) of each assay versus concentrations prepared by the dilution of each formulation.

Nitric oxide scavenging activity

Nitric oxide is implicated in inflammation, cancer and other pathological conditions. A potential determination of oxidative damage is the oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments. Sodium nitroprusside in aqueous solution at physiologic pH (7.4) spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions, which can be determined by use of Griess reagent. The method of Akiri SVC et al 2010 (18) was adopted to determine the nitric oxide radical scavenging activity of O/W sunscreen cream. Two milliliter of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of the equally diluted sample of formulation (1–5 % v/v). The standard sample was processed in same manner and all the mixture were incubated at 25°C. After 150 min, 0.5 ml of incubated solution was withdrawn and mixed with 0.5 ml of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was subjected for incubation at room temperature for 30 min. The absorbance was measured at 540 nm. The amount of nitric oxide radical was calculated by following this equation:

$$\% \text{ inhibition of NO} = [\text{Abs (control)} - \text{Abs (standard)}] / \text{Abs (control)} \times 100$$

Scavenging of Hydrogen peroxide

The ability of the formulations to scavenge hydrogen peroxide was estimated according to the method of Bhuiyan MAR. *et al* 2009⁽¹⁹⁾. A solution of hydrogen peroxide (25mmol/l) was prepared in phosphate buffer (pH 7.4). The sample of diluted formulation (1–5 % v/v) and diluted standard cream sample was added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was taken after 10 min against a blank solution having phosphate buffer in absence of hydrogen peroxide. For each concentration, a separate blank sample was used. The percentage scavenging activity of hydrogen peroxide by sample and standard formulations were calculated using the following formula,
$$\% \text{ scavenging activity [H}_2\text{O}_2] = [\text{Abs (control)} - \text{Abs (standard)}] / \text{Abs (control)} \times 100.$$

RESULTS

Free radicals are produced in body system as a result of oxidative reactions. This free radical may cause damage to cells and are responsible for aging and other disease condition like cancer, Alzheimer's disease that is life threatening. Flavonoids are chemical moieties which are usually antioxidant in nature ⁽²⁰⁾. Flavonoids scavenge free radicals by forming a stable radical that can react with another flavonoid radical to produce two non-radicals. Free radical scavenging activity of each extract was evaluated to obtain the IC50 were calculated (fig 2).

The diluted sample of F5 exhibited maximum antioxidant activity but it was lstandard O/W cream by all three methods. The F55 sample posed slightly more antioxidant activity than all the test sample and standard cream. The formulation with calendula oil in

concentration of 1%, 2%, 3%, 4% and 5% were prepared and numbered F1, F2, F3, F4 and F5 and further 0.5gm of each sample diluted with 10ml of water was subjected for free radical scavenging activity evaluation including diluted sample of standard cream diluted with similar pattern applicable for test sample. The diluted sample was named F11, F22, F33, F44 and F55 and the standard diluted sample was named as FSS. Dose-response curve of DPPH radical scavenging assay, H₂O₂ assay and NO assay method of all the samples are presented in fig 1. By the DPPH assay method, the F11, F22, F33, F44 and F55 showed 29, 43, 61, 75 and 86% antioxidant activity respectively, whereas blank and FSS sample posed 5% and 69% respectively. IC₅₀ value by DPPH assay method was 0.24 ± 0.02 (v/v). When focusing on suppression of NO, it may be partially associated with NO scavenging, as the herbal cream diluents reduced the amount of nitrite produced from the sodium nitroprusside *in vitro*. The free radical scavenging of NO by the test samples was observed in dose dependent manner. The F55 and FSS sample posed maximum activity 70.25% and 87.51% respectively in comparison to all other test samples. The IC₅₀ value by NO assay method for formulations was found to be 0.38 ± 0.014 (v/v). Small amounts of reactive oxygen species (ROS), as hydroxyl radicals (HO·), hydrogen peroxide radicals are constantly generated in aerobic organisms in response to both external and internal stimuli.

Scavenging of H₂O₂ by diluted samples of sunscreen cream may be related to their flavanoid content, which can easily release electrons to H₂O₂, thus neutralizing it to water. The samples were having a potent free radical scavenging activity in a concentration-dependent manner. F55 and FSS when evaluated for

H₂O₂ free radical scavenging activity, 56.78% and 52.65% inhibition was found. The IC₅₀ value for scavenging of H₂O₂ was 0.45 ± 0.03 (v/v).

DISCUSSION

Skin exposure to ionizing and UV radiation generates free radicals in excessive quantities that quickly overwhelm tissue antioxidants and other oxidant-degrading pathways⁽²¹⁾. Uncontrolled release of these free radicals are involved in the pathogenesis of a number of human skin disorders including sunburn, dermatitis etc. Therefore the cosmetic products, which are used for these conditions, must be having enough potential to quench the free radicals⁽²²⁾. In the present study, the O/W sunscreen cream was formulated incorporating the calendula oil in concentration ranging 1-5% and further the samples were diluted with aqueous phase. The diluted sample of test as well as standard cream were subjected to free radical scavenging activity by three methods and the concentration dependent antioxidant activity was found in formulations. It was observed that diluted sample of formulation F5 having 5% (v/v) concentration of calendula oil is more potent in quenching free radical as well as no significant difference was observed for standard sample in antioxidant activity. It was concluded that in the formulated cream, due to very good antioxidant activity, it may be used to treat the ailments which are generated from free radicals as sunburn etc.

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Table 1. Formula for Herbal O/W sunscreen cream ⁽¹⁶⁾

Ingredients	Concentration
Oleagenous phase	
Stearyl alcohol	15%
Beeswax	8%
Sorbitan monooleate	1.25%
Calendula oil	1 to 5% *
Aqueous phase	
Sorbitol solution 70% USP	7.5%
Polysorbate 80	3.75%
Methyl paraben	0.025%
Propyl paraben	0.015%
Purified Water, q.s. ad	100%

*For the formulation F₁,F₂,F₃,F₄ and F₅, the concentration of calendula oil was 1%,2%,3,5,4% and 5%.

Fig 2. Concentration dependent antioxidant activity of diluted O/W sunscreen cream by DPPH, NO and H₂O₂ assay method