Trop J Nat Prod Res, April 2020; 4(4):141-145 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Formulation and Evaluation of Sunscreen Cream Using *Detarium senegalense* Oil as Base

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ARTICLE INFO

Article history:
Received 12 March 2020
Revised 06 April 2020
Accepted 22 April 2020
Published online 30 April 2020

ABSTRACT

Exposure to solar radiation has the beneficial effects of stimulating the cutaneous synthesis of vitamin D and providing radiant warmth. Unfortunately, when the skin is subjected to excessive radiation in the ultraviolet range, deleterious effects may occur such as acute sunburn or solar erythema. This study aimed to formulate and evaluate sunscreen creams with photo protective and antioxidant activities using *Detarium senegalense* oil as a base. Six cream formulations (DC 1 to DC 6) were prepared using fusion method and characterized for pH, rancidity, thermal stability, viscosity, organoleptic tests and antioxidant properties. *In-vitro* determination of sun protection factor (SPF) by UV-spectrophotometer was carried out on all the formulations. The pH readings of the creams were found to be in the range of 5.40 and 6.80. DC 6 formulation had the highest spreadability factor of 14.22 ± 1.07 mm^2g^{-1} other formulations ranged from 11.45 ± 0.94 to 13.01 ± 1.11 mm^2g^{-1} for DC 1 to DC 5. The formulated creams showed excellent *in vitro* sun protection ability with SPF ranging from 11.97 for DC 2 to 15.88 for DC 4. Increase in the concentration of detarium oil in the formulation led to an increase in SPF. Topical application of the sunscreen antioxidant cream formulated with the oils of *Detarium senegalense* and *Citrus sinensis* will help protect the skin from damage caused by harmful ultraviolet radiation from the sun, as well as protect the skin from oxidative stress.

Keywords: Sunscreen cream, Detarium oil, Sun protection factor (SPF).

Introduction

Many topical dosage forms are intended for epicutaneous delivery of ingredients.[1] Some may also be inhalational, such as asthma medications, or applied to the surface of tissues other than the skin, such as eye drops applied to the conjunctiva, or ear drops placed in the ear, or medications applied to the surface of a tooth.[2] Sunscreen, also known as sun cream or sunblock, is a topical product that absorbs or reflects some of the sun's ultraviolet (UV) radiation and thus helps protect against sunburn, especially for fair-skinned individuals.[3,4] Diligent use of sunscreen can also slow or temporarily prevent the development of wrinkles and sagging skin. Depending on the mode of action, sunscreens can be classified into physical sunscreens (those that reflect the sunlight) or chemical sunscreens (those that absorb the UV light).[4,5]

Various systemic agents in the form of antioxidants, vitamins and minerals, designated as systemic sunscreens, have emerged as new photo-protective measures. The main goals of sunscreens are to protect against UV-B radiation and long wavelength UV-A radiation, scavenge reactive oxygen species (ROS), activate cellular repair systems and DNA repair.[6] Utilization of readily available oils possessing ethnopharmacological importance relating to anti-inflammatory activity, anti-ageing as well as skin regeneration is relevant for formulation of an ideal sunscreen topical formulation. Incorporation of natural free radical scavengers which can regress skin damage as a result of over exposure to UV-B radiation and long wavelength UV-A radiation is also essential in the design of the ideal sunscreen topical formulation.[7,8]

Detarium senegalense JF Gmelin is a leguminous tree in the subfamily Detarioideae. Unlike most members of the family, it produces globular fruits.[9] Oil from the seed of *D. senegalense* has antimicrobial activity and contains active principles such as linoleic and oleic acids which constitute 74.9% of the total oil content.[9-11] The oil has been utilized for its nutritional and organoleptic potentials, however its utilization as a pharmaceutical excipient will provide a novel base for topical product formulation. Citrus oil is rich in ascorbic acid - a water-soluble free radical scavenger,[12] vital for producing collagen, which is the substance responsible for giving skin it's firmness and elasticity. It is also useful for correcting pigmentation problems and reducing free radicals whilst providing UVA/UVB protection, decreasing pigmentation, reducing redness, and increasing collagen production.[12,13] It also acts as one of the most powerful antioxidants available for skin care, and improves the appearance of skin, prevents wrinkles, and is essential in cell proliferation.

The proposed formulation has the full potentials of serving as a good sun screening topical formulation which will protect the skin from harmful UV radiation from the sun. The chosen oils for this study, possess very key functions of moisturizing, softening, hydrating, and serving the skin antioxidant properties. These are desired properties in novel topical and cosmetic products.

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Citation: Ilomuanya MO, Ekerebe Z, Cardoso-Daodu I, Sowemimo A. Formulation and Evaluation of Sunscreen Cream Using *Detarium senegalense* Oil as Base. Trop J Nat Prod Res. 2020; 4(4):141-145. doi.org/10.26538/tjnpr/v4i4.5

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Materials

Stearic acid (Surfachem, UK), Polyethylene glycol (PEG) 200 (Niram chemicals, India), cetyl alcohol (Niram chemicals, India), Carbopol® 940 (Shree organics, India), Disodium Ethylenediaminetetraacetate EDTA (Ava chemicals, India), methyl paraben and propyl paraben (Sigma–Aldrich St. Louis, USA), Triethanolamine (TEA) (DBS Chemicals, India), detarium oil, *Citrus sinensis* oil (Neroli® essential oils USA), Water used in all the tests was Milli-Q water (Millipore, USA). All other chemical reagents and solvents were of analytical grade and were used for this research without further purification.

Extraction of the oils

The dried seeds of *D. senegalense* purchased from herb sellers in Oyingbo Market in Lagos State, Nigeria, were identified by Mr. Daramola at the Herbarium of the Department of Botany and Microbiology, University of Lagos. A voucher specimen (FHI 56829) was prepared and deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria.

D. senegalense seeds were oven-dried at 50°C overnight and ground to powder. The ground plant material (1 kg) was extracted cold with 3 L Petroleum ether (60 to 80°C) for 48 h. The extract was filtered and concentrated *in vacuo* at 30°C using the rotary evaporator (BUCHI Rotavapor™ R-100 Rotary Evaporator System) and stored in amber colored bottles at 8°C.

Formulation development

The components used were in a generally recognized as safe (GRAS) status. An oil in water-based cream was formulated via emulsification process. Briefly, the oil phase (PEG 200, detarium oil, stearic acid) and an aqueous phase were separately heated in a water bath to 80°C (Table 1). Afterwards, the aqueous phase was gradually added into the oil phase with constantly stirring. Carbopol 940 dispersed in water was used as the thickening agent and was introduced into the cream base. The mixture was stirred while cooling until the cream congealed at room temperature.

Evaluation of cream formulations

The formulated creams were evaluated for physical appearance, microscopic studies color change, extrudability, pH and viscosity utilizing the methods of Sarruf and D'Almeida.[8] Spreadability factor for all the creams was performed on samples immediately after preparation and after 90 days, in triplicate. A circular mold plate of glass (diameter = 20 cm, width = 0.2 cm) with a central orifice of 1.2 cm diameter, was placed on a glass support plate and positioned over millimetric graph paper. The formulated cream (0.5 g) was introduced into the orifice of the die plate and the surface levelled with a spatula. The plaque mold was carefully removed, and a glass plate of known weight was placed over the sample. After one minute, the diameter in opposing positions (as covered by the sample) was read with the aid of the graph paper scale and the subsequent average diameter calculated. This procedure was repeated successively adding other plates in one-minute intervals. Spreadability factor (S_f) was calculated as the total area (mm^2) divided by the weight applied (g).

$S_f = A/W$……Equation 1

Utilizing the method of Singh *et al*.,[1] with some modification, the formulations were tested for thermal stability to evaluate if there was oil separation from the creams when challenged at 60-70% RH and 37 ± 1°C in accelerated stability testing chamber. One gram of cream was introduced into a test tube and stored at 60-70% RH 37°C ± 1°C.

Rancidity

Rancidity occurs when free fatty acids are liberated during oxidation.[8] The formulations were tested using phloroglucinol solution. To 10 mL of sunscreen cream, 10 mL of concentrated hydrochloric acid and 10 mL of phloroglucinol solution were added and agitated for one minute. If a pink color developed, then the cream would be deemed as being rancid.

Microbial limit test

Using the World Health Organization (WHO) guidelines for product evaluation [14], microbial analysis was carried out for all the sunscreen cream formulations. The biological load was calculated as shown in Equation 2

$$\text{Number of bacteria} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of inoculum}}$$

……. Equation 2

Antioxidant activity of the cream using DPPH Assay

The radical scavenging activity of the sunscreen formulations against 1,1-diphenyl1-2-picrylhydrazyl (DPPH) radical via UV absorbance at 517 nm, utilizing ascorbic acid as standard and ethanol as control was carried out. The formulations were assayed spectrophotometrically using a modification of the method described by Omari *et al*.[15] The formulation (100 mg) was extracted with 100 mL absolute ethanol. To a methanol solution of DPPH (100 mmol/L, 2 mL), 2 mL of the test sample dissolved in ethanol was added at different concentrations (5-25 mg/mL). Absorbance was recorded at 517 nm after 30 min. The scavenging activity was calculated as shown in Equation 3. Ascorbic acid was used as a standard.

$$\% \text{ Scavenging activity} = \frac{(\text{Absorbance}_{517\text{control}} - \text{Absorbance}_{517\text{sample}})}{\text{Absorbance}_{517\text{control}}} \times 100$$

…… Equation 3

Table 1: Formulation table for ingredients for the sunscreen cream

Ingredients	DC 1	DC 2	DC 3	DC 4	DC 5	DC 6
Titanium dioxide	-	0.5	1.0	-	-	-
Stearic acid (g)	2	2	2	2	2	2
PEG 200 (g)	1	1	1	1	1	1
Cetyl alcohol (g)	0.5	0.5	0.5	0.25	0.25	0.25
Carbopol 940 (g)	0.5	0.25	0.5	0.5	0.25	0.5
Disodium EDTA (mL)	0.1	0.1	0.1	0.1	0.1	0.1
Methyl paraben (mL)	0.3	0.3	0.3	0.3	0.3	0.3
Propyl paraben (mL)	0.08	0.08	0.08	0.08	0.08	0.08
Triethanolamine (mL)	0.2	0.2	0.2	0.2	0.2	0.2
Detarium oil (mL)	7.5	7.5	10	10	7.5	15
Citrus sinensis oil (mL)	3.0	4.5	3.0	4.5	3.0	4.5
Distilled water q.s to (mL)	100	100	100	100	100	100

ISSN 2616-0684 (Print)
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In-vitro Determination of Sun Protection Factor (SPF) by UV-spectrophotometer

To 1000 mg of sunscreen cream, 100 mL of ethanol was added and transferred into a 200 mL volumetric flask. The flask was kept on ultra-sonication for 10 minutes, and the contents filtered using a 0.45 µm Millipore filter. An aliquot of 5 mL was transferred to a 25 mL volumetric flask and the volume was adjusted with ethanol to a final volume of 25 mL. The absorption spectra of samples in solution were obtained in the range of 290-320 nm in 1 cm quartz cell. Ethanol was utilized as blank. The absorption data were obtained in the range of 290-320 nm every 5 min interval and three determinations were made at each point. A marketed sunscreen product was also evaluated. The SPF of the formulated sunscreen formulations and the marketed formulations were calculated using Equation 4. The aliquot prepared were scanned between 290-320 nm and the obtained absorbance values were multiplied with the respective EE (λ) and I (λ) values. Then, their summation was taken and multiplied with the correction factor (CF)

$$SPF = CF \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad \text{.....Equation 4}$$

Accelerated stability testing

ICH guidelines (40ºC/75% RH) were followed in the accelerated stability testing of the sunscreen formulations. The creams were packed in amber coloured jars and kept in an accelerated stability chamber with set temperature and relative humidity. The formulations were subjected to accelerated stability testing at both room temperature and at 40°C and parameters were recorded on day 1, 30 and 90. The formulations were evaluated microscopically as well as for physical appearance, colour change, extrudability, pH and viscosity.

Statistical analysis

The data were expressed as mean ± SD. Differences between means were evaluated using one-way analysis of variance ANOVA) and Tukey's post hoc test. Significant difference was set at P value < 0.05.

Results and Discussion

Herbal medicines are considered safer than allopathic medicines as they are associated with less adverse effects such as contact irritation, local swelling, photosensitivity, itching, scaling, skin peeling and redness.[5,9] The oils of *Detarium senegalense* and *Citrus sinensis* have significant antioxidant activity and it is a well-known fact that natural antioxidants have beneficial effects on the aging process of the skin, sun protection and skin cancer.[9] Many other studies confirmed that an acute exposure of the human skin to excessive UV radiation (sun light) leads to oxidation of cellular biomolecules which cause cellular aging of the skin. This cellular aging can be prevented by a prior antioxidant treatment.[12-15] Hence, the increasing demand for herbal cosmetic products in the market.

The prepared creams were white to slightly off-white in color depending on the composition of opacifying components. They had pleasant citrus smell due to their citrus oil composition. The microstructures of the cream formulations were studied under a light microscope and their respective micrographs revealed the granular features of the creams (Figure 1). The bonding networks as well as intermolecular spaces between molecules as shown in Figure 1 were evenly distributed and staining showed that the creams composed of water-in-oil emulsions. There was absence of coalescence in the micrographs studied.

Evaluation of cream formulations

The pH of the creams was measured using a digital pH meter and the pH readings of the creams were found to be in the range of 5.40 and 6.80. This suggests that the prepared creams are less likely to cause skin irritation. Spreadability consists in the expansion of a semi solid formulation on a surface after a certain time, and its determination is important because topical products should easily spread on the skin surface.[16, 17]

During storage the spreadability of the creams may be altered especially at higher temperatures. Variations in spreadability results from the accelerated stability testing where the cream samples are exposed to different temperature ranges in order to assess the stability of the creams. Variations in spreadability are due to differences in temperatures at which the samples are tested, and for any formulation, the lower the viscosity, the greater its spreadability. DC 6 had the highest spreadability factor 14.22 ± 1.07 mm^2g^{-1}, the other formulations ranged from 11.45 ± 0.94 to 13.01 ± 1.11 mm^2g^{-1} for DC 1 to DC 5. There was decrease in spreadability for all the formulations with prolonged storage at day 30 and 90 (Table 2).

The creams were thermally stable and did not show any sign of phase separation after being exposed to accelerated stability testing at 60-70% RH 37°C ± 1°C. There was however a change in the color of some of the creams. DC 1, DC 2 and DC 4 showed color change but the spreadability, texture and smell of the formulations were not affected. The pH was seen to have been reduced in these formulations compared to the initial evaluation at day 0. All the creams were non-greasy and easily removed by washing with water.

Rancidity

Oxidation as a result of free fatty acid liberation is the main cause of rancidity. Reaction of these free fatty acids with Phloroglucinol solution gives a pink color. No pink color was observed in all the formulations, hence the absence of rancidity.

Standard acceptable limits for the presence of microorganisms in non-sterile pharmaceutical preparations is 10^1 cfu/mL and 10^2cfu/mL for total combined yeast and mold count (TYMC) and total aerobic microbial count (TAMC), respectively.[14] Table 3 shows that the results for the microbial limit test for all six formulations met the acceptable criteria for TYMC and TAMC. Formulations DC 1 to DC 5 had total aerobic microbial counts (TAMC) of 1×10^2 cfu/mL and DC 6 had TAMC of 2×10^2 cfu/mL. Formulation DC 1 had total yeast mold counts (TYMC) of 1×10 cfu/mL. The other formulations did not show any yeast or mold growth. Microbial limit tests were also carried out for specific susceptible pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. No growth was found in any of the inoculated plates; thus, the standards were met by all the creams.

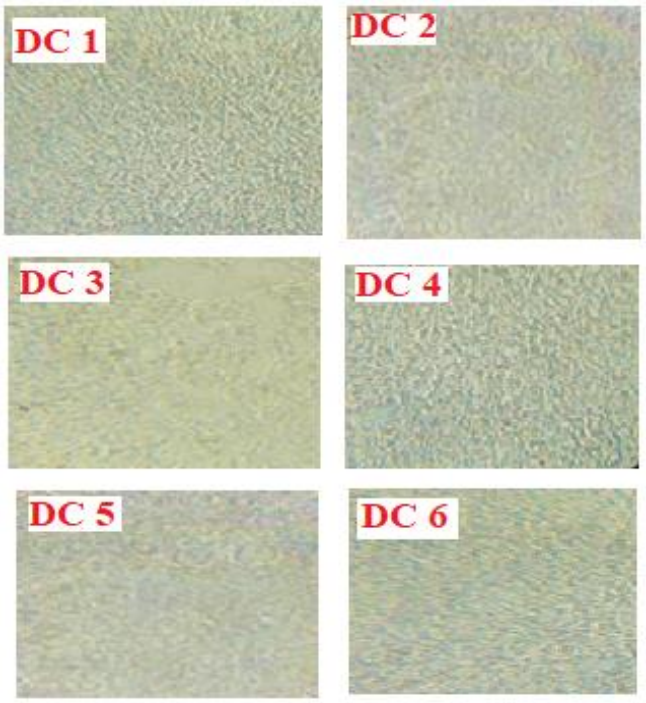

Figure 1: Photomicrographs of the varying detarium cream formulations

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The presence of specific pathogens would have otherwise meant that the creams were unsafe for use and could cause bacterial infections on the skin. All six creams conformed to standard as they were prepared aseptically to avoid contamination of the creams during formulation.

Antioxidant activity of the cream using DPPH Assay

The DPPH radical scavenging activity was assessed for different concentrations of the Detarium oil. DPPH radical reacts with suitable reducing agents which lose color stoichiometrically and the number of electrons consumed are measured spectrophotometrically at 516 nm. The radical scavenging activity of the detarium oil was 73.13% at a concentration of 100 mg/mL. Thus, showing that detarium oil possessed DPPH radical scavenging activity. The percentage DPPH radical inhibition was higher in all formulations than detarium oil alone due to the additive effect of citrus oil which already has confirmed DPPH radical scavenging activity. Hence as the *Detarium senegalense* oil performs its skin-protective function, the *Citrus sinensis* compliments its effect with a good skin-repairing antioxidant and antibacterial property.

In-vitro determination of Sun Protection Factor (SPF) by UV-spectrophotometer

The formulated creams showed excellent *in vitro* sun protection ability with SPF ranging from 11.97 for DC 2 to 15.88 for DC 4. Increase in the concentration of the Detarium oil in the formulation led to an increase in SPF. After storage for 90 days, it was observed that DC 1 and DC 4 which showed color change unrelated to formulation rancidity. DC 1 and DC 4 however had a higher SPF factor on storage despite the color change which was observed.

Table 2: Accelerated stability testing on the polyherbal face creams

Time in days	Formulation	pH	Dynamic Viscosity mPas at 40rpm	Spreadability factor (S_f) (mm^2g^{-1})	Homogeneity and Appearance	SPF
DAY 0	DC 1	6.33 ± 0.05	6134 ± 11.2	12.34 ± 1.02	G/NCC	13.42
	DC 2	6.24 ± 0.11	6844 ± 9.7	11.45 ± 0.94	G/NCC	11.97
	DC 3	5.40 ± 0.09	7854 ± 10.1	11.76 ± 2.3	G/NCC	15.97
	DC 4	5.57 ± 0.07	6944 ± 6.6	11.54 ± 2.06	G/NCC	15.88
	DC 5	6.23 ± 0.05	5993 ± 7.3	13.01 ± 1.11	G/NCC	13.93
	DC 6	6.80 ± 0.01	6999 ± 12.2	14.22 ± 1.07	G/NCC	13.87
DAY 30	DC 1	6.30 ± 0.10	6102 ± 10.2	12.34 ± 1.02	G/NCC	13.42
	DC 2	6.24 ± 0.09	6800 ± 15.3	11.45 ± 0.94	G/NCC	11.97
	DC 3	5.40 ± 0.11	7805 ± 11.2	11.76 ± 2.3	G/NCC	15.97
	DC 4	5.57 ± 0.23	6940 ± 5.4	11.54 ± 2.06	G/NCC	15.88
	DC 5	6.10 ± 0.16	6000 ± 7.1	13.01 ± 1.11	G/NCC	13.93
	DC 6	6.70 ± 0.01	6901± 11.7	14.22 ± 1.07	G/NCC	13.87
DAY 90	DC 1	6.30 ± 0.10	6102 ± 10.2	11.94 ± 1.12	G/CC	14.99
	DC 2	6.24 ± 0.09	6800 ± 15.3	11.01 ± 0.79	G/NCC	11.97
	DC 3	5.40 ± 0.11	7805 ± 11.2	11.10 ± 1.5	G/NCC	15.97
	DC 4	5.57 ± 0.23	6940 ± 5.4	10.99 ± 1.89	G/CC	16.87
	DC 5	6.10 ± 0.16	6000 ± 7.1	12.91 ± 1.03	G/NCC	13.93
	DC 6	6.70 ± 0.01	6901 ± 11.7	13.01 ± 1.1	G/NCC	13.87

KEY: G – Good; B – Bad; CC – Color Change; NCC – No Color Change

Table 3: Microbial limit test of the *Detarium senegalense* oil-based sunscreen cream

Formulation	TAMC (cfu/mL)	TYMC (cfu/mL)	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus aureus*
DC 1	1×10^2	1 × 10	Absent	Absent	Absent
DC 2	1×10^2	Absent	Absent	Absent	Absent
DC 3	1×10^2	Absent	Absent	Absent	Absent
DC 4	1×10^2	Absent	Absent	Absent	Absent
DC 5	1×10^2	Absent	Absent	Absent	Absent
DC 6	2×10^2	Absent	Absent	Absent	Absent

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

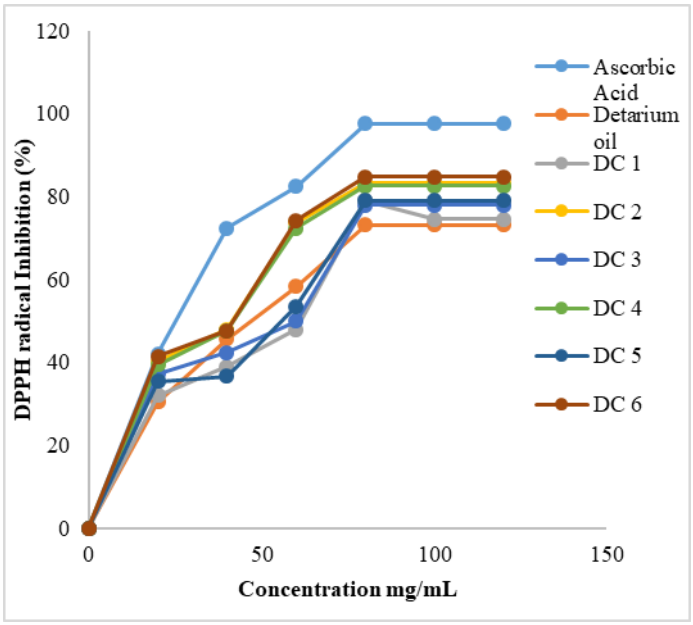

Figure 2: DPPH Radical Scavenging activity of the sunscreen cream formulations utilizing ascorbic acid as the antioxidant standard.

Conclusion

The study confirmed the antioxidant activity of *Detarium senegalense* oil by determination of its scavenging activity using DPPH Assay. Detarium oil, however, was used in combination with citrus oil, based on the synergism of their antioxidant activities as well as the sunscreen effect of the oil base. Topical application of the developed detarium oil-based sunscreen antioxidant cream will help protect the skin from damage caused by harmful ultraviolet radiation from the sun, as well as protect the skin from oxidative stress.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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