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Photoabsorbent, Antioxidative, and Anti-inflammatory Properties of Three Traditional Cosmetic Seed Oils

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ABSTRACT

The use of sunscreens helps protect the skin against effects of ultraviolet rays emitted by the sun such as, oxidation, burns, and inflammation. These sunscreens are mostly made of several chemical molecules that can induce undesirable reactions. For that, we aimed by the current study to evaluate the UV absorption, antioxidative action and anti-inflammatory effects of three oils from seeds of *Brassica rapa*, *Raphanus sativus* as well as *Cucurbita maxima* for possible use as sunscreen. The results obtained indicate that the three oils showed more absorption in UVB than UVA where we find that Brassica oil induce a maximum absorption of 90% in UVB and 75% in UVA compared to sunscreen SPF 50. In addition, an important antioxidant activity was observed by DPPH assay with maxima more than 80%**,** the IC_{50} for oils were 1.2% for *Brassica* and *Raphanus* and was 1.5% for *Cucurbita*. Furthermore, the three oils exhibited an inhibitory effect on spleen cell proliferation reaching a maximum inhibition of 39%. These finding, for the first time, shed light on the UV-absorption properties of seed oil from *Brassica, Raphanus* and *Cucurbita.* These properties allow the development of a new sunscreen formulation using oils from these seeds for skin treatment and protection.

***Keywords*:** Anti-inflammatory, Antioxidant, Oil, Sunscreen, UV radiation.

Introduction

The ultraviolet (UV) radiations emitted by the sun and many artificial sources used in industry and commerce, are divided into three types, notably UVA, UVB and UVC. Their low wavelength gives UV rays more energy, going as far to cause burns on the human body. If UVA rays are beneficial by increasing the production of vitamin D3 by irradiating 7-dihydrocholesterol, UVB radiations are more dangerous because their energy could cause inflammatory reactions and accelerate ageing of the skin by loss of elasticity and by the accumulation of oxidizing molecules.[1] Prolonged and repetitive exposure of skin to UV could also favourite dysregulation of the genetic material of cells which can induce skin cancers.[2] The use of sunscreens helps us to protect the skin against the harmful effects of UV radiation by absorbing (partially or totally) UV. Most of the commercially available sunscreens are made from several chemical molecules formulated to achieve protection. But natural products remain the main source of new UV blockers that can protect skin against undesirable UV effects. For this study, we were interested in three seeds traditionally used by Moroccans in their cosmetics.[33] We have studied here the seeds of *Brassica rapa var.rapa* of *Raphanus sativus* as well as of *Cucurbita maxima. Brassica rapa var.rapa* belongs to the family of Brassicaceae. This family is known to contain numerous compounds with antioxidative properties.[3,4,5,6] *Raphanus sativus* is a part of the same family, Brassicaceae, commonly known as radish, and is widely consumed as a vegetable or condiment in human diets throughout the world.[7]

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which described antioxidant,[6] anti-inflammatory and antitumor activities,[7,9] antimicrobial antiviral and antimutagenic as well.[10]
Finally, *Cucurbita maxima* is included in the family Cucurbitaceae which was characterized by different effects like antioxidant activity,[11,12,] and has been identified as an exceptional prevention against hypertension and carcinogenic diseases.[12]
These oils were traditionally used by Moroccans to protect their face and hair. This study aimed to evaluate the cosmetic properties of these oils by exploring the UV absorption potential of the oils, their antioxidant effects and their anti-inflammatory action on immunity in cells.

Materials and Methods

Animal materials
Zemmouri male rabbits weighing 2.5 kg was used for all investigations *in-vitro*. Rabbits were distributed in cages under a 12h light/dark cycle in a temperature-controlled room (22 to 24°C). Rabbits had free access to standard chow and water. All studies were carried out according to ethical rules and guidelines of animal care as prescribed by national ethical standards.

Preparation of extracts
Seeds of *Brassica rapa var L rapa* (turnip), *Raphanus sativus, and Cucurbita maxima* were purchased from herbalists and fully identified by botanists. The voucher specimen of each plant was provided under ID-numbers (BLMUP). All preserved specimens were deposited in the herbarium of Department of biology, Faculty of Sciences and techniques, University of Sidi Mohamed Ben Abdellah, Fez. Then washed twice using distilled water and desiccated at 40°C until stabilization of weight. Afterwards, they were ground to a fine powder for extraction. The oil powder was separated using petroleum-ether extraction. After, evaporation (of the solvent using a rotary evaporator) the lipids were stored at -20°C until use.

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UV Absorbance

The UV absorbance of the three oils was measured *in-vitro* by preparing diluted samples at 1, 5, and 10% concentrations in ether. Then absorbance values were determined from 290 nm to 400 nm wavelength corresponding to the UVB and UVA regions (*Rayleigh UV-9200)*. Under the same conditions, we determined the UV absorbance of a commercial sunscreen with a sun protection factor (SPF) of 50.

DPPH assay

The antioxidative effects were measured using a scavenging test for oil seeds using 2.2-diphenyl-1-picrylhydrazyl (DPPH) with slight modifications.[14,15,16,17] In short, the oil was diluted at 1, 5, and 10% in methanol. Then 2 ml of each dilution was mixed with 2 ml of DPPH solution (0.5 mM in Methanol). The absorbance of the reaction mixture was measured after 30 min incubation in the dark at room temperature at 517 nm. Each condition was made in triplicate (n=3) and at least three experiments were repeated (N>3) at different times. For comparison, we have used BHT and ascorbic acid as positive standards. The antioxidant activity was calculated as follows:

Antioxidant activity % = [(Abs (control) – Abs (sample)) / Abs (control)] × 100

The control was realized by the DPPH solution alone as the maximum of absorption.

Immunity Cell culture

Cell suspensions used in this study were obtained from the rabbits after being sacrificed. The spleen was removed aseptically from animals and cell suspensions were prepared by pressing the organs through a fine wire mesh as described in the previous study.[18-20] These Cells were washed by RPMI and the red blood cells were lysed by 154 mM Ammonium Chloride. The number of viable cells was determined microscopically by the trypan blue 0,1% exclusion test.

The culture used RPMI medium supplemented with 2 mM glutamine, 10% of serum,antibiotics (ampicillin 100 U/ml and streptomycin 100 mg/ml), and antifungal (Fluconozole 2mg/ml).

Cell proliferation assay

Cell proliferation was measured by the MTT assay according to,[21,22] and as described in Daoudi. [23,2418] In short, cells were plated at 150,000 cells/well in 96 well plates and incubated at 37°C in a humidified chamber under an atmosphere of 95% air and 5% CO_2 for 72 hours. Oil extracted were added (at desired concentrations) to cells before their incubation. At incubation time, 10 µL of MTT solution (5 mg/ml in PBS) were added and cells incubated three hours before addition of DMSO to dissolve the formazan formed. Finally, the optical density was measured through wavelength at 570 nm using the spectrophotometer (Bio Tek L800).

Statistical analysis

Each experimental condition was realized in triplicates (n=3). Data were expressed as the mean ± standard deviation (S.D.). Statistical analyses were carried out using the student's t test. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

The purpose of this study was to explore the possible use of three oils as sun protection. For that, we have evaluated the UV absorption, antioxidative action and anti-inflammatory effects of three oils from seeds of *Brassica rapa, Raphanus sativus* as well as *Cucurbita maxima.*

UV absorption properties

Figures 1 and 2 display the UV absorption of oils extracted from seeds of *Brassica rapa var rapa, Raphanus sativus, and Cucurbita maxima.* These results indicated that the three oils showed more absorption of UVB than UVA. In UVB zone, we observed that *Brassica* oil induced an absorption equal to that observed with sunscreen SPF 50 with a maximum which reached 3.05. This high absorption of *Brassica* oil was followed by *Cucurbita* oil which reached 3.02 and finally *Raphanus* oil which was less with a partial absorption of UVB and a maximum of 2.4.

In UVA zone, we observed less absorption of these oils compared to UVB. But *Brassica* oil exhibited more effect and showed a partial absorption of UVA with a maximum of 2.35 where the other oils didn't show any significant absorption compared to sunscreen SPF 50 which fully absorbed in UVA and UVB zones with highly values.

It observed that *the three* oils had a significant UVB absorption potential, but weak UVA protection. These results are reported for the first time with these oils and it is in accordance with other research, which confirmed that some plant oils contain natural sunscreens that are considered to be able to block the UV light equal to that observed by a commercial sunscreen of SPF 50. This property is in accordance with other plant oils such as sesame oil, which is claimed to resist to 30% of UV rays, whereas coconut, peanut, olive, and cotton seed oils block out about 20%.[2] Oil of Raphanus displayed an UV-absorption important in UVB zone and non-significant in UVA zone.

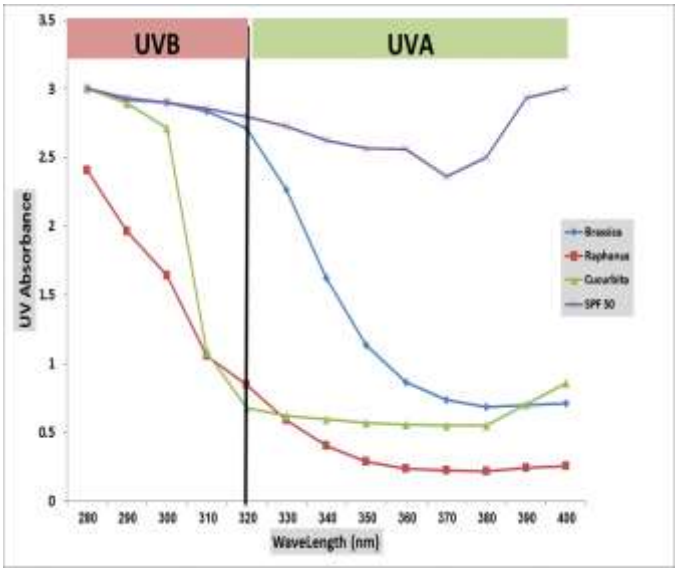

Figure 1: The UV absorption spectrum of the three oils seeds

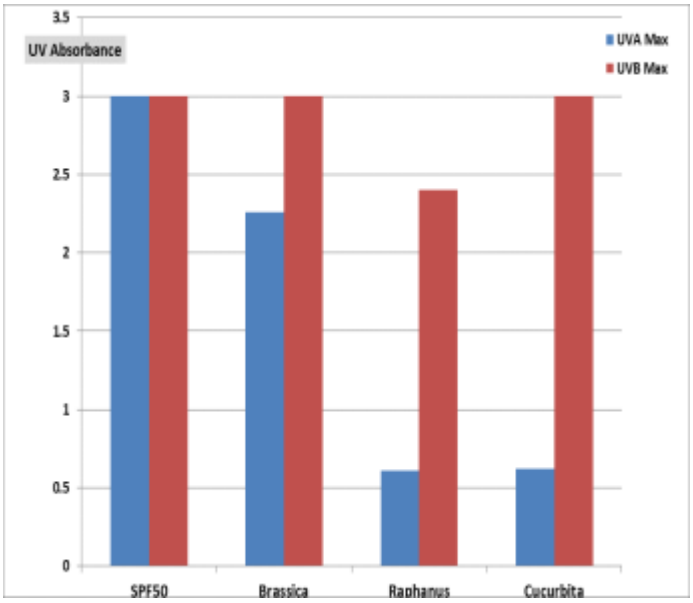

Figure 2: Maxima absorbances of the three oils seeds compared to sunscreen of SPF 50

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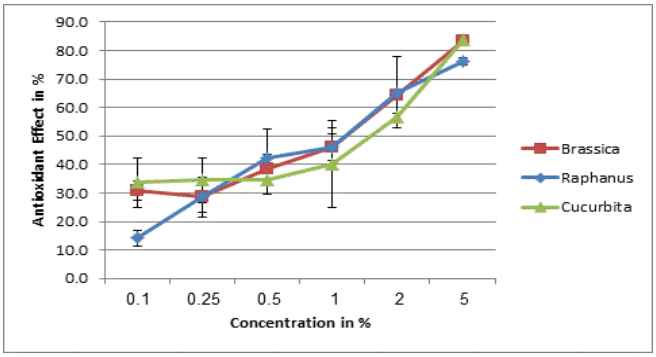
This oil possessed an antioxidant effect and anti-inflammatory action. These properties were reported here for the first time and in literature, we recorded a photoprotection, antioxidative and anti-inflammatory effects only for the methanolic extract of radish seeds and roots. [27,37] *Cucurbita* oil exhibited an important absorption in UVB zone however in UVA zone it didn't show any significant absorption, our finding is in accordance with result obtained from other species such as *Cucurbita* pepo oil seed that blocks only 22% of the hottest UVB. [28] In addition, the SPF value obtained with aqueous extracts of *C. reticulata, C. moschata and C. ternatea* were 10.82, 11.54 and 23.13 respectively in other studies that provide evidence of sun protective activity.[29]

Antioxidant effect of oils

Figure 3 shows the antioxidative effect of the oils of the three seeds *Brassica rapa var rapa, Raphanus sativus,* and *Cucurbita maxima* compared to BHT, ascorbic acid, and vitamin E. It observed that the three oils exhibited an important antioxidant effects that increases as a function of the concentration from 0.1% to 5%. We reached for the three oils maxima more than 80%. These antioxidative effects were similar to maxima recorded with standards (BHT, Ascorbic acid and E-Vit). But the IC_{50} were high for oils than standards. IC_{50} for oils were 1.2% for *Brassica* and *Raphanus* and was 1.5% for *Cucurbita* compared to Standard's IC_{50}s which were 0.55 %, 0.065% and 0.085% for Vit-E, BHT and ascorbic acid. Moreover, the oil of *Brassica Rapa* had an important antioxidant activity, which can fight against the oxidation caused in the skin cells by ultraviolet solar radiations. This antioxidant capacity of these oil increases as a concentration-dependent manner. These results are reported for the first time. However, Turnip edible parts as flower buds, leaves, stems and roots also exhibited an important antioxidant potential. [3,25,26,37] Further, *Cucurbita* oil show an important antioxidative effect, the IC_{50} was 1.5% high for oils than Standard's IC_{50}s that were 0.55 %, 0.065% and 0.085% for Vit-E, BHT and ascorbic acid. This inhibition indicate that these oils blocked the cell proliferation and consequently immunity reactions induced by the three immunity cell populations. This leads to suppression of inflammatory reactions which are a part of immunity reactions.



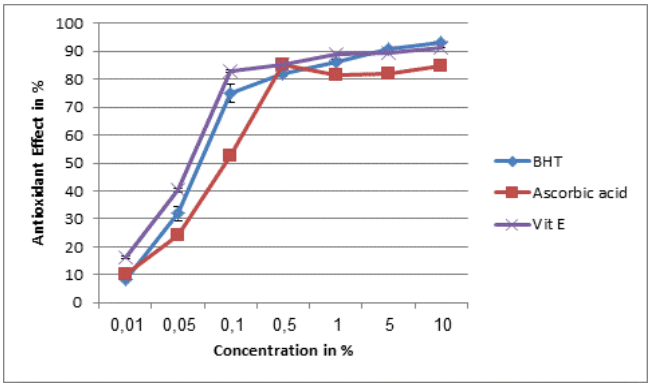


Figure 3: Antioxidative effects of the three oils seeds compared to BHT, Ascorbic acid and vit E.

Anti-inflammatory effect

Figure 4 describes the result obtained with the three oils on the proliferation of spleen cells. Spleen cells are a mixture of immunity cells composed of B and T-lymphocytes and macrophages. We observed that oils exhibited an inhibitory effect on spleen cell proliferation. These inhibitory effects were observed with the low concentrations (0.25%) and reached a maximum of inhibition of 39% for Brassica oil, the same for proliferation of thymocytes (Figure 5).

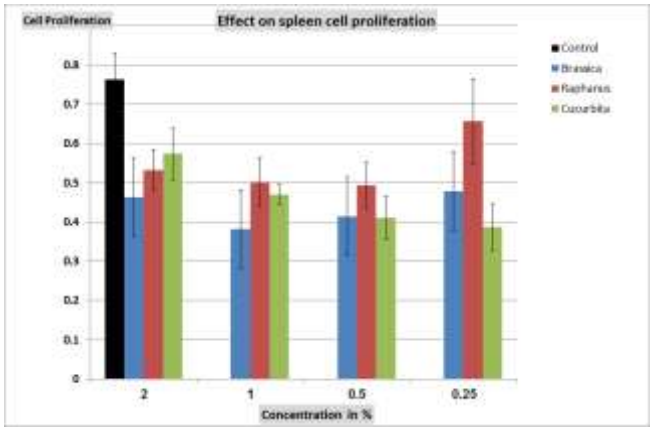


Figure 4: Effect of the three oils seeds on the proliferation of spleen cells.

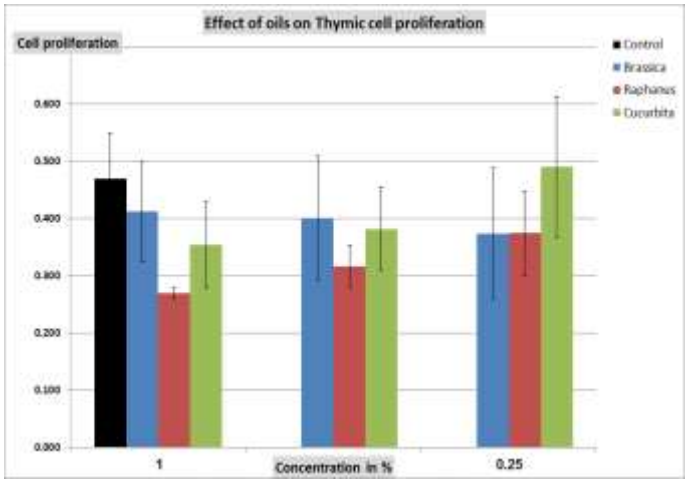


Figure 5: Effect of the three oils seeds on the proliferation of thymocytes.

These results indicate that the addition of *Brassica* lipids reduces the multiplication of B lymphocytes by more than 50%. This indicates an immunosuppressive activity and therefore anti-inflammatory effect. This anti-inflammatory effect was probably related to the unsaturated acid erucic acid mostly present in Brassica rapa seeds as demonstrated by Velasco and Becker 1998. [36] It was indicated before that the unsaturated fatty acids could block the synthesis of inflammation mediators. [36] The inhibitory effect of *Cucurbita* oil on spleen cell proliferation is compatible with research carried out by. [30,31,32] that *Cucurbita maxima Linn* seed oil showed an anti-inflammatory activity to more than 89.68 ± 0.78%.

Conclusion

The results obtained in this study demonstrates an important potential photoprotection of these oils against UV rays combined to antioxidative effects and anti-inflammatory actions.

These properties, in addition to lipophilic oil properties, which protect skin against desiccation, showed a possible use of the three seed oils in the development and production of a new sunscreen formulation for skin treatment and protection against solar rays.

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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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