



Antioxidant Properties of Some Plants Extracts Used As Natural Sunscreen in the Formulated Cream

[Propriétés antioxydantes de quelques extraits de plantes utilisés comme écran solaire naturel dans la crème formulée]

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Abstract

The aim of the present research is to study the antioxidant properties of eight seeds [carrot, *Moringa oleifera*, soya bean, coconut, *Cucurbita maxima* (Ccm), palm walnut (Pmw), *Vigna unguiculata* called niebe and *Citrullus lanatus* (Ctl)] extracts using radical DPPH as model in view of their possible application as antisolar agents. Ethyl acetate seed extracts were prepared with methanol as solvent and the absorbance were recorded after reaction with the radical DPPH using UV-vis spectrophotometer. The EC₅₀, the antiradical power (ARP) and the stoichiometry of eight seeds extracts were determined. It was noticed that three of the tested herbal extracts namely carrot, Pmw and niebe showed some antioxidant properties. The carrot extracts gave the better results with an EC₅₀, an ARP and a stoichiometry of 1.12, 0.89 and 0.45 respectively. Five seeds extracts (Ccm, moringa, Ctl, coconut and soya) didn't show antioxidant properties. Thus, the carrot extracts are most active whereas five samples did not show reactivity towards radical DPPH.

Keywords: Antioxidant properties, EC₅₀, antiradical power (ARP), *Citrullus lanatus*, *Moringa oleifera*

Résumé

Le but de la présente recherche est d'étudier les propriétés antioxydantes de huit extraits de graines [carotte, *Moringa oleifera*, soja, noix de coco, *Cucurbita maxima* (Ccm), noix de palme (Pmw), *Vigna unguiculata* appelée niebe et *Citrullus lanatus* (Ctl)] en utilisant le radical DPPH comme modèle en vue de leur possible application en tant qu'agents antisolaires. Les extraits de graines en acétate d'éthyle ont été préparés avec du méthanol comme solvant et l'absorbance a été enregistrée après réaction avec le radical DPPH à l'aide d'un spectrophotomètre UV-vis. La CE₅₀, le pouvoir antiradicalaire (ARP) et la stoechiométrie de huit extraits de graines ont été déterminés. Il a été constaté que trois des extraits de plantes testés, à savoir la carotte, le Pmw et le niebe, présentaient des propriétés antioxydantes. Les extraits de carotte ont donné les meilleurs résultats avec une EC₅₀, un ARP et une stoechiométrie de 1.12, 0.89 et 0.45 respectivement. Cinq extraits de graines (Ccm, moringa, Ctl, noix de coco, soja) n'ont pas montré de propriétés antioxydantes. Ainsi, les extraits de carotte sont les plus actifs alors que cinq échantillons n'ont pas montré de réactivité vis-à-vis du radical DPPH.

Mots-clés : Propriétés antioxydantes, EC₅₀, pouvoir antiradicalaire (ARP), *Citrullus lanatus*, *Moringa oleifera*

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1. Introduction

The skin is the body's first line of defense for external exposure. The harmful effects of solar radiation are caused predominantly by the ultraviolet (UV). An over exposure of human skin to ultraviolet light produces several adverse effects including mutagenicity, immune depression of the skin, photodermatoses, sunburn cells, premature skin aging and an increased risk for skin cancers. Solar ultraviolet radiation is divided into three categories UV-A (400-320 nm), UV-B (320-280) and UV-C (280- 100 nm). The most energetic radiation UV-C has been filtered out by the atmosphere before reaching earth. UV-B radiation is not completely filtered out by the ozone layer and is responsible for causing the adverse effects of the UV radiation. UVB radiation may promote a deficit in the immunologic functions of the skin in the addition to the anomalies in the DNA. UVA radiation reaches the deeper layers of the epidermis and the dermis and is responsible for causing premature skin aging. Due to these facts, sunscreens substances are incorporated nowadays into everyday products such as moisturizers, creams, lotions, shampoos, mousses, and other hair and skin preparations and the application of sunscreen formulation to the skin changes the way the body reacts to the sun rays (Mbanga et al., 2015; More et al., 2013).

There are chemical filters, physical blocker and natural filters that have the UV properties. The UV filters must be safe. Inorganic sunscreens are cosmetically unacceptable because of their opaque quality and being occlusive. It was reported that the salicylates (organic sunscreen), the first agent used in sunscreen preparations, have been increasingly for allergic and contact dermatitis, phototoxic and photo-allergic reactions, contact urticaria producing skin rash notable for pale red, raised, itchy bumps and even solitary cases of severe anaphylactic reactions and also showed ability to interfere only in selected pathways of multistage process of carcinogenesis (Mbanga et al., 2015; Kale et al., 2011; More et al., 2013).

There are persons suffering from skin hypersensitivity who don't want to use chemical sunscreens due to concern about skin exposure to unknown chemicals. Although a variety of hypoallergenic cosmetic products have been introduced for customers with sensitive skin, there are still limited options in sunscreen agents.

At this effect, the development of herbal sunscreen agents which are effective with less or no side effects is wanted. Wholes herbal extracts consist

of numerous compounds that together provide better effects on the skin. Chemical research showed that the plants are rich source of glycosides, linoleic acid, flavonoids. Nowadays, because of benefits of products containing natural compounds and compliance of user of these products, use of the natural compounds that can absorb ultraviolet radiation is of great interest (Shenekar et al., 2014; Mbanga et al., 2015; More et al., 2013).

It is evident that the plant has great potentials in treating a number of ailments where the free radicals have been reported to major factors contributing to the disorders. Earlier studies have also shown that the plant possesses good potential related to various skin diseases such as anti-inflammatory, antibacterial, antifungal; wound healing (Mbanga et al., 2015; More et al., 2013).

The dermo-cosmetic natural simply proposes to discover and learn how to use the capacities of the plants to get for our skin the simple ingredients which the skin needs. The plants can play thus multiple roles of which that of natural filters anti-UV through their high content in antioxidants (vitamin C, vitamin E, glutathion, uric acid, β -carotene and some enzymes), (Shenekar et al., 2014; Vertuani et al., 2013). One herbal extract may show antioxidant, anti-inflammatory, emollient, melanin-inhibiting, antimutagenic, antiaging properties. Herbal sunscreens products are safe, widely accepted by consumers and also work in various ways, playing multiple roles in improving the process of carcinogenesis (Mbanga et al., 2015; More et al., 2013).

Thus, we resorted to nature to find the solution with the operational limits of the synthetic filters for people with skin sensitive by evaluating activity anti-UV of the extracts of eight local plants to through the determination their antioxidant capacity. This parameter is a significant factor of the effectiveness of the plant extracts which can be used in dermo-cosmetic.

2. Matériel et méthodes

2.1. Recation DPPH radical-ascorbic acid and seeds extracts

In this study, we used the extracts of plants obtained with the ethyl acetate after delipidation. Radical DPPH used is a Merk's product, the ethyl acetate; the methanol and the ascorbic acid are products pro analyzes and are provided by BDH prolabo. Measurements of absorbance of radical

DPPH are made with a spectrophotometer, models Hitachi U-3900 H.

2.2. Experimental procedure

For studying the reaction between the seeds extracts and the radical DPPH, a methanolic solution of radical DPPH of unknown concentration is prepared. For the ascorbic acid (standard solution) and seeds extracts, a range of concentrations of DPPH in methanol is prepared (C₁ concentrations, C₂, C₃... in ppm). We then mixed 0.5 ml of methanolic solutions of the ascorbic acid or of seeds extracts of various concentrations to 3.5 ml of methanolic solution of the radical DPPH. Finally, we recorded the absorbances of radical DPPH for these various mixtures at 515 nm.

3. Resultats

3.1. A scorbic acid solution and carrot extracts

Table 1. Absorbances and percentages of radical DPPH in it reaction with the ascorbic acid and carrot extracts

C.V _{AAc} /C.V _{DPPH}	Ascorbic acid		C.V _{extracts} /C.V _{DPPH}	Carrot extracts	
	Absorbances	%		Absorbances	%
	Radical DPPH	Radical DPPH		Radical DPPH	Radical DPPH
0.000	1.066	100.00	0.000	0.808	100.00
0.123	0.781	73.26	0.408	0.625	77.35
0.246	0.500	46.90	0.816	0.492	60.89
0.369	0.317	29.74	1.224	0.373	46.16
0.492	0.154	14.44	1.632	0.354	43.81
0.614	0.045	4.22	2.041	0.238	29.45
0.737	0.040	3.75	2.449	0.084	10.40
0.860	0.022	2.06	2.857	0.073	9.03

While carrying on graph the percentage of the radical DPPH according to ratio C.VAAC/C.VDPPH, we obtained the figure 1 that represents the curve of EC₅₀ of the ascorbic acid.

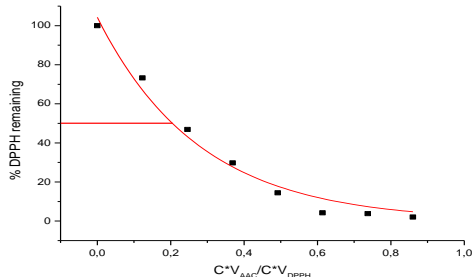


Figure 1. Curve of determination of EC₅₀ of the ascorbic acid

While carrying on graph the percentage of the radical DPPH according to ratio C.V carrot extracts / C.VDPPH, we obtained the figure 2 illustrating the curve of EC₅₀ of carrot extracts.

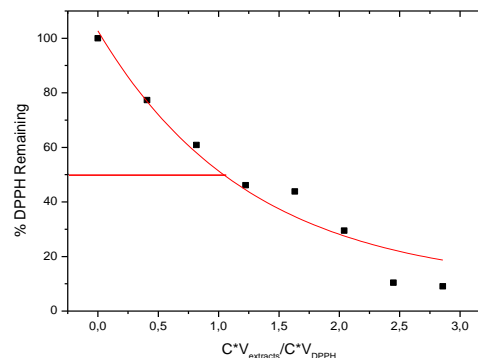


Figure 2. Curve of determination of EC₅₀ of the carrot extracts

3.2. Three seeds extracts

Table 2. Absorbances and percentages of radical DPPH in it reaction with three seeds extracts

C.V _{extracts} /C.V _{DPPH}	Niebe		Pmw		Soya	
	Absorbances	% radical DPPH	Absorbances	% radical DPPH	Absorbances	% radical DPPH
0.000	0.546	100.00	0.546	100.00	0.546	100.00
0.096	0.411	75.27	0.400	73.26	0.519	95.05
0.191	0.383	70.15	0.382	69.96	0.507	92.86
0.382	0.351	64.29	0.378	69.92	0.495	90.66
0.765	0.331	60.62	0.367	67.22	0.483	88.46
1.529	0.318	58.24	0.308	56.41	0.477	87.36
3.058	0.296	54.21	0.260	47.62	0.462	84.62
6.116	0.255	46.70	0.140	25.64	0.449	82.23

The curves of EC₅₀ of niebe, palm walnut (Pmw) and soya extracts represented in figure 3

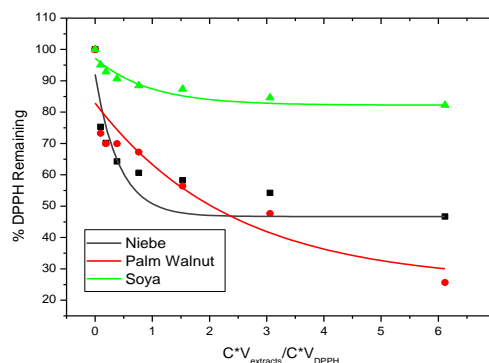


Figure 3. Curves of determination of EC₅₀ of three seeds extracts

3.3. Four seeds extracts

Table 3. Absorbances and percentages of radical DPPH in it reaction with four seeds extracts

C.V _{extracts} /C.V _{DPPH}	Cem		Moringa		Ctl		Coconut	
	Absorb.	% radical DPPH	Absorb.	% radical DPPH	Absorb.	% radical DPPH	Absorb.	% radical DPPH
0.00	0.546	100.00	0.546	100.00	0.546	100.00	0.546	100.00
0.096	0.457	83.70	0.477	87.36	0.493	90.29	0.504	92.31
0.191	0.438	80.22	0.461	84.43	0.486	89.01	0.500	91.58
0.382	0.422	77.29	0.444	81.32	0.482	88.28	0.483	88.46
0.765	0.413	75.64	0.409	74.91	0.468	85.71	0.466	85.35
1.529	0.399	73.08	0.387	70.88	0.444	81.32	0.449	82.23
3.058	0.394	72.16	0.369	67.58	0.436	79.85	0.438	80.22
6.116	0.392	71.79	0.350	64.10	0.419	76.74	0.408	74.73

The figure 4 shows the curves of EC_{50} of Ccm, Moringa, Ctl and coconut.

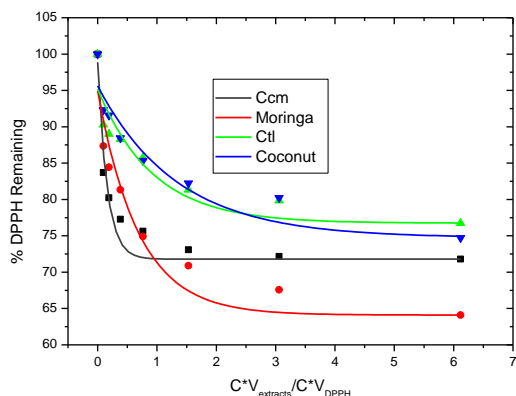


Figure 4. Curves of determination of EC_{50} of four seeds extracts

The absorbances recorded of the methanolic solutions of the DPPH radical vary from 0.781 to 0.022 in their mixtures with the standard solution of ascorbic acid and the various seeds extracts. The percentages of the DPPH radical were found to range between 100 and 2.06 % for the whole of the seeds extracts and the standard. The ratios of concentrations in ascorbic acid and in seeds extracts vs DPPH concentrations vary from 0.00 to 6.116 for all the samples.

The ascorbic acid, the carrot, the Pmw and niebe gave curves which allowed the determination of EC_{50} as showed in the figures 1 - 3 whereas for the other samples (soya, Ccm, Moringa, Ctl and coconut), the estimate of EC_{50} was not possible (figure 4).

The values of EC_{50} ; antiradical power (ARP) and the stoichiometry of ascorbic acid, the extracts of carrot, Pmw and of niebe are included in table 4 below.

Table 4. EC_{50} , ARP and the stoichiometry of the ascorbic acid and the seeds extracts

Samples	EC_{50}	ARP	Stoichiometry
Ascorbic acid	0.23	4.35	2.17
Carrot	1.12	0.89	0.45
Pmw	2.61	0.38	0.19
Niebe	4.37	0.23	0.12
Ccm	-	-	-
Moringa	-	-	-
Ctl	-	-	-
Coconut	-	-	-
Soya	-	-	-

The table 4 showed that more the EC_{50} is weak, more the antiradical power is high (acid ascorbic). The sample having a high EC_{50} gave place to a weak antiradical power (niebe).

The ascorbic acid and the carrot extracts showed, during their reaction with the DPPH radical, a great decrease of the absorbance of DPPH radical (figures 1 and 2).

The Pmw and niebe gave place to a weak decrease (figure 3). The other samples (soya, Ccm, moringa, Ctl and coconut) gave place to high percentages (higher than 50%) of the DPPH radical.

4. Discussion

The standard solution used in this study of the reaction with the DPPH radical is the ascorbic acid like in Habrant (2008) and Anitha (2012) works.

The ascorbic acid gave as result ($EC_{50} = 0.23$) with a stoichiometry close to 2. In its thesis, Habrant (2008) found a value of $EC_{50} = 0.24$ for the ascorbic acid.

The results of EC_{50} obtained made it possible to classify the extracts in three categories:

- The carrot extracts are the most active product of the series with $EC_{50} = 1.12$; (table 4, 2nd line). Indeed, its stoichiometry is 0.5. This result is particularly interesting, since all of the samples of this study, it is the carrot which showed a great EC_{50} . Mbanga et al. (2015) reported that the carrot extracts can contain more flavonoids and other phenolics compounds as the most important components than the other extracts seeds. Lokapure et al. (2013) affirmed that the vitamins A, C and E are very powerful antioxidants. Shweta et al. (2011) affirmed through their work that the carrot contributes to the formation of the new cells and supports the reduction of the wrinkles and that the carrot is rich in vitamins A and C which are antioxidants which prevent the formation of the free radicals by trapping them or by facilitating their decomposition by promotion. Shweta et al. (2011) supported from their epidemiologic and in vitro studies on the medicinal plants and leguminous plants that the components of the plants having an antioxidant activity such as those of carrot are able to exert protective effects against the oxidative stress of the biological systems ;
- Pmw and niebe have respectively EC_{50} 2.61 and 4.37, stoichiometries 0.2 and 0.1 respectively (table 4, 3rd and 4th lines) ;
- The Ccm samples, moringa, Ctl, coconut and soya did not show a great reactivity with respect to radical DPPH and stoichiometry thus could not be given (table 4, 5th to 9th lines). These

extracts are thus to be the least active. These measurements are thus not exploitable.

The flavonoids and phenolics compounds are more abundant in the plants, especially edible plants. Some edible plants have anti-UV properties because a close connection exists between the total flavonoids, the total phenolic and of the antioxidants activities, anti-inflammatory drugs and anti-carcinogenesis of plants (Kessler et al., 2003; Gonzalez et al., 2008; El Bedaway et al., 2010; Saraf & Kaur, 2010). Thus, El Bedaway et al. (2010) noted in their work that the total contents in flavonoids in the extracts of Ginger were correlated with the antioxidant activity, which indicated that the flavonoids were the principal compounds responsible for this antioxidant activity. Gonzalez et al. (2008) underlined the fact that the oral administration of antioxidants protects surface from the whole skin without this skin being affected while being washed, while rubbing or while perspiring. Thus, as all the samples of this study are edible plants, these extracts of plants can be a source of the flavonoids and their oral consumption contributes enormously to the protection of the skin and have a positive incidence on the skin.

In their work, Rishi and Sneha (2012) found the following ratios between the EC₅₀ of their samples and the EC₅₀ of the ascorbic acid (standard), namely 2.8, 11.2 and 52 for *Gloriosa superba*, bulbs of *Urginea indica* and sheets of *Urginea indica* respectively. They concluded in the light of their results that the concentrations of flavonoids and the phenolic compounds are high in the first and the second samples. In this work, the ratios between the EC₅₀ of carrot, Pmw and niebe like samples and the EC₅₀ of the ascorbic acid as standard are respectively 4.9, 11.3 and 19. Thus, we can conclude as the preceding authors that those samples contain flavonoids and phenolic compounds with high concentrations and that they have anti-UV properties.

Of all the extracts which were the subject of this study, the carrot extracts in first contain more of the flavonoids and the phenolic compounds in the light of the results of EC₅₀, APR and stoichiometry found. Anita (2012), supported in her work that the carrot has the antioxydant capacity and the capacity to defend the human organism to the action of the free radicals and to prevent the degenerated disorder of the oxidative stress.

In more carrot contains the apigenin, a very effective flavonoid in the prevention of radiations UVA and UVB. The results of works of Anita (2012), Gonzalez et al. (2008) and Rishi & Sneha (2012) are in agreement with the results of this study where the carrot is classified as the head of series.

The results obtained in this study showed a decrease of the absorbance of radical DPPH (tables 1,

2 and 3) when the ratios of concentrations of the seeds extracts vs the concentration of radical DPPH increases. Almey et al. (2010) found the yellow DPPH2 in their work like in this work.

The results of this study showed that the EC₅₀ varied in opposite direction of the antiradical power like those of Habrant (2008).

5. Conclusion

The study of the reaction of the seeds extracts with radical DPPH showed through the results obtained the existence of the antioxidant activity and the antiradical power of the seeds extracts. The carrot extracts are most active with a antiradical power of 0.89 and a stoichiometry of 0.45 whereas five samples did not show reactivity tested with respect to radical DPPH.

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