Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

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Version of record first published: 22 Sep 2010

To cite this article: Elena Cremenescu, Maria Giurginca, Andrei Giurginca & Aurelia Meghea (2008): Anti-UV, Antioxidant Activity and Cytotoxicity of Selected Natural Extracts for Cosmeceuticals, Molecular Crystals and Liquid Crystals, 486:1, 193/[1235]-202/[1244]

To link to this article: http://dx.doi.org/10.1080/15421400801917999

Mol. Cryst. Liq. Cryst., Vol. 486, pp. 193/[1235]–202/[1244], 2008

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400801917999

Anti-UV, Antioxidant Activity and Cytotoxicity of Selected Natural Extracts for Cosmeceuticals

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Selected natural extracts formulated as nanostructured glycerol – water mixtures have been in vitro tested in order to establish the optimum balance among cosmetic efficacy/human health and ecosafety. In our work, cytotoxicity and antioxidant activity have been studied on the natural extracts of bilberry, red rose, raspberry, hazelnut, wild strawberry, marigold and blackberry. Cytotoxicity effects have been estimated on the cell culture system from human fibroblast. The antioxidant activity has been estimated by chemiluminescence technique (CL) and by chemical determination of flavones and the tannin contents. In order to obtain cosmetic formulations, we selected bilberry, blackberry, red rose and raspberry extracts. These data have been completed by determination of stability in thermo and photooxidative conditions (irradiation UV at 290–400 nm domain). The behavior of extracts in these conditions justified their utilization in cosmeceuticals with photoprotective properties.

Keywords: antioxidant activity; cytotoxicity test; plant extracts; spectral determination (FT-IR; UV-VIS and CL); SPF index

INTRODUCTION

Over the time, the skin suffers transformations due to the physiological processes and also due to the environmental factors, both being responsible for the malfunctions of the defense and regeneration natural mechanism of the skin [1].

This is the reason for interventions devoted to maintain a healthy skin by abatement or eliminating the aging effects but also the

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hazardous effects of the environmental factors (solar radiation with all its components), free radicals, pollution, smoking, improper alimentation [2].

Among these factors, short-term or long-term skin changes due to the solar radiation had attracted the attention of the dermatologists and made necessary new investigations for new cosmeceuticals needed to reduce the noxious actions of the UV solar radiation (erithema, excessive keratinization, excessive production of melanine, etc.)

Photo-ageing of the skin, resulting from prolonged sun exposure without a proper protection takes place gradually in four successive stages:

- light photo-ageing without keratozis but with minor wrinkles;
- moderate photo-ageing with actinic keratozis and obvious wrinkles;
- advanced photo-ageing with strong keratosis and moderate scars;
- severe photo-ageing with advanced wrinkles and scars and possible skin cancers [3].

The natural mechanisms through which the organism counteracts these effects are limited, and the need to use cosmeceuticals is continuously increasing as, according to the international legislation, they should contain natural active substances aiming to diminish the amount of free radicals involved in this process. Thus, the photoprotection is achieved by an active antioxidant action [4].

Inorganic or organic (synthesis or natural) photo-protective substances (filter or sunscreen) have the ability to reflect or disperse the UV radiation and to selectively absorb in the 290–400 nm domain.

The content of organic compounds in cosmeceutical formulations has a restricted use according to the actual legislation, therefore the search for natural products containing active principles, with antioxidant and photo-protective capacity but without sub-lethal effects at the cell level [5], is highly promoted.

Our article presents the anti-UV and antioxidant activity of several cosmetic formulations containing selective plant extracts and evaluates their cytotoxic effects on human fibroblasts.

EXPERIMENTAL

Materials

The main plant species used for the extracts are presented in Table 1 along with the main active principles contained.

TABLE 1 Active Principles in the Investigated Extracts

Extract type	Active principle
1. Bilberry (*Vaccinium myrtillus*)	Quinine, sugars, organic acids (oxalic, malic, citric), ascorbic acid (vitamin C), tannins
2. Hazelnut tree (*Corylus avelana*)	Phenol acid (cafeic + chlorogenic acids), sugars, anthocyanins
3. Wild strawberry (*Fragaria vesca*)	Fragarol, quercitin, sugars, ascorbic acid (vitamin C), tannins
4. Blackberry (*Rubus* sp.)	Flavonoids, organic acids, ascorbic acid (vitamin C), tannins
5. Strawberry (*Fragaria* sp.)	Flavonoids, vitamins B, carotenoids
6. Raspberry (*Rubbus idaeus*)	Organic acids, ascorbic acid (vitamin C), flavonoids, tannins
7. Red rose petals (*Rosa* sp.)	Flavonoids, sugars, terpenoides, tannins, cyanidine

The extracts were obtained following the method used in the reference [6] by maceration and solid-liquid extraction.

Reagents

- for FT-IR analysis: spectral KBr (Merck);
- for UV-VIS-NIR analysis: MbO (Merck);
- for chemiluminescence analysis (CL): luminol:H_2O_2 system, TRIS-HCl 0.2 M at pH = 8.6 and solvent of distilled water with pH = 7.0 ± 0.2.

Apparatus and Investigation Techniques

- spectrometer FT-IR 620 (Jasco, Japan) in the 4000–400 cm^{-1} domain;
- spectrometer UV-VIS-NIR, V570 (Jasco, Japan) in the 200–2000 nm domain), using the ILN-472 accessory for diffuse reflection and the soft for the determination of the sun protection factor (SPF) [7];
- chemiluminometer Turner-Design (TD 20/20, USA);
- CALORIS oven (Romania) with adjustable temperature for exposure at 40–80°C;
- UV irradiation room (Ultra-Lum, USA) for exposition at 40–65°C;
- The cytotoxcity (Neutral Red) test was carried out on human fibroblasts; it is based on the ability of living cells to incorporate this colorant with weakly cationic character and accumulate it in liposomes in order to bind it in the anionic sites from the liposomal matrix. The test was performed in the fresh MEM culture medium (Modified *Eagle* Medium) for 24 h × 37°C; the extracted colorant

after washing the viable cells was spectrocolorimetrically determined for quantitative information. From the concentration – cytotoxicity curves, the cytotoxicity index IC_{50} was evaluated and the concentration of extract corresponding to 50% dead cells. The test was conducted at concentrations of 0.1–0.5% tested extract [8].

RESULTS AND DISCUSSION

During the last 25 years, plants had held the attention of researchers and solutions are sought in order to obtain very stable and high quality products. For this aim, the most important aspect is to extract from plants active principles with a certain destination.

The original extracts investigated in our article are of glycerin – water type. They were structurally characterized by searching for their characteristic bands through IR and UV-VIS spectroscopy, before and after accelerated ageing in thermooxidative conditions and UV irradiation.

Structural Characteristics of the Extracts in the IR Domain

The spectra of the initial extracts present some common bands in the 3400–3300 cm^{-1} and 1800–1600 cm^{-1} domains:

- in the 3400–3450 cm^{-1} region, the specific band for the –OH and –NHR groups is situated at 3380 cm^{-1} showing a certain degree of association; after thermooxidation, the band widens pointing out to new types of associations and shifts toward 3350 cm^{-1}.
- in the 1800–1600 cm^{-1}, the initial extracts present two bands with different intensity at 1710 and 1645 cm^{-1}, the last one having the greater intensity. The 1710 cm^{-1} band comes from acid or estheric structures of the –COOH/–COOR type while that from 1645 cm^{-1} is given by the anions of the organic acids (COO^{*}). During the thermooxidative process, the 1710 cm^{-1} band increases in intensity, surpassing that from 1645 cm^{-1}; at the same time, a new band is formed at 1762–1767 cm^{-1} given by cetonic and/or lactonic groups following the oxidation of the –OH groups [9].

The spectra of the extracts exposed to UV irradiation at 40°C were recorded in the 4000–400 cm^{-1}. As an example, we present the IR spectrum of the raspberry extract (Fig. 1). The behavior at the UV irradiation of all the extracts is similar to that at thermooxidative destruction. This aspect may result from the participation of some extract compounds to the same type of destructive reactions through free radical mechanism.

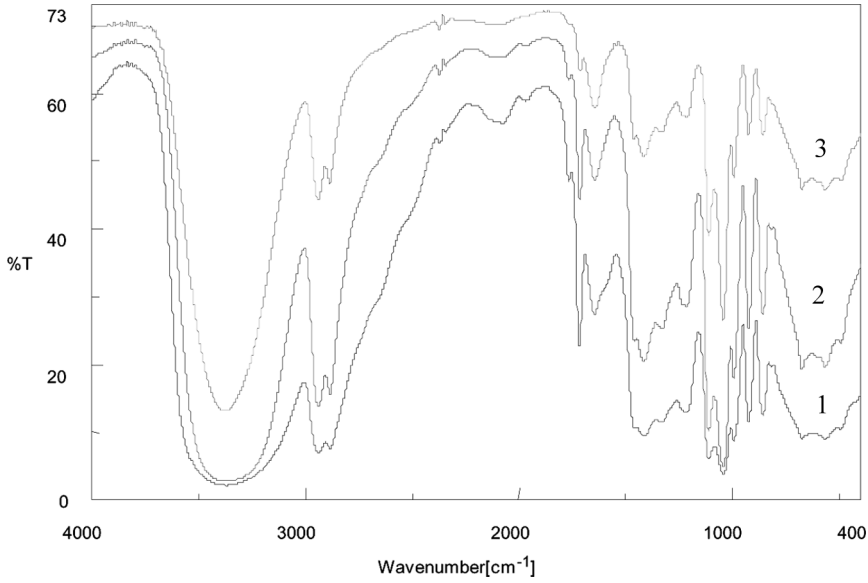

FIGURE 1 IR spectra of blackberry extract (initially – green; at temperature (4 h × 80°C) – blue; UV irradition (2h × 65°C) – red

Spectral Characteristics in the UV-VIS-NIR Domain

The extracts were characterized in the 200–2300 nm domain, initially, than after thermooxidative ageing for two hours at 80°C and UV irradiation for two hours at 40°C.

The spectra were recorded by diffuse reflection using the special cell and magnesium oxide. The main spectral characteristics are attributed to the electronic transitions $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$, as follows:

- in the 200–400 nm region, the transition are attributed to acid, quinonic and amidic groups from flavonoids, polyphenols and their derivatives; the extracts containing groups with extended conjugation (bilberry, wild strawberry, hazelnut tree and raspberry) present bands from tannins in the 350–620 nm domain;
- in the NIR domain, nearly all the extracts present bands in the 1200–1750 nm and 1950–2300 nm regions coming from the combining valence and deformation vibrations of some free or associated –CH, –OH, –NH and –C=O groups.

The exposure at 80°C does not affect the spectra of the extracts but only their viscosity (in a smaller degree), aspect resulting from their good stability.

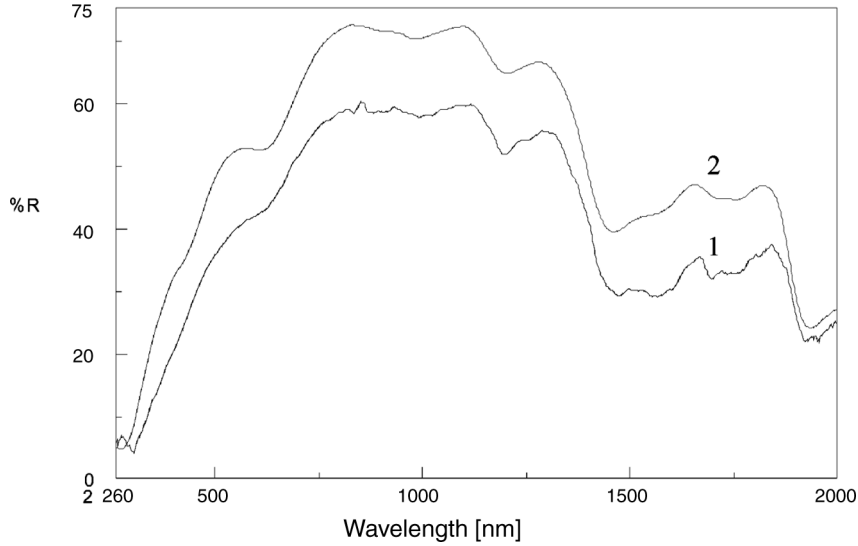

FIGURE 2 UV-VIS-NIR spectra of the red rose extract initially (black) and after (green) UV irradiation.

By UV irradiation, the main characteristic bands exhibit batochrome shifts of 15–30 nm and new bands appear in the 600–670 nm region. These come from the appearance of new oxidative products with extended conjugation. This aspect was noted especially at the red rose extract, a plant with a high content in flavones, polyphenolic acids and tannins. As an example, we present the spectrum of the red rose extract (Fig. 2).

Antioxidant Characteristics of the Selective Plant Extracts

All the extracts were analyzed by chemiluminescence in order to evaluate the antioxidant activity, its evolution being followed also after the thermooxidative ageing and UV irradiation. As a standard the system luminol + H_2O_2 at pH = 8.6 in the presence of TRIS-HCl was used [10]. The data are presented in Table 2 along with the content in flavones and tannins of the extracts.

From their analysis, we remarked the following aspects: as the extract viscosity and the concentration of active principles increased, in some cases the antioxidant activity increased, especially after the UV irradiation; the conjugated quinonic structures (present in the flavonoids, anthocians and tannins, whose antioxidant activity was proved) may have also contribute to this increase [11,12]. It is the case

TABLE 2 Content in Flavones and Tannins of the Extracts and the Evolution of the Antioxidant Activity

Extract type	Flavones (rutosid), %	Tannins (pirogalol), %	Antioxidant activity, %		
			Initially	2h × 80°C	2h × 40°C/UV
1. Bilberry	0.033	0.125	58.8	27.3	58.2
2. Hazelnut tree	0.014	0.038	66.2	49.1	70.7
3. Wildstrawberry	0.007	0.187	43.0	25.0	11.9
4. Blackberry	0.047	0.029	35.7	54.3	51.1
5. Strawberry	0.021	0.146	66.9	45.3	42.0
6. Raspberry	0.041	0.214	26.4	67.1	86.1
7. Red rose	0.059	0.602	64.7	80.0	64.2

of the red rose, which has the highest content in flavones and tannins and presents an antioxidant activity that remains high even in destructive conditions. The same behavior is characteristic for the extracts of hazelnut, bilberry and blackberry which have a high content of tannins.

Also particular is the evolution of the antioxidant activity of the raspberry extract which increases its protective capacity under UV irradiation. This aspect was attributed to an enhancing effect of the flavones, tannins and polyphenolic acids from its composition.

The extracts behavior at thermooxidation and UV irradiation points out to their stability and justifies their use in cosmeceuticals with specific destinations, including photo-protective activity.

Biological Characterization of the Extracts in vitro

The test Neutral Red made on normal cells allowed us to differentiate the viable, injured or dead cells after the altering of the surface of the liposomal membrane.

Working at three concentrations from the domain 0.1–0.5% plant extract, we established the degree of cytotoxicity and the cytotoxicity index (Table 3).

From the experimental data, we note the following:

- all the extracts have an average cytotoxicity degree, relatively constant at higher concentrations (0.25–0.50%), which decreases, sometimes significantly, at the 0.1% concentration.
- the hazelnut tree extract present an important cytotoxic activity;
- the viability for the normal cells at 0.1% concentration is satisfying, excepting the hazelnut tree extract which did not exceed 50% from the standard value;

TABLE 3 Variation of the Cell Viability in Relation with the Extract Concentration

Extract type	Extract concentration, %	Cell viability, %	IC_{50}, %
1. Bilberry	0.10	93.68	
	0.25	41.63	0.358
	0.50	41.33	
2. Hazelnut tree	0.10	42.99	
	0.25	39.67	–
	0.50	37.07	
3. Wild strawberry	0.10	100.00	
	0.25	39.20	0.332
	0.50	33.10	
4. Blackberry	0.10	96.64	
	0.25	54.05	0.380
	0.50	38.56	
5. Strawberry	0.10	100.00	
	0.25	47.21	0.397
	0.50	42.59	
6. Raspberry	0.10	100.00	
	0.25	48.01	0.380
	0.50	40.41	
7. Red rose	0.10	73.30	
	0.25	41.84	0.281
	0.50	34.32	

- the bilberry, wild strawberry, strawberry, blackberry and raspberry had a viability value comparable with that of the standard (with $\pm 5\%$ variations), while the red rose extract had 25% less than the standard.

Based on experimental data performed from the cytotoxicity test (Neutral Red), but also on their use in the traditional medicine, we opted to use the bilberry, blackberry, red rose and raspberry extracts for the nanostructured complexes with antioxidant and photoprotective capacity.

The nanostructured complexes containing the extracts of the selected plants were included in some cosmeceuticals. The SPF index and its variation after UV irradiation at 1 h at 65°C, was determined for each formulation (Table 4).

It is obvious the variants including carotens and nanostructured complexes from plant extracts, in the presence of TiO_2, significantly improve the anti UV protection.

The increase of the SPF index after irradiation was attributed to the participation of some components from the nanostructured complex,

TABLE 4 SPF Index for the Cosmeceuticals

Code	Content	SPF value		ΔSPF
		Initially	1 h × 65°C/UV	
EC1	Organic synthesis compounds (OSC)	9.7	11.0	+1.3
EC10	OSC + carotens + TiO_2	23.2	25.7	+2.5
EC10 + EC11	Nano complex 1	28.1	33.7	+5.6
EC10 + EC12	Nano complex 2	28.5	32.5	+4.0

respectively from the selected plant extracts, in the photophysical-photochemical processes taking place by the selective absorption of the UVA-UVB radiations. The TiO_2 powder has an important sunscreen role as it has protective capacity on the entire 290–400 nm domain, a high stability at the UV radiation, without toxic effects on the skin [13].

CONCLUSION

The detailed characterization of some original plant extracts for cosmeceuticals has been presented.

The extracts, obtained in a pilot phase, were spectrally characterized in the IR and UV-VIS domains, initially and after accelerated ageing at temperature (80°C for 4hours) and UV irradiation (40°C for 2 hours), establishing the variation of the antioxidant activity under these conditions.

The cytotoxicity data, obtained by the Neutral Red test, showed that the bilberry, blackberry, red rose and raspberry can be used to prepare nanostructured complexes with photo-protective and antioxidant capacity.

The nanostructured complexes were included, together with TiO_2 ultra-fine powder, in cosmeceuticals and the SPF index was determined comparatively with a product containing organic synthetic compounds, pointing out the superiority of the couples natural extracts – TiO_2.

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