Journal of Photochemistry and Photobiology B: Biology 120 (2013) 142–147

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Efficacy of *Punica granatum* L. hydroalcoholic extract on properties of dyed hair exposed to UVA radiation

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A R T I C L E I N F O

Article history:
Received 19 July 2012
Received in revised form 16 December 2012
Accepted 17 December 2012
Available online 5 January 2013

Keywords:
Punica granatum L.
Photo-oxidation
UVA radiation
Hair
Color fading
Mechanical properties

A B S T R A C T

The solar radiation promotes color fading of natural and dyed hair by free radical generation, which oxidize the pigments, and it has been proposed the incorporation of antioxidants in order to reduce the alterations of hair color. Due to its high content of polyphenols and tannins, which are potent antioxidants, the hydroalcoholic extract of *Punica granatum* L. (pomegranate) was used in this research. Hair care formulations containing pomegranate extract were applied to red dyed hair tresses, and these were exposed to UVA radiation. Non-ionic silicone emulsion presenting color protection properties were also used for comparison purpose between the results obtained with different treatments, including silicone in combination with the pomegranate extract. The pomegranate extract at 5.0% and 10.0% w/w was effective in preventing the hair color fading in 37.6% and 60.8%, respectively, but the association of hydroalcoholic extract and non-ionic silicone emulsion is not encouraged. Mechanical properties were not affected by UVA radiation, since significant differences in breaking strength were not observed. Considering the conditions which the tresses have been exposed, it was concluded that the pomegranate extract at 10.0% w/w in hair care formulations are effective in reducing color fading of red dyed hair.

1. Introduction

Human hair is composed mainly of keratin, a group of proteins which account for 65–95% of hair weight. The cuticle is the outer layer, its role is to protect the hair shaft against environmental and chemical damages. The keratinized scales present in this layer are responsible for brightness and tactile properties. Cortex is the layer located below the cuticle and confers resistance to the hair fiber due to its crystallized α-keratin fibrils organization. In this layer, the melanin (natural pigment) and the artificial hair dyes are located. The medulla, another component of hair fiber, may or may not be present, and its role is not clearly defined [1], although it is known that it shows air cavities in its constitution which can distribute the strength of the fiber in a less uniform way, without affecting its mechanical properties [2].

There are several factors that affect hair properties, like chemical and physical treatments such as bleaching, coloring or brushing, or even prolonged exposition to sun radiation. Hair fibers exposed to sunlight may present physical and chemical changes. As physical changes, dryness, reduced strength, rough surface texture, color fading, decreased luster, stiffness, and brittleness can be mentioned. As chemical changes, shifts in hair proteins, lipids and pigments may occur [3].

Hair photodamage may be caused by UVA, UVB and visible radiations, and the effects of different wavelength ranges vary. The UVB and UVA radiations interact negatively with hair proteins, while the visible light promotes melanin granules degradation (photo-bleaching) [4]. According to Nogueira and Joekes [5], UVB radiation is the main responsible for hair protein loss and UVA promotes color changes. The UVB is largely absorbed by the cuticle, while UVA reaches through the cuticle layers and promotes oximelanin production, which is related to the bleaching of the natural hair. It also promotes the generation of oxyradicals, such as superoxide and hydroxyl, which oxidize hair dyes molecules and modifies its color characteristics [1]. Artificial hair color fades at a much faster rate. Therefore, the exposure time required to cause natural hair fibers photodamage is longer than the required to have the same effect on dyed fibers [6].

In order to reduce or delay the color change from the natural or dyed hair fibers, the use of silicones, UV filters (natural or synthetic) and antioxidant additives in cosmetic formulations have been

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http://dx.doi.org/10.1016/j.jphotobiol.2012.12.011

proposed [6–8]. Some silicone blends like dimethiconol/dimethicone can protect hair color during washing procedures, while resins like trimethylsiloxysilicate and propylphenylsilsesquioxane are able to reduce color changes caused by exposure to a simulated sunlight provided by a weatherometer. Therefore, silicones may be used in hair formulations to ensure longer hair color maintenance [7].

Antioxidants are substances which avoid free radicals formation and could be used in dye formulations, as well as in subsequent treatments, for preventing hair color fading. Following the worldwide trend of using plant extracts and derivatives in cosmetic formulations, natural oxidants properties and applications have been widely studied [8].

Pomegranate (*Punica granatum* L.) is a fruit from the Persian region and it's cultivated from the Mediterranean to the Middle East. Due to its characteristics, it has medicinal purposes, being used for strep throat, hoarseness and fever, as well as antiviral and antiseptic. Plants and fruits medicinal use, such as pomegranate, is possible due to the bioactive substances, with potential biological activity, present in extraction products [9].

All parts of pomegranate present antioxidant activity, but it is known that the peel have greater antioxidant activity than the juice, which in turn is greater than the seeds [10]. Pomegranate is rich in polyphenols whose content varies during different stages of maturation. Full-ripe fruits have lower polyphenols than unripe, therefore to obtain extracts rich in polyphenols it is necessary to use immature fruits [11]. Furthermore, parameters related to sample preparation and extraction process need to be controlled in order to obtain a quality extract in terms of polyphenol content and antioxidant characteristics, like solvent type or solvent mixtures used, temperature, particle size, drying process, etc. [12].

Pomegranate contains a complex mixture of anthocyanins and hydrolysable tannins (HT). The anthocyanins are mono- and di-glycosides of cyanidin, pelargonidin, and delphinidin. Pomegranate leaves, peel, and fruit contain over 18 individual HT structures that are classified into gallotannins, ellagitannins (ellagic acid esters of D-glucose with one or more galloyl substitutions), and the most unusual gallagyl esters such as pomegranate-specific punicalagin (two anomers) and punicalin. It also contains oligomeric ellagitannins with two to five glucose core molecules cross-linked by dehydrodigalloyl esters, as found in other plants. So, hydrolyzable tannin compounds may number in the hundreds [13]. Fischer et al. [14] detected 48 phenolic compounds in pomegranate, which were presented as nine anthocyanins, two gallotannins, 22 ellagitannins, two gallagyl esters, four hydroxybenzoic acids, seven hydroxycinnamic acids and one dihydroflavonol. Thus, ellagitannins were the predominant phenolics. As polyphenols, including tannins, present high antioxidant activity, they could be used in order to reduce color fading of natural or dyed hair.

Tannins are usually defined as polyphenolic compounds that precipitate proteins, which is fundamental to their observed biological activities in human and veterinary medicine. It is known that tannins relative affinities for different proteins can vary as much as 10,000-fold. Tannins act as multidentate ligands, promoting protein cross-linking and consequent precipitation, therefore it is expected that tannins with high molecular weight should precipitate proteins more effectively, although some data suggest that this rule does not apply to all tannins [15]. Given the tannin affinity to protein structures, the interaction of such compounds with hair keratin is expected.

Thus, based on the pomegranate extract characteristics and tannins properties, the aim of this research was to evaluate the pomegranate extract effectiveness, incorporated to hair care formulations, in protecting the color and mechanical properties of dyed hair tresses exposed to UVA radiation.

2. Material and methods

2.1. *P. granatum L. extracts*

P. granatum L. peel was split and dried in a circulating air oven at 105.0 ± 2.0 °C. The vegetal material was then triturated and passed through a 20-mesh sieve. Six different extracts were prepared with the pericarp of pomegranate: one aqueous, four hydroalcoholics and one alcoholic. The plant hydroalcoholic extracts were obtained in the following proportions: 20, 40, 60 and 80% (v/v) ethanol (Synth®) in distilled water. About 2.0 g of the vegetal material were soaked with sufficient amount of extraction fluid and subsequently it was placed in a percolator (separation funnel) over a small amount of cotton. A piece of filter paper and glass balls with a diameter of, approximately, 1.0 cm were placed over the vegetal material inside the separation funnel. The extraction fluid was added (about 25 mL) and the preparation remained in maceration for 24 h at room temperature (22.0 ± 2.0 °C) and subsequently it was percolated at a maximum speed of 1 drop/s, collecting 20.0 mL of pomegranate extract.

2.1.1. *Antioxidant activity evaluation*

The antioxidant activity of pomegranate extracts was assessed using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) provided by Sigma–Aldrich. A 40 μL aliquot of pomegranate extract was diluted into 50 mL with distilled water. An aliquot of 0.5 mL was transferred to test tubes, adding 2.5 mL of 100 μM ethanolic solution of DPPH. The negative control was carried out using 0.5 mL of distilled water. The test tubes were stored for 30 min protected from light and the absorbance was measured with a spectrophotometer (Shimadzu UV-1203) at 517.0 nm. Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), purchased from Sigma–Aldrich (97% purity), is a vitamin E derivative widely used for assessing antioxidant activity and the absorbance results were calculated in Trolox equivalent antioxidant activity (TEAC). Serial dissolution of Trolox standard were used to build a calibration curve.

2.1.2. *Quantification of tannins*

The tannin content (expressed as pyrogallol equivalents) was determined by the method described in the Brazilian Pharmacopoeia [18], which is based on the colorimetric reaction of polyphenols with Folin–Ciocalteu reagent, purchased from Haloquímica, and differential precipitation of tannins with skin powder.

2.2. *Hair samples*

Caucasian virgin dark brown hair tresses of 15.0 cm in length, purchased from Bella Hair® (Brazil), were used. The hair tresses were initially washed in order to remove any traces of cosmetic or dirt according to a standardized methodology, and then soaked in warm water at 40.0 ± 1.0 °C for 30 s. It was applied 4.0 mL of sodium lauryl sulfate solution (2.0% w/w), for 1 min, with gentle movements, with further rinsing of the tresses, for 1 min, with warm water at 40.0 ± 1.0 °C. The excess of water was first removed by passing the tresses three times between the fingers and then they were dried on paper towel, at room temperature (22.0 ± 2.0 °C) for 24 h [16].

After drying, the tresses were bleached with ammonium persulfate and hydrogen peroxide (30 vol) (Amend®), mixed in a 1:1 (w/w) ratio. The samples were kept in this solution for 40 min, followed by a washing procedure (described above), with further treatment with a commercial oxidative red hair dye (Amend®), color 6.66, composed by oleyl alcohol, sodium sulfite, erythorbic acid, behentrimonium chloride, stearalkonium chloride, propylene glycol, pentasodium pentetate, parfum (fragrance), amodimethicone, silk amino acids, hydrolyzed keratin, ammonium hydroxide and aqua (water). The dye was mixed with hydrogen peroxide (30 vol) in a ratio of 1:1 (w/w) and applied to hair tresses in a ratio of 1:1 (w/w). The reaction occurred for 40 min. After this period, the tresses were washed according to the methodology described, and left to dry at room temperature.

2.3. Hair care formulations development

A hair care formulation was prepared according to the composition in Table 1. Pomegranate extract and non-ionic silicone emulsion [divinyldimethicone/dimethicone copolymer (and) C12–C13 pareth-23 (and) C12–C13 pareth-3] (Dow Corning®) were incorporated into the formulation, according to Table 2. The pH was adjusted to a 4.5–5.5 value.

Hair care formulations (Table 2) were applied to the hair tresses, in a 1:0.3 (hair:formulation) ratio, before each UVA radiation exposure. The applying procedure was performed with gentle movements assuring that the product distribution was uniform.

2.4. Exposure to artificial UVA radiation

The hair tresses were exposed to artificial UVA radiation emitted by the equipment Qsun (QLAB, XE1), with Daylight Filter Q and irradiance of 0.35 W/m^2 at 340 nm. The test was performed at 40 °C and with a 50% relative humidity. The tresses were exposed for two periods of time of 96 h each and 0.12 MJ/m^2 total dose of incident radiation. Before each exposure, the standardized washing procedure was performed and the hair care formulation was reapplied on the tresses.

2.5. Color changes

Color measurements were performed using Hunter Miniscan Labs® XE Plus (CIELAB – Universal Software v. 4.01). The measurement was performed in five replicates, using the middle portion of the tresses. Hunter *L-a-b* parameters were measured for time 0 and after irradiation time t. The color shifts of the samples were determined using Eq. (1) [4]. The equipment provides the color parameters based on three vectors: dL^* parameter represents the difference in brightness (with positive values standing for clearer and negative values meaning darker), da^* defines the difference in color-coordinated green–red (being positive if the hair shows redness and negative for greenness), db^* defines the difference in color-coordinated blue–yellow (with yellowness presented by positive numbers and blueness by negative). All these color parameters can be summarized in dE^* that indicates the total difference in color [17].

$$dE^* = \{(L_0 - L_t)^2 + (a_0 - a_t)^2 + (b_0 - b_t)\}^{1/2} \quad (1)$$

The percentage of total color protection offered by pomegranate extract with or without non-ionic emulsion of silicone was calculated according to Eq. (2) [4].

$$\%\text{Protection} = 100 \cdot (dE_B - dE_A)/dE_B \quad (2)$$

Legend: dE_B – total color variation of the tresses treated with base formulation; dE_A – total color variation of the tresses treated with the formulation containing pomegranate extract and/or non-ionic silicone emulsion.

2.6. Mechanical properties

Analysis of breaking strength was performed in texturometer TAXT2 Analyzer® model, operating at a clutch speed traction of 300 mm/min, distance of 80 mm, 25.0 kg load and sensitivity of 0.49 N. Five fibers from each treatment measuring 10.0 cm each were used for the tests. Their diameters were measured with micrometer Mitutoyo®, in three positions (root, middle portion and tip), and the mean value was used to calculate the total area of the hair fiber.

2.7. Statistical analysis

Possible significant differences in the results were analyzed by one-way ANOVA and the differences between treatments were identified by Tukey's test ($\alpha = 0.05$).

3. Results and discussion

3.1. *P. granatum* L. extracts characterization

It was found that the polarity of the extraction fluid influenced the antioxidant activity of pomegranate extract. The 40% (v/v) ethanol in distilled water as extraction fluid provided the highest antioxidant activity (136.4 mM TEAC), followed by 60% (v/v) ethanol in distilled water (117.6 mM TEAC) and 80% ethanol in distilled water (v/v) (87.1 mM TEAC) (Fig. 1).

The profile between the percentage of ethanol in the extraction fluid and tannin content expressed in pyrogallol followed the same pattern observed in the antioxidant activity (Fig. 2). The highest

Table 1
Qualitative and quantitative composition of base hair care formulation.

INCI[a] component	Proportion (% w/w)
Oil phase	
Cetearyl Alcohol (Aquatec)	4.5
Glycol Distearate (Clariant)	1.5
Cetrimonium Chloride (Natural Farma)	3.0
Methylparaben (PharmaSpecial)	0.15
Propilparaben (PharmaSpecial)	0.10
BHT (PharmaSpecial)	0.05
Aqueous phase	
Disodium EDTA (Vital Especialidades)	0.05
Acqua	90.65

[a] INCI: International Nomenclature of Cosmetic Ingredient.

Table 2
Proportion of non-ionic silicone emulsion and pomegranate extract in hair care formulations.

Componente INCI[a]	Proportion of components incorporated (% w/w)					
	EC	ED	ECS	EDS	SIL	BASE
Divinyldimethicone/dimethicone copolymer (and) C12–C13 pareth-23 (and) C12–C13 pareth-3 (Dow Corning®)	–	–	5.0	5.0	5.0	–
Punica Extract (40% ethanol in distilled water)	5.0	10.0	5.0	10.0	–	–

BASE: Base hair care formulation; EC: Base formulation containing 5.0% (w/w) pomegranate extract; ED: Base formulation containing 10.0% (w/w) pomegranate extract; ECS: Base formulation containing 5.0% (w/w) pomegranate extract and 5.0% (w/w) non-ionic silicone emulsion; EDS: Base formulation containing 10.0% (w/w) pomegranate extract and 5.0% (w/w) non-ionic silicone emulsion; SIL: Base formulation containing 5.0% (w/w) non-ionic silicone emulsion; (–) not added.
[a] INCI: International Nomenclature of Cosmetic Ingredient.

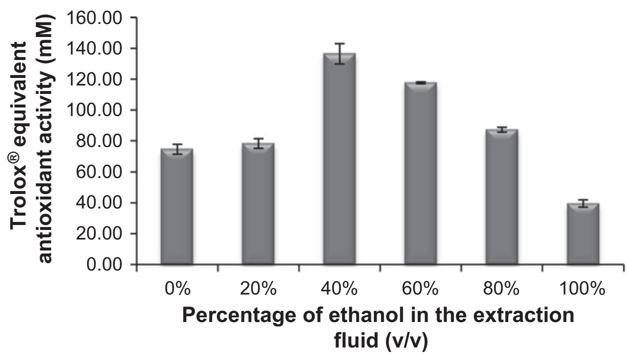

Fig. 1. Trolox® equivalent antioxidant activity in function of percentage of ethanol in the extraction fluid.

content of tannins (1.64 g pyrogallol/g extract) was observed in the 40% (v/v) hydroalcoholic extract. This fact is directly related to the results obtained in the antioxidant activity analysis, as the tannins are polyphenols and present such activity.

3.2. Color and brightness

COLIPA (European Cosmetic, Toiletry and Perfumery Association) recommends the incorporation of plant extracts to cosmetic formulation in a concentration up to 10.0% (w/w) [19]. Therefore, it was decided to develop formulations containing the maximum proportion of *P. granatum* L. extract recommended (10.0%) and also an intermediate proportion (5.0%) in order to evaluate the effect of this variable in the color and mechanical properties protection effectiveness under UVA radiation exposition. The proportion of non-ionic silicone emulsion used in the formulations (5.0%) was defined according to the manufacturer's instructions.

As seen in Fig. 3, the tresses treated with base formulation had a higher brightness difference (dL^*) compared to other treatments. Thus, UVA radiation promoted bleaching of hair fibers treated with base formulation at higher intensity, which indicates that pomegranate extract and non-ionic silicone emulsion protected the hair tresses from the effects of UVA radiation in its brightness (dL^*). The formulations with 5.0 and 10.0% w/w of pomegranate extract (EC and ED, respectively) protected the bleaching of the tresses in 41.4% and 58.1%, respectively, while the other treatments, except base, showed the dL^* values statistically equal.

The UVA radiation promoted an intense reddening of the tresses treated with BASE, ECS and EDS formulations. The low values of reddening obtained with the non-ionic silicone emulsion treatment, however, were statistically equal to those obtained with the pomegranate extract at 5.0 and 10.0% (w/w) treatments.

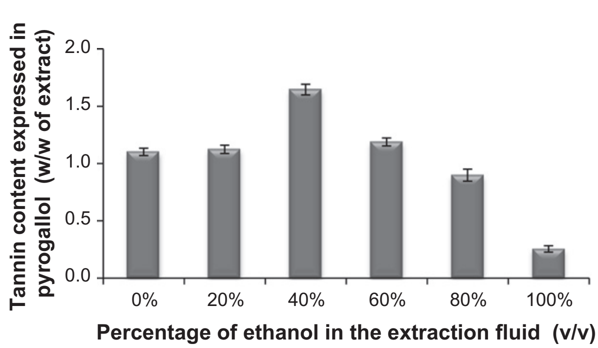

Fig. 2. Relation between tannin content expressed in pyrogallol and percentage of ethanol in the extraction fluid.

The increase in the parameter db^* could be attributed to the deposit of tannins from the pomegranate extract on the hair fiber, specifically on the protein keratin. However, the increased concentration of pomegranate extract in hair care formulations was related to lower tendency to yellowing, suggesting that this color change (db^*) can be related to the oxidation of dye molecules or even other changes caused by UVA radiation.

The dE^* parameter is calculated in order to establish a value that represents the total color change. It is considered as perceptible change of color dE^* values greater than 1.0 [6]. So, all tresses showed differences in color after the period of exposure to UVA radiation, except those treated with the formulation SIL ($dE^* < 1.0$). The variation in the brightness was an essential parameter for color change provided by dE^*. However, as seen in Fig. 3, the variation of the parameter db^* (yellow–blue coordinate) indicated that there was a trend to yellowing of the tresses during exposure to UVA radiation.

The formulations containing pomegranate extract promoted less variation in the total color of the hair fibers (dE^*), compared to the base formulation, which suggests that pomegranate extract protected the color of the tresses. This fact is probably seen due to the antioxidant activity of its components (tannins), preventing the formation of free radicals and the consequent oxidation of the hair dye.

Meinert et al. [8] showed beneficial effects of antioxidant incorporation in formulations, by protecting hair color fading. Commercially available antioxidants like 0.05% white tea extract, 0.05% Oxynex®, 2,5% GSP-T (antioxidant complex which combines water-soluble Swiss grape seed procyanidins and oil-soluble natural tocopherol) and 0.05% rosemary, incorporated into a pre-sun formulations were effective in protecting the hair fibers brightness (dL^*), one of the factors that affect the dE^* parameter (total color change).

The preparations containing 5.0 and 10.0% w/w pomegranate extract (EC and ED) were able to preserve dE^* value in 37.6% and 60.8%, respectively (Fig. 4). The color protection provided by pomegranate extract at 10.0% (w/w) were statistically equal to non-ionic silicone emulsion at 5.0% (w/w) which indicates the high potential of this extract in this kind of formulation.

The non-ionic silicone emulsion at 5.0% w/w showed the expected result [7] protecting the color of the tresses in 83.5% and the brightness in 72.0% compared to the base formulation. However, the association between pomegranate extract and non-ionic silicone emulsion did not show the expected effect, since a lower percentage of total color protection was observed in the tresses treated with formulation 5.0 and 10.0% w/w of pomegranate extract in combination with silicone at 5.0% w/w (33.8% and 18.6%, respectively). The silicone acts involving the hair fiber in a protective film [7], while pomegranate extract can protect the hair color by preventing the oxidation of dye molecules, due to its high antioxidant activity. Though a synergistic protective effect was expected, that was not observed.

After observing these results, some tests were carried out in order to try to explain the type of interaction between the pomegranate extract and non-ionic silicone emulsion. It was observed that non-ionic silicone emulsion is not soluble in ethanol, an important component of the hydro alcoholic pomegranate extract. Because of this incompatibility, the silicone was not able to form the protective film, responsible for its hair color protection characteristic, around the hair fiber. Furthermore, the antioxidant activity of molecules in the pomegranate extract was probably impaired, reducing its hair color protection activity. Thus, the non-ionic silicone emulsion proved to not be compatible with the type of pomegranate extract prepared, and so, the aqueous or glycol extracts in combination with this non-ionic silicone emulsion could be used in further research.

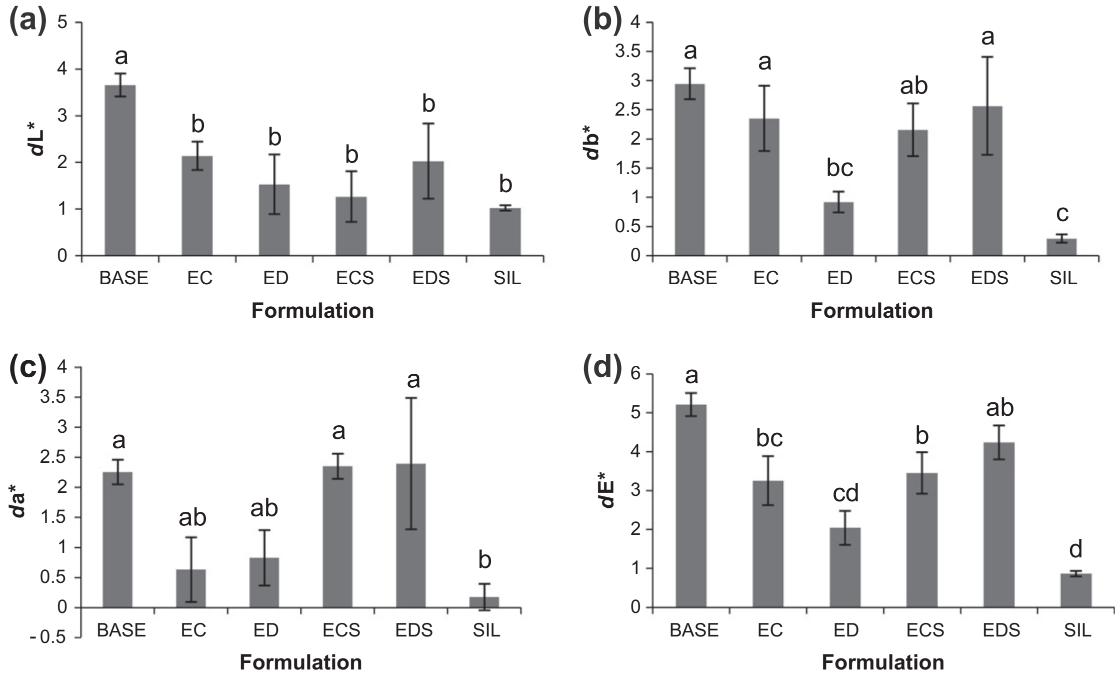

Fig. 3. Brightness dL^* (a) and color parameters db^* (b), da^* (c) and dE^* (d) of the hair tresses treated with different formulations and exposed to artificial UVA radiation. *Legend:* BASE: Base hair care formulation; EC: Base formulation containing 5.0% (w/w) pomegranate extract; ED: Base formulation containing 10.0% (w/w) pomegranate extract; ECS: Base formulation containing 5.0% (w/w) pomegranate extract and 5.0% (w/w) non-ionic silicone emulsion; EDS: Base formulation containing 10.0% (w/w) pomegranate extract and 5.0% (w/w) non-ionic silicone emulsion; SIL: Base formulation containing 5.0% (w/w) non-ionic silicone emulsion. dE^*: total color variation; dL^*: brightness variation; da^*: variation in coordinated red-green, db^*: variation in coordinated yellow–blue. Means that do not share a letter are significantly different.

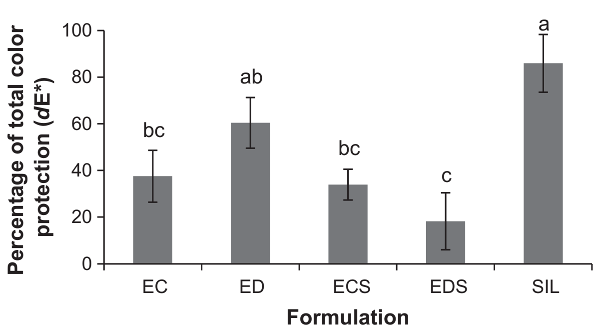

Fig. 4. Percentage of total color protection (dE^*) provided by the formulations. *Legend:* BASE: Base hair care formulation; EC: Base formulation containing 5.0% (w/w) pomegranate extract; ED: Base formulation containing 10.0% (w/w) pomegranate extract; ECS: Base formulation containing 5.0% (w/w) pomegranate extract and 5.0% (w/w) non-ionic silicone emulsion; EDS: Base formulation containing 10.0% (w/w) pomegranate extract and 5.0% (w/w) non-ionic silicone emulsion; SIL: Base formulation containing 5.0% (w/w) non-ionic silicone emulsion. Means that do not share a letter are significantly different.

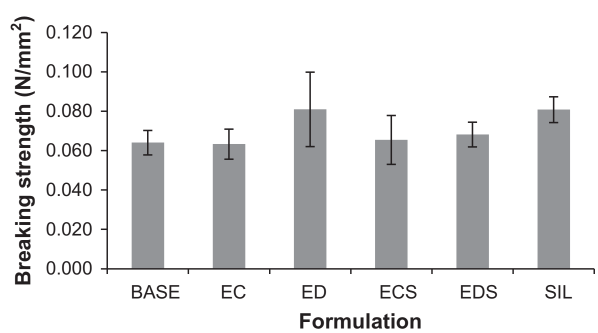

Fig. 5. Breaking strength of the tresses exposed to UVA radiation. *Legend:* BASE: Base hair care formulation; EC: Base formulation containing 5.0% (w/w) pomegranate extract; ED: Base formulation containing 10.0% (w/w) pomegranate extract; ECS: Base formulation containing 5.0% (w/w) pomegranate extract and 5.0% (w/w) non-ionic silicone emulsion; EDS: Base formulation containing 10.0% (w/w) pomegranate extract and 5.0% (w/w) non-ionic silicone emulsion; SIL: Base formulation containing 5.0% (w/w) non-ionic silicone emulsion.

3.3. Breaking strength

In response to the application of a load, the hair fiber is stretched approximately 2% of its initial length (elastic property). With a moderate load, the distension is about 25–30% of the length (plastic property). Applying the force steadily, the fibers stretch in proportion to the load until rupture occurs [21]. Fig. 5 presents the results of breaking strength test of the tresses treated with the different hair care formulations exposed to UVA radiation.

The breaking strength values showed no statistically significant differences, which suggests that UVA radiation does not have influence on hair mechanical properties, as also shown by Nogueira et al. [20].

4. Conclusions

According to the assay of UVA radiation exposition and the experimental conditions applied, the *P. granatum* L. extract at 5.0 or 10.0% (w/w) was concluded to be able to protect the color of the dyed red tresses in 37.6% and 60.8%, respectively, compared to the base formulation. The non-ionic silicone emulsion alone guaranteed high color protection of dyed tresses (83.5%), while the association between pomegranate extract and non-ionic silicone emulsion was not related to a better protection because of the incompatibility between non-ionic silicone emulsion and alcohol presented in pomegranate extract. Thus, pomegranate extract at 10.0% (w/w) and non-ionic silicone emulsion at 5.0% (w/w), when not associated in the same formulation, could be incorporated to hair care formulations in order to protect the color fading of dyed red hair exposed to UVA radiation. The mechanical properties had no significant differences, as shown in the breaking strength tests for the different formulations, suggesting that UVA radiation affects the color but not the mechanical properties of dyed hair.

Acknowledgements

We thank to Vitor Augusto Alves to his contribution and to Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp) for financial support.

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