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Development and Characterization of Novel Bigel-Based 1,4-Naphthoquinones for Topical Application with Antioxidant Potential

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Abstract
Discovering new antioxidant agents and optimizing their processing is a necessity to treat skin diseases. The assessment of quality and antioxidant activity of topical formulations based on 5,8-dihydroxy-1,4-naphthoquinone (M1) and 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (M2) was carried out for the first time for this purpose. Stability studies including evaluation of pH, viscosity, microscopic observation, and microbiological quality were determined. M1 and M2 were examined for their antioxidant capacities after their incorporation into bigels. Obtained data suggested that BG-M1 and BG-M2 have a good quality, a natural pH of the skin (4.5–6.0), and no sign of microbial development. Sensory analysis was also performed, and no negative assessment of unpleasant bigel properties was found. M1 and M2 have shown antioxidant activity toward DPPH radical (data are expressed as IC_{50}), 19×10^{-3} mg/mL for M1 and 35×10^{-3} mg/mL for M2, respectively. After their formulations, they have maintained this ability, for BG-M1 = 3.0 and BG-M2 = 1.1 mg/mL. Also, they demonstrated interesting ABTS scavenging with 1.5×10^{-3} and 2.5×10^{-3} mg/mL for M1 and M2, respectively. They kept this potential after formulation, since, BG-M1 = 1.7 mg/mL and BG-M2 = 4.9 mg/mL. Likewise, pure molecules revealed notable β-carotene bleaching inhibition (0.3 and 0.1 mg/mL for M1 and M2, respectively), which was maintained after their formulation into bigels (2.9 and 10.3 mg/mL for BG-M1 and BG-M2, respectively). These developed bigels maintaining the antioxidant potential of M1 and M2 could be used as the provision of a barrier to protect skin.

Keywords 5,8-Dihydroxy-1,4-naphthoquinone · 2,3-Dichloro-5,8-dihydroxy-1,4-naphthoquinone · Physicochemical characterization · Sensory analysis · Antioxidant · Bigel formulation

1 Introduction

Oxidative stress appears when the balance between pro-oxidant and antioxidant species is broken in favor of the pro-oxidant state. Probably, the rupture of this balance could be due to the promotion of reactive oxygen species (ROS), oxygenated free radicals, or both [1]. Although some ROS physiologically act as cellular messengers in redox signaling, several studies indicate that they are involved in the development of cardiovascular diseases [2]. It has been also proved their implication in many pathologies such as atherosclerosis [3], diabetes type 2, inflammation [4, 5], and cancer [6, 7]. Furthermore, ROS may be damaging and may play a causative role in aging and several diseases associated with it, which often manifest adversely in the skin [8–11]. Folk therapy has gained a big interest, and several plant preparations are used for various disorders including those of the skin [12, 13]. According to the World Health Organization

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(WHO), in the country side of developing countries, 80% of people use traditional therapies. The difficulty in extracting active ingredient from a plant in on the one hand, and its low productivity on the other hand, promotes resorting to the use of pure compounds. 1,4-Naphthoquinones (1,4-NQ) are secondary metabolites present in nature and constitute a large family of ubiquitous and varied substances, from simple molecules to complex structures. A big number of quinones has been characterized in plants, and they might be found naturally in dark walnut tree (*Juglans nigra*), in the leaves of *Lawsonia inermis* (*Henna*) [14, 15], and others have been synthetized. Inside each of its classes, variations around the basic chemical skeleton essentially concern the degree of hydroxylation, glycosylation, methylation, or oxidation, and the possible connections to other molecules. 1,4-NQ are valuable molecules possessing scavenging properties toward radical oxygen species [16].

This potential makes quinones interesting for the treatment of various diseases like cancer [17], inflammation [18], or antiaging purposes [9]. Unfortunately, these compounds are characterized by their low stability, making them very sensitive to light and heat. 1,4-NQ present also a poor biodisponibility mainly due to their low water solubility.

The objective of the current investigation is to formulate for the first time bigel-based alginate with active 5,8-dihydroxy-1,4-naphthoquinone (M1) and 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (M2) for topical application. Further, their characterization and antioxidant evaluation, in comparison with free quinones, will be performed.

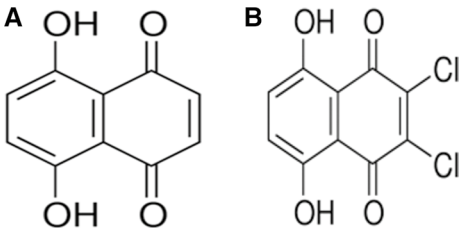

Fig. 1 Chemical structures of **a**: 5, 8-dihydroxy-1,4-naphthoquinone (M1), and **b** 2,3-dichloro-5, 8-dihydroxy-1,4-naphthoquinone (M2)

2 Materials and Methods

2.1 Materials

5,8-Dihydroxy-1,4-naphthoquinone (Fig. 1a), 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (Fig. 1b), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS), potassium persulfate, NaH_2PO_4, Na_2HPO_4, $K_2S_2O_8$, acid alginic, span 65, beta carotene, linoleic acid, Tween 40, chloroform, distilled water, dimethylsulfoxide (DMSO), $K_3Fe\ (CN)_6$, trichloroacetic acid, and $FeCl_3$ were purchased from Sigma-Aldrich, France, and the stock solution of M1 and M2 was prepared in DMSO and stored at 4 ± 0 °C in the dark. Sweet almond oil was purchased from (Amazon, Toulouse, France). All chemicals used were of the highest grade commercially available.

2.2 Methods

2.2.1 Development of Bigel Base and Bigel with Active Molecules

The composition of blank bigel and the bigel with M1 and M2 is shown in Table 1. Hydrogel and organogel (50/50) (w/w) were prepared separately and stirred with an ultra-turrax (6000 rpm) at 60 ± 0 °C. The organogel was prepared with the sweet almond oil as apolar solvent, and span 65 as organogelator. The complex was prepared at 60 ± 0 °C and then cooled over night at 4 ± 0 °C. To make the hydrogel, alginate was chosen at concentration of 3% (w/v) and mixed with water, using a mechanical homogenizer (300 rpm) for 60 min. The active molecules were then added continuously

Table 1 Composition and proportion of different samples: blank bigel, bigel with M1 and M2, and cetavlon®

Samples	Compounds	Formulation (w/w) Bases (for 10 g)	Formulation (%) Bigel with AI[a]
Hydrogel	Alginate	0.3	50
	Water	9.7	
Organogel	Span 65	2.0	50
	Sweet almond oil	8.0	
5,8-Dihydroxy-1,4-naphthoquinone (M1)			1.0
2,3-Dichloro-5,8-dihydroxy-1,4-naphthoquinone (M2)			1.0
Cetavlon®	Alcohol	3.3	
	Paraffin	6.6	
	Cetrimide	0.05	0.5

[a]*AI* active ingredient

stirred to the organogel before adding the aqueous phase. The amount of M1 and M2 in the bigels were kept constant at 1% (w/w). Blank bigel was prepared in the same manner without adding active compounds. Prepared bigels were stored at 25 ± 2 °C in well-closed glass containers protected from light. The developed bigels were evaluated for their organoleptic properties (homogeneity, texture, and freshness) and pH [19].

2.2.2 Physicochemical Characterization

2.2.2.1 pH Measurement The pH was determined at 27 ± 0 °C by measuring that of a tenth dilution of each formulation in distilled water using the pH meter (Bante Instruments 950). Under the same conditions, the pH of each excipient was measured [19]. All the measurements were performed in triplicate.

2.2.2.2 Rheological Study The flow properties of the bigels were studied using a rheometer (Thermo Scientific HAAKE RS75 Rheo Stress) at 25 ± 2 °C to determine the character of the formulation obtained [20, 21]. Three recordings were taken per sample.

2.2.2.3 Differential Centrifugation The resistance of the formulated bigels to centrifugation was tested in a 2-mL graduated cylinder, once at 4000 rpm for 10 min and at 25 ± 2 °C and then again at 5000 rpm for 10 min as well, using a centrifuge (Eppendorf AG 22331 Hamburg) as described by Cockton and Wynn [22].

2.2.2.4 Microscopic Observations The microstructure of formulated bigels, in terms of particle size distribution, number and shape were studied via 3D digital microscopic analysis [21]. Samples were prepared by spreading a very thin layer of the bigel on the specimen slide and pressing it well with a cover slip. Micrographs were obtained using the microscope model VHX 3D (kayence VHX), equipped with objective 40× and taken on undiluted samples.

2.2.2.5 Formulation Stability (Safety Assay) Bigels with 1% of quinones were evaluated immediately after preparation and after lapse of almost 12 months of storage at the temperature of 25 ± 2 °C. Microbiological examination was carried out in conformity with requirements of European Pharmacopoeia for non-sterile products [23–25].

2.2.2.6 Sensory Analysis The sensory analysis (hedonic test type) of the prepared formulations was carried out with a panel of untrained subjects (60 people) under well-defined conditions to ensure an objective and a neutral evaluation as possible of the prototype of formulated bigels [26]. It refers to the qualitative properties of the formulation, perceived by the customer and which must be adapted to the intended application. Thus, evaluated parameters were the following: homogeneous appearance, non-oily, ease of spreading, short penetration time, and freshness.

2.2.3 Antioxidant Activity

2.2.3.1 DPPH Test Antioxidant-scavenging activity was studied using DPPH assay as described by [27] with some modifications. 20 µL of various dilutions of each sample was mixed with a 0.2 mM methanolic DPPH solution. After 30 min of incubation at 25 ± 2 °C, the absorbance at 524 nm was recorded as $A_{(\text{sample})}$. For the $A_{(\text{blank})}$, we applied the same experimentation for a solution devoid of the test material, and then we recorded the absorbance. Then, for each solution, we calculated the free-radical-scavenging activity as percent inhibition as the following equation:

$$\%\ \text{Inhibition} = 100 \times \left[\left(A_{(\text{blank})} - A_{(\text{sample})}\right)/A_{(\text{blank})}\right]. \quad (1)$$

The IC_{50} is the concentration required for the test formulation to cause a 50% decrease in DPPH concentration. Ascorbic acid was used as reference. All the measurements were performed in triplicate.

2.2.3.2 ABTS Assay BG-M1, BG-M2, standard ointment, and blank bigel were subjected to evaluation of their ability to scavenge free radicals by using ABTS radical assay as described by [27] with some modifications. A solution of ABTS (7 mM) was mixed with 2.5 mM of $K_2S_2O_8$, followed by storage for 16 h in the dark at 25 ± 2 °C. The mixture was then diluted with water, and the absorbance was determined at 734 nm. 20 µL of ABTS diluted was added to each sample. The capacity of free radical scavenging was expressed by IC_{50}, which indicates the required concentration to scavenge 50% of ABTS radicals. The same equation described previously for the DPPH assay was used to calculate this capacity. Ascorbic acid was used as reference. All measurements were performed in triplicate.

2.2.3.3 Reducing Power Assay The ability of the tested samples to reduce Fe^{3+} was assessed by the method of Oyaizu [28] with slight modifications. 20 µL of samples was mixed with 0.2 M phosphate buffer (pH 6.6) and 1% $K_3Fe(CN)_6$. After incubation at 50 ± 0 °C for 20 min, trichloroacetic acid (10%) was added, and the mixture was centrifuged at 650×*g* for 10 min. Finally, 50 µL of the upper layer was mixed with distilled water and 0.1% of aqueous $FeCl_3$, and then absorbance was measured at 700 nm.

2.2.3.4 β-Carotene Bleaching Test A modification of the method described by Koleva et al. [29] was employed.

β-carotene (2 mg) was dissolved in 20 mL chloroform, and to 4 mL of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under reduced pressure at 40 ± 0 °C and 100 mL of oxygenated ultra-pure water was added, then the emulsion was vigorously shaken. An aliquot (150 µL) of the β–carotene/linoleic acid emulsion was distributed in each of the wells of 96-well plate, and solutions of the test samples (10 µL) were added. Three replicates were prepared for each sample. The plates were incubated at 50 ± 0 °C for 120 min. Absorbance was measured immediately ($t = 0$ min) and after incubation ($t = 120$ min) using a model EAR 400 plate reader (LabSystems Multiskan MS) at 470 nm. The β-carotene bleaching ability of the samples was determined using following formula:

$$\text{Inhibition } (\%) = \left[\left(A_{s\,(120)} - A_{c\,(120)}\right) / \left(A_{c\,(0)} - A_{c\,(120)}\right)\right] * 100. \quad (2)$$

where $A_{c\,(0)}$ and $A_{c\,(120)}$ are the absorbance values of the control at ($t = 0$ min) and ($t = 120$ min), respectively, and $A_{s\,(120)}$ is the sample absorbance at 120 min. The results were expressed as IC_{50} values (mg/mL).

2.3 Statistical Analysis

All data were expressed as mean ± standard deviation of triplicate measurements. The confidence limits were set at $p < 0.05$. Correlations were carried out using the correlation and regression in the Microsoft® EXCEL program. Data analysis procedure (ANOVA) was performed into evaluate results.

3 Results

3.1 Determination of pH

The skin is covered with a hydrolipidic film, which determines the skin pH. Thus, determination of a topical formulation's pH is an important factor for its efficiency. The blank bigel (BG) which is a preparation devoid of the active molecule is tested to insure its skin tolerance. Cetavlon® has also been evaluated as a reference; it is a pharmaceutical formulation (cream) with a synthetic active compound (cetrimide) with antioxidant activities [30]. As shown in Table 2, the pH of freshly prepared control was 5.8 ± 0.2, whereas the pH of BG-M1 and BG-M2 were 5.3 ± 0.1 and 5.4 ± 0.1, respectively, which is within the range of skin pH. The pH value of cetavlon® was also suitable for skin (5.5 ± 0.2).

Table 2 Physicochemical indices of tested samples including blank bigel, bigels with M1 and M2, and cetavlon®

Samples	BG	BG-M1	BG-M2	Cetavlon®
Physical stability[a]	+	+	+	+
Formulation stability[b]	+	+	+	+
pH	5.8 ± 0.2	5.3 ± 0.1	5.4 ± 0.1	5.5 ± 0.2

[a]+: stable (no phase separation)

[b]+: stable (no microbial development)

3.2 Rheological Study

The flow property of a formulation is one of its major characteristics. Thus, measuring such feature is very essential. The viscosity values of bigels present a non-Newtonian shear-thinning flow behavior (pseudoplastic fluid), identified by a weakening in the viscosity with a reciprocal rise in the shear rate, as shown in Fig. 2c, which presents the viscosity as a function of shear rate. The shear thinning property is a typical and suitable with the semisolid systems.

3.3 Physical Stability

In order to characterize the prepared bigels, various criteria were used, namely the physical stability. In this study, centrifugation test was performed for all samples stored at 25 ± 2 °C. Results are represented in Table 2. No phase separation after centrifugation was seen in any of the samples.

3.4 Microscopic Observations

Prepared bigels (control) were characterized by the presence of micrographs and a porous structure, which promote their use in the field of drug system delivery (Fig. 2b). All samples present a tridimensional network constituted by an interconnected porosity, and the pores were regular in size for blank bigel and bigel with active molecules and BG-M2. All formulations have shown small uniform droplets regular in terms of size and number. Bigels have kept their microstructure even after incorporation of active M1 (Fig. 3a) and M2 (Fig. 3b).

3.5 Formulation Stability

Microbial quality of the developed formulation was observed at the end of experiments (one year after its preparation). There was no change in the color, aspect, and microbial growth of the formulation during the storage time (Table 2).

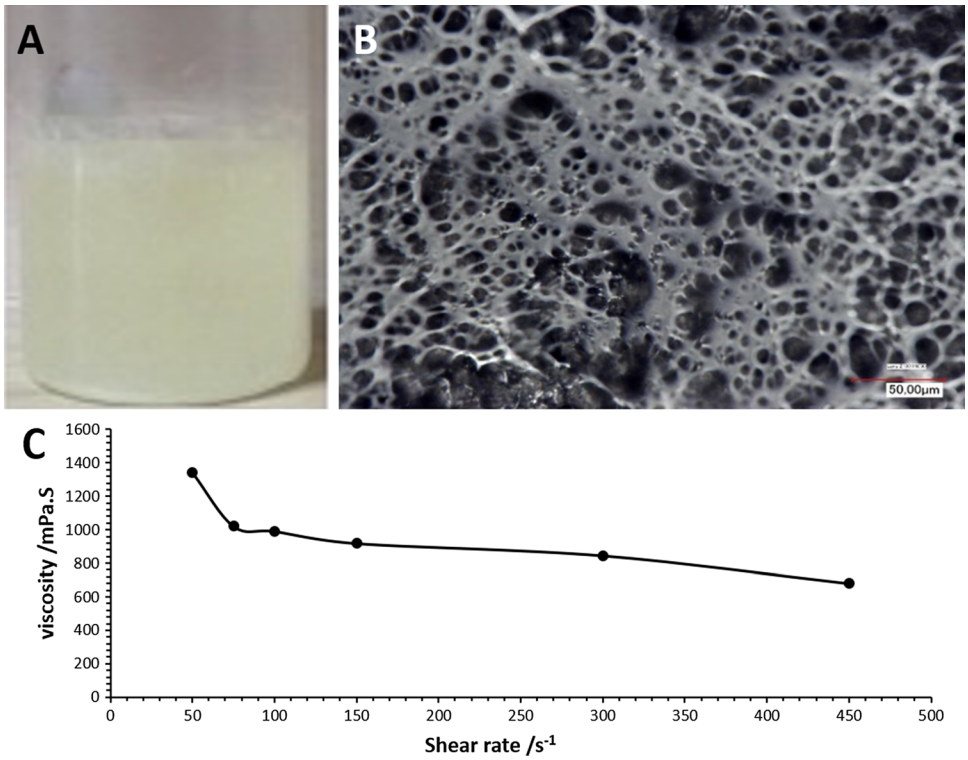

Fig. 2 Characterization of blank bigel; **a** developed bigel (alginate and organogel), **b** microscopic observation 3D (50×); **c** effect of shear rate on the viscosity

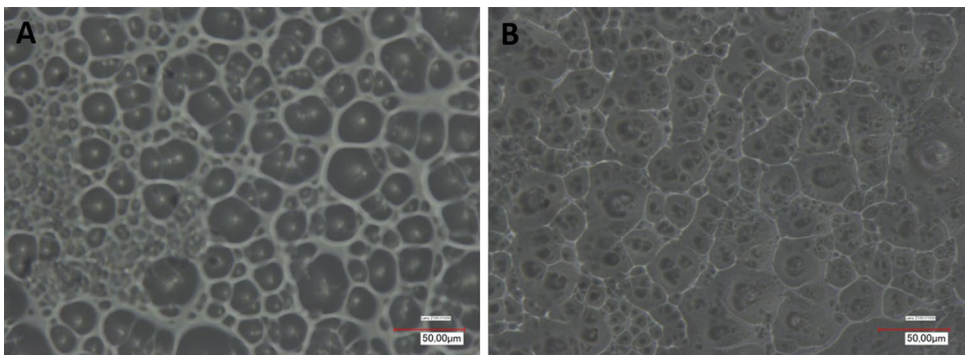

Fig. 3 Microscopic observations of developed bigels; **a** BG-M1, **b** BG-M2. Reference bar corresponds to 50 µm

3.6 Sensory Analysis

Sensory evaluation was developed to collect sensory description of prepared formulations from consumers. Obtained data performed on a panel of 60 people are represented in Fig. 4. Several organoleptic properties were evaluated on the formulated bigels (BG-M1 and BG-M2): before (homogeneous appearance), during (ease of spreading, penetration time), and after application (oily/fatty, freshness). The homogeneity of bigels was well perceived (7.4 and 7.1/10 for BG-M1 and BG-M2, respectively) by the panel. During application, spreading was perceived as easy (7.6 and 6.6/10 for BG-M1 and BG-M2, respectively) with a satisfying penetration time (7.0 and 6.5/10 for BG-M1 and BG-M2, respectively). After application, the bigel seemed to

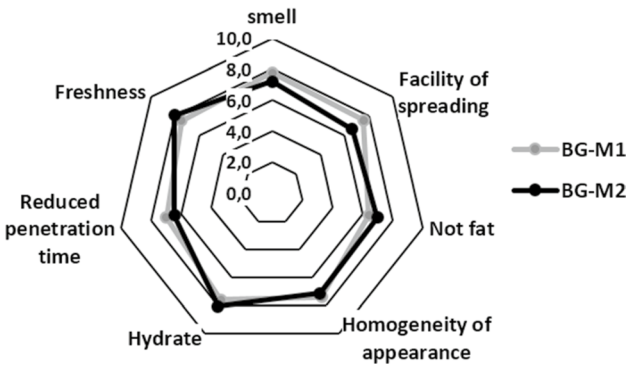

Fig. 4 Data of sensory analysis of bigels made on a panel of 60 people

be non-oily (6.4 and 7/10 for BG-M1 and BG-M2, respectively) with a sensation of hydration perceived as favorable (7.6 and 8.1/10 for BG-M1 and BG-M2, respectively) and a feeling of freshness (7.5 and 8.1/10 for BG-M1 and BG-M2, respectively).

3.7 Antioxidant Assays

The radical-scavenging capacity of samples was evaluated with the DPPH and ABTS assays as represented in Table 3. All samples have never been tested for their antioxidant potential by previous investigators. Obtained data suggested a good scavenging capacity of free M1 and M2 toward DPPH radicals, for M1 = $(19 \pm 0.0) \times 10^{-3}$ mg/mL and for M2 = $(35 \pm 0.0) \times 10^{-3}$ mg/mL. Even, after being incorporating in alginate bigels, they kept this potential. In fact, developed bigels have reported good radical-scavenging activity via DPPH assay with IC_{50} equal to 3.0 ± 0.4 and 1.1 ± 0.2 mg/mL for BG-M1 and BG-M2, respectively.

The capacity of samples to scavenge free radicals was also, determined by another method based on decolorization of the solution of ABTS° + cation, at a specific absorbance (734 nm) for a precise time of incubation. Free quinones have showed a high antioxidant effect by scavenging ABTS radical with IC_{50} equal to $(1.5 \pm 0.0) \times 10^{-3}$ and $(2.5 \pm 0.0) \times 10^{-3}$ mg/mL for M1 and M2, respectively. The potential of free molecules against ABTS radical had decreased, when incorporated into bigels with IC_{50} equivalent to 1.7 ± 0.1 and 4.9 ± 0.4 mg/mL for BG-M1 and BG-M2, respectively. Similarly, concerning the reducing power, formulated bigels presented interesting capacities with IC_{50} equivalent to 4.1 ± 0.1 and 4.9 ± 0.2 mg/mL for BG-M1 and BG-M2, respectively, but less important than those of free quinones, which showed IC_{50} equal to $(2.3 \pm 0.3) \times 10^{-3}$ and $(1.1 \pm 0.1) \times 10^{-3}$ mg/mL for M1 and M2, respectively. As well, developed formulations revealed less important β-carotene bleaching inhibition (IC_{50} equal to 2.9 ± 0.1 and 10.3 ± 0.5 mg/mL for BG-M1 and BG-M2, respectively) than pure molecules (IC_{50} equal to 0.3 ± 0.0 and 0.1 ± 0.0 mg/mL for M1 and M2, respectively).

4 Discussion

The pH of prepared bigels with or without active molecules was found to be around 6 which is suitable for topical application as the skin pH of normal subjects has a range of 4.5 to 6.0 as defined by Jennifer et al. [31]. All tested preparations were found to enhance a safe application to the skin without irritation problems.

Biphasic formulation (organogel and hydrogel) affects the rheology of the bigel itself by its viscosity and chemical ingredients [32]. The rheological characteristics of bigels investigated in the current study are similar to those of semisolid systems gels used in cosmetic or pharmaceutical applications [33–35]. This formulation guarantees a maximum area and simple utilization during a topical application. Those data are in line with Singh et al. [36] who found that bigels for topical delivery of metronidazole showed a non-Newtonian behavior.

Centrifugation was used as a helpful means to determine the physical stability of formulations [37]. Data showed no sign of separation phase, which reflect a proper homogenization speed during bigel development, and proved that formulated bigels were stable considering phase separation as a parameter of stability.

These formulations were also observed by microscope 3D. They showed uniformly distributed droplets with a network of apolar phase of organogel. Hence, this observation confirmed the formation of oil in water type of bigel, as confirmed previously by the rheological study. The dispersion of microparticles may reflect the possibility that big droplets have broken into smaller ones when higher shear force was applied. The flow of microparticles may be resulting on the decrease of viscosity [36].

Formulation stability of prepared bigels revealed no microbial growth during the experimentation period. That verified the requirements of European Pharmacopoeia [23–25], and determined the antimicrobial property of BG-M1 and BG-M2, which could protect the bigels against microorganism contamination. As formulated bigels have

Table 3 *In vitro* antioxidant activity of samples as IC_{50} (mg/mL) via scavenging abilities, reducing power, and β-carotene bleaching test

Samples/activities	Scavenging activity		Reducing power	β-carotene bleaching activity
	ABTS	DPPH		
BG-M1	1.7 ± 0.1	3.0 ± 0.4	4.1 ± 0.1	2.9 ± 0.1
BG-M2	4.9 ± 0.4	1.1 ± 0.2	4.9 ± 0.2	10.3 ± 0.5
BG	–	–	–	–
Cetavlon®	–	–	15.4 ± 1.1	–
Free M1	$(1.5 \pm 0.0) \times 10^{-3}$	$(19 \pm 0.0) \times 10^{-3}$	$(2.3 \pm 0.3) \times 10^{-3}$	0.3 ± 0.0
Free M2	$(2.5 \pm 0.0) \times 10^{-3}$	$(35 \pm 0.0) \times 10^{-3}$	$(1.1 \pm 0.1) \times 10^{-3}$	0.1 ± 0.0

Data are expressed as mean ± standard deviation ($n = 3$); –: absence of activity

not developed microbes and alginate had no antibacterial activity, it might be due to the presence of free quinones M1 and M2 within these formulations, which enable them to have antimicrobial properties.

Sensory analysis was evaluated in order to define the customer preferences. Obtained results were relatively pleasant for the general aspect of the formulations. For tested preparations, the bigel was obtained by mixing the organogel and the hydrogel at 50/50 w/w. These proportions must not be modulated in order to preserve these organoleptic properties. Indeed, this proportion is the one that is responsible for touch perceived acceptable as well as the penetration time during and after application.

During this work, antioxidant potential of formulated bigels and free compounds were evaluated. We found that free quinones M1 and M2 have shown antioxidant activities through the DPPH assay; $(19 \pm 0.0) \times 10^{-3}$ mg/mL for M1 and $(35 \pm 0.0) \times 10^{-3}$ mg/mL for M2. Even, when introducing into formulated bigels, they have kept their scavenging capacities against DPPH radical with IC_{50} equal to 3.0 and 1.1 mg/mL for BG-M1 and BG-M2, respectively. Also, the scavenging ability toward ABTS radical cation of free M1 and M2 had decreased for more than thousand times, after processing, as shown in Table 3, which represented also the reduction of their iron reducing power for almost the same values.

Moreover, the capacity of samples to inhibit the auto-oxidation of polyunsaturated fatty acids was performed using the β-carotene bleaching assay. Free M1 showed an inhibition almost ten times higher than prepared BG-M1, likewise, free M2 had reported a β-carotene bleaching inhibition one hundred times more interesting than developed BG-M2. This might be due to interactions between pure molecules and the bigel matrix, thus covering the chromophore of M1/M2 or causing changing of its position, shape or conformation, resulting in a decrease of the activity. Also, this phenomenon might be explained by the formation of a complex between pure molecules and fatty acids of organogel, which created a suitable environment making free M1 and M2 lazy when exerting their effect.

It is important to highlight that despite the antioxidant potential's decrease of free molecules (against the ABTS and DPPH radicals, reducing power and β-carotene bleaching inhibition), formulated bigels have preserved their antioxidant power which was more important than that of cetavlon®. The absence of any antioxidant effect of blank bigel (BG) guarantees that the bigel's antioxidant potential is specific to the molecules themselves. This could be explained by the fact that active compounds have found a suitable environment with matrix of bigel, so they were able to preserve their antioxidant power.

During this study, we succeeded to formulate bigels for topical application with a high quality and good stability protecting skin from oxidative stress damages mediated aging and UV radiations. Several studies have reported the antioxidant potential of plants preventing oxidative disorders [38] and reducing ROS production involved in skin damages [39, 40]. As our knowledge could be certain, this is the first work describing the antioxidant effect of M1 and M2 on the one hand, and their formulation within alginate bigel for topical application, on the other hand. Therefore, comparison with other works could be taken just with molecules of the same family. Our data is in accordance to what was reported by Soussi et al. [41], who have demonstrated the potent antioxidant effect of phenolic compounds of *Juglans regia*, and data found by Casagrande et al. [42], that quercetin, a flavonoid, which kept its antioxidant and showed protective effect against UVB-induced oxidative stress in hairless mice after processing. Other investigators have developed alternative delivery systems for pure compounds, such as Mohammad et al. [43], who have formulated rutin in nanoemulsion system.

In this study, we formulated preparations in which water and oil content in formula contains more than 70% by weight of the bigel. Microscopic observations showed that all formulations (bigel control and bigel with active molecules) were semisolid preparations with distribution of polar phase and apolar one. Similarly for the BG, the BG-M1 and BG-M2: there was no change occurred in color up to the observation period of more than 12 months. This showed the stability of bigels at 25 ± 2 °C.

5 Conclusion

Performed examinations of formulated bigels with M1 and M2 proved a significant antioxidant potential and suitable chemical and formulation stability. The prepared bigels showed non-Newtonian behavior, which reflecting their Pseudoplastic tendency, resulting in the formation of a coherent film covering the skin surface. This property is valuable and critical for a better fortification of the skin surface. Bigels have also shown a good spreadability, no evidence of phase separation, good consistency, high stability parameters, and no microbial growth during the experiment period. Sensory analysis of the formulations showed that prepared bigels were pleasant by the panel of 60 people. These developed bigels maintaining or improving the antioxidant potential of M1 and M2 could be used as the provision of a barrier to protect skin.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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