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Formulation and Control of a Topical Emulsion, Containing Algerian Vitis vinifera L. Leaves Extract

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Abstract

Topical emulsions find their applications in wrinkle reduction (skin aging), acne treatment and, sebum secretions regulation. For various dermatological affections, many topical formulations like sunscreens and anti-aging creams are prepared using plant-based ingredients

The objective of this study was to formulate an emulsion containing *Vitis vinifera L.* leaves extract, and to evaluate its stability and antioxidant activity in vitro. A spectrophotometric assay of the main phenolic groups for 10 *Vitis vinifera L.* leave samples was performed. O/W emulsions were prepared using a nonionic surfactant polysorbate 80.1,1-diphenyl-2-picrylhydrazyl assay was performed to evaluate plant extracts and emulsions antioxidant activity.

The Physical stability of the emulsions stored at 25 °C and 40 °C for 60 days was assessed based on various physico-chemical characteristics including color, creaming, liquefaction, centrifugation pH, and electrical conductivity.

The emulsions showed good physical properties and pharmaceutical stability. The polyphenol-rich-plantderived extract and the emulsion showed good antioxidant activities.

this research allowed the development of an emulsion based on *Vitis vinifera L*. extract, which can be proposed for topical use. However, in vivo studies are recommended to confirm the antioxidant action of this cream.

Key Words: Plant, emulsion, antioxidant, stability, parameters.

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1. Introduction

Emulsions are thermodynamically unstable dispersed systems, of two immiscible phases, one of them dispersed in the other as fine droplets by using an interface surfactant (Seiller and Martini 1996). They have potential applications in paint, food, cosmetic and pharmaceutical industries (Al-Achi 2020). They have received particular interest as Drugs Delivery Vehicle because they increase their bioavailability (Abdulbagi and Rajab 2020; Zhao et al. 2020). Emulsions are the most frequently used galenic form in dermocosmetology, due to their physical, chemical, and biological compatibility with the skin. Their main advantage is that they increase solubility, bioavailability, and skin absorption of active substances (Lu and Gao 2010). Oil-in-water or O/W emulsions are commonly used as bases for water-soluble substances in cosmetology (Lim et al. 2020). A benefit value can be brought to these formulations by including components with a specific cosmetic effect, especially those containing antioxidants as an active ingredient (Noon et al. 2020). Due to their biological and therapeutic properties, plant extracts and their derived products are often included in emulsions and several other pharmaceutical preparations (Huma et al. 2020). In the last decade, there has been a lot of interest in the antioxidant potential of natural polyphenol in health field (Pandey and Rizvi 2009).

Red vine, one of the most widespread fruit crops, is a plant rich in polyphenols (Baydar et al. 2004). Several studies have reported that red vine leaves contain organic acids, phenolic acids. flavonols. tannins. procyanidins, anthocyanins, (Orhan et al. 2009; Selka et al. 2019). Most therapeutic properties of this plant and especially of its leaves are related to phenolic compounds (Xia et al. 2010). The use of plant phenolic extract in new cosmetic product development such as emulsions or creams has already been mentioned (Zillich et al. 2015; Selka et al. 2016). Plant polyphenols can be used as sunscreens, whitening, and anti-aging agents (González et al. 2008).

Emulsion stability is an important factor that determines the safety of its use, this type of

formulation is thermodynamically unstable, so it may be subject to deterioration over time because of various physicochemical mechanisms such as creaming, flocculation, coalescence, phase inversion and/or Ostwald ripening (Ushikubo and Cunha 2014, Ralla et al. 2020). This study aimed to develop a *Vitis vinifera L.* leaf extract emulsion based, and to study its physicochemical stability and its in vitro antioxidant property.

2. Material and Methods

2.1. Apparatus: Centrifuge Sigma 4-16K, Germany; Spectrophotometer OPTIZEN 3220, South Korea; Vortex mixer VWR, Germany; Rotary Evaporator Buchi RII, Germany; Homogenizer ultra-turrax T18, Germany; Microscope Leica DM300, Germany; pH meter Inolab WTW level 2, Germany; Conductivity meter Inolab WTW level 1, Germany.

2.2. Plant material: Red vine leaves sampling was carried out in autumn 2020 (September-October), during this period Vitis vinifera L. leaves begin to take a reddish color, and their polyphenol content is at its highest level (Katalinic et al. 2013). The collection was carried out in various areas of Algeria; region selection was based on the international organization of oenology and viticulture (OIV) report, classifying the most known regions for viticulture through the northern Algerian area from East to West (OIV 2017)

The region of El-Bayadh does not appear on The OIV report, it was introduced following the recent development that knows the viticulture in this region despite its particular climate. Geographical situations and bioclimatic stages of the different stations are represented in Table 1.

Region	Sample	Collecting	Latitude	Longitude	Altitude	Sector	Bioclimatic
	code	date	(North)	(West)	(M)		stage
Terga -	E3	October	35.41'	-1°17'	28	Oranese interior	Semi-arid
Aïn Temouchent		2015				plain : O2	
Bir El Djir - Oran	E1	October	35.72'	-0°55'	164	western coastline	Sub-humid
		2015				: 01	
Dellys –	E6	October	36.87'	3° 93'	184	central coastline :	Sub-humid
Boumerdes		2015				A1	
Aïn Soltane -	E5	October	36.07'	4° 81'	946	Constantine High	Semi arid
Bordj-Bou-		2015				Plains H2	
Arreridj							
Stidia –	E4	October	35.83'	0° 006'	26	western coastline	Sub-humid
Mostaganem		2015				: 01	
Dhayet El-Bagra	E10	October	32.35'	0° 60'	810	Saharian : SS1	Saharian
El-Bayadh		2015					
Si Mahdjoub -	E2	October	36.28'	2° 76'	789	Tellian Atlas : A2	Sub-humid to
Medea		2015					humid
El Bouni –	E9	October	36.85'	7° 74'	20	Numidian : K3	Sub-humid to
Annaba		2015					humid
Beni	E7	October	35.39'	0° 14'	563	Tellian Atlas : 03	Sub-humid to
Chougrane -		2015					humid
Mascara							
Mitidja - Blida	E8	October	36.49'	2° 84'	199	central coastline :	Sub-humid
		2015				A1	

Table 1. Geographical situations and bioclimatic stages of the sampling stations

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The collected samples were identified by Dr M.Chelghoum in the botany laboratory of Sidi Bel Abbes pharmacy department in Algeria and confirmed by the Botany Institute of Liege University in Belgium.Harvest materials were shade-dried, at room temperature. The drying time was about 10 days for the different samples, which were afterwards stored in Kraft paper bags. **2.3. Microscopic observation of leaves powder:** Grapevine leaves powder was placed separately on slides, each slide was mounted 2-3 drops of chloral hydrate, and each slide was covered with a coverslip, and then examined under a microscope at different magnifications. The found elements were noted and photographed.

2.4. Phenolic compound extraction: 40 g of crushed leaves were placed in a flask

containing 100 ml of methanol-water mixture (80 - 20) v/v and 0.1 ml/ml of 37% hydrochloric acid to avoid polyphenols oxidation. Extraction was performed by reflux at a temperature of 60°C for 30 minutes. The extracts were filtered and centrifuged at 3000 rpm for 20 min at 25°C, the supernatants were concentrated using a rotary evaporator at room temperature, and the crude extract was kept at low temperature in amber glass vials until use (Benmeziane et al. 2014).

2.5. Determination of the total phenolic content: The total polyphenol content of leaves extracts was determined by the Folin-Ciocalteu method.400 microliters of methanolic extract were mixed with 1.6 ml of 7,5% sodium carbonate solution (Na2CO3) and with 2 ml of freshly prepared Folin-Ciocalteu reagent (diluted (1:10). The mixture was vortexed and incubated at room temperature for 90 min, the absorbance was measured at 765 nm. A calibration curve was obtained using gallic acid solution at different concentrations. The result was expressed as mg Gallic acid equivalents/ g dry plant material. All operations were performed in triplicate (Benmeziane et al. 2014).

2.6. Determination of the total flavonoid content: Flavonoid content was determined using the method described by Ying and Wan (2012).1 ml of diluted extract was mixed with 3 ml of distilled water and with 300 μ l of 7% sodium nitrite (NaNO2) solution. This was incubated for 5 min. Later, 300 µl of 10% aluminum chloride (AlCl3), and 1 ml of sodium hydroxide (1N) were added to the mixture. After 6 min of incubation, the total mixture was placed in visible а spectrophotometer and the absorbance reading was taken at a wavelength of 510 nm. A calibration curve was obtained using catechin solution at different concentrations. The result was expressed as mg catechin equivalents/ g dry plant material. All operations were performed in triplicate (Ying and Wan 2012).

2.7. Determination of condensed tannins content: Condensed tannins content was estimated using the vanillin HCl method.50 μ l of diluted methanolic extract was mixed with 1500 μ l of vanillin/methanol solution (4%, w/v) and with 750 μ l of 37% hydrochloric acid (HCl). After 20 minutes of incubation at room temperature, the absorbance of the mixture was measured at 500 nm. A calibration curve was obtained using catechin solution at different concentrations. The result was expressed as mg catechin equivalents/ g dry plant material. All operations were performed in triplicate (Julkunen-Tiitto 1985).

2.8. Preparation of emulsions: A galenic formulation, following the work of Rasul and Akhtar (2012) and Khan, Akhtar et al (2013) was carried, based on grapevine leaves extract (Rasul and Akhtar 2012; Khan et al. 2013).The extract used for emulsion development was chosen according to the crude extract amount and its polyphenol content.Two O/W emulsions type (control formulation and formulation containing the extract) were prepared according to the work of Khan, Akhtar et al. (2013), the qualitative quantitative composition of the and emulsions is summarized in Table 2.

The preparation was carried out in four steps:

-Oily phase excipients mixing (liquid paraffin, stearic acid, cetostearyl alcohol, beeswax, sorbitan monooleate) at a temperature of 70°C.

-Aqueous phase excipients mixing and addition of grapevine leaves extract at the temperature of 70°C.

-Addition of the aqueous phase to the oil phase on a drop-to-drop basis under continuous stirring at 1000 rpm for 10 min.

Control emulsion was also prepared by the same method above but without plant extracts (the active ingredient). Homogenization of the emulsion with a homogenizer at 13000 rpm for 2 min (Khan et al. 2013)

Ingredients	Role	Control emulsion	Active emulsion
liquid paraffin	Lipophilic phase	27%	27%
Stearic acid	emulsifier	5%	5%
Sorbitan monooleate	Lipophilic surfactant	1.2%	1.2%
Beeswax	thickener	4%	4%
Cetostearyl alcohol	viscosifier	5%	5%
Polysorbate 80	Hydrophilic surfactant	6.8%	6.8%
Plant extract	active ingredient	-	5%
Water	Hydrophilic phase	51%	46%

Table 2. Composition of emulsions	s weight by weight (%,w/w)
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(Rasul and Akhtar 2012; Khan et al. 2013)

2.9. Characterization of emulsions: Various controls were carried out on the freshly prepared emulsions:

-Macroscopic examination: organoleptic characteristics (appearance, odor, color, and consistency) physical stability (creaming, sedimentation, and phase separation) (Smaoui et al. 2017; Huma et al. 2020)

-Emulsion Type: using the dye method where two dyes were used including methylene blue as a water-soluble dye and Sudan red III as a lipophilic dye.

-Microscopic examination: colored emulsion drop was analyzed using an optical microscope to study the globule size homogeneity and to detect flocculation and coalescence phenomena (Khan et al. 2013)

-Centrifugation stability: centrifugation test was done by adding 2 g of the colored emulsion into centrifugation tubes to be centrifuged at 25°C and speed of 3000 rpm for 30 min.

-pH measurement: emulsions pH was measured using a calibrated pH meter, after dilution (1:10) in neutral distilled water, (Wehrlé 2012; Brossard et al. 2016).

-Conductivity measurement: this test was performed to highlight a potential phase change. The electrical conductivity was measured in μ S/cm using a calibrated conductivity meter.

-Macroscopic examination, pH, and

conductivity measurements were performed during 60 days on the emulsions kept at 25°C and 40°C. These tests were carried out on D1, D7, D14, D21, D30, D40, and D60 (Khan et al. 2013).

2.10. Mathematical analysis: The percentage changes for the individual values of pH and conductivity, taken every week, were calculated by the following formula;

Percentage Change = [(A - B) / B]*100 Here; A = Individual value of any parameter on D1, D7, D14, D21, D30, D40, D60 B = Zero hour value of that parameter (freshly prepared emulsions)

2.11. Evaluation of the Antioxidant Activity: Extract and emulsions Antioxidant Activity was evaluated by DPPH (2,2diphenyl-1-picrylhydrazil) standard methods . 50 μ l of different concentrations of the extract was added to 1.950 ml of freshly prepared DPPH- methanolic solution (0.025 g/l). At the same time, a negative control was prepared by mixing 50 μ l of methanol with 1.95 ml of the methanolic DPPH solution. After 30 min of incubation in the dark at room temperature, the absorbance was measured at 515 nm (OPTIZEN 3220, South Korea). All operations were performed in triplicate.

The radical scavenging activity was

calculated as a percentage of DPPH discoloration using the following equation: DPPH radical scavenging $\% = [(A0 - A1)/A0] \times 100.$

Where A0 is the absorbance of the DPPH solution and A1 is the absorbance of the sample. The antiradical activity was expressed as IC50 (mg/mL), the extract dose required to cause a 50% decrease of the absorbance at 515 nm. A lower IC50 value corresponds to a higher antioxidant activity. Emulsion antioxidant activity was determined by the same method. 50 µl of different concentrations of each emulsion (base and with extract) previously diluted (1:100) were mixed with 1.950 ml of freshly prepared DPPH- methanolic solution (0.025 g/l). The negative control was prepared the same way as for the extract (50 µl of methanol with 1.95 ml of the DPPHmethanolic solution) (Sánchez-Moreno 2002; Khan et al. 2013). This operation aimed to define if the emulsion was able to keep the antioxidant activity of the extract compared to control formulation.

3. Results and Discussion

3.1. Microscopic observation

Microscopic observation of leaves powder revealed the following characteristic elements (Figure 1): Unicellular long and flexuous trichome (A), Parenchymatous cells containing calcium oxalate raphides (sharp needle shape) (B) fragments of cutinized epidermis with calcium oxalate star-shaped druses (C).



Figure 1: Characteristics of *Vitis vinifera L.* leaf powder, (A) Unicellular trichome, (B) Parenchymatous cells containing calcium oxalate raphides , (C) Calcium oxalate starshaped druses

powder In this work, microscopic characteristics of red vine leaves, agree with the data related by French Pharmacopoeia 11th edition, which mentions the presence of unicellular, long, and tapered trichomes with a truncated base and numerous dispersed calcium oxalate raphides or located in cells. French Pharmacopoeia 11th edition also fragments mentions of parenchyma containing calcium oxalate star-shaped druses like found in this study (French Pharmacopoeia 2014).

3.2. Total phenolic, flavonoid, and condensed tannin contents

Table 3, compares total phenolic, flavonoid, and condensed tannin contents for the different samples.

Table 3: Total polyphenol, flavonoids, and condensed tannins content of grapevine leaves

Sample area	Total polyphenol content *	Flavonoïd content**	Condensed tannins content **
Aïn Temouchent	391±40	10,62±0.23	5,30±0.04
Oran	923±88	9,87 ±0.15	5,14±0.16
Boumerdes	842±76	7,93±0.43	4,15±0.03

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Bordj-Bou- Arroridi	246±18	4,36±0.13	3.45±0.13	
Mostaganem	299±14	7,02±0.04	5,00±0.05	
El-Bayadh	316±13	5,74±0.25	1,81±0.04	
Medea	423±22	4,10±0.07	2,32±0.04	
Mascara	491±72	7,20±0.14	4,19±0.04	
Annaba	499±82	2.07±0.08	0,85±0.03	
Blida	558±88	4,15±0.12	1,82 ± 0.11	
* : mg Eq in Gallic acid / g dry plant ** mg Eq in Catechin / g Dry plant Means ± SD (n=3)				

The highest contents of total phenolic were found in Oran and Boumerdes samples, which were respectively 923±88.06 and 842±76.02 mg Eq in gallic acid / g dry plant material. Aïn Temouchent and Oran samples contained also the highest flavonoid contents, which were 10.62-±0.23 and 9.87±0.15 mg Eq in catechin/g Dry plant material, respectively.

The highest condensed tannin contents were found in Ain Temouchent and Oran samples with values of 5.30±0.04 and 5.14±0.16 mg Eq in catechin/g Dry plant respectively. All Samples material. contained more flavonoids than condensed tannins. Overall, Oran sample was the richest in total phenolic, flavonoid and condensed tannin compared to the others. Therefore, this extract was used as the active ingredient in the emulsion. A global comparison of polyphenols, flavonoid, and condensed tannin contents indicates that the highest flavonoid and condensed tannin concentrations do not necessarily correspond to the highest phenolic concentrations, so their distribution differs from one region to another. The obtained results confirmed high polyphenol contents of Vitis vinifera L. leaves. These results were in agreement with those found by Yu, Lim et al (2014) who determined the average polyphenol content of Vitis labruscana L.H.Bailey. leaves, which was in the order of 328.5±1.0 mg Eq in gallic acid / g dry plant material (Yu et al. 2014).

The work of Pastrana-Bonilla, et al (2003) is among the first studies on red grapevine leaves, from which they were able to determine an average polyphenol content of 351.6 mg Eq in gallic acid / g dry plant material, this content was obtained from ten cultivars of muscadine (*Vitis rotundifolia Michx*) from southern Georgia in the United States. The results of this study also agree with this present work (Pastrana-Bonilla et al. 2003).

Other studies such as that of Güler and Candemir (2014), who worked on five Vitis vinifera L. leaves samples from the Manisa region in Turkey found lower polyphenol levels with an average of 14.25 6 mg Eq in gallic acid / g dry plant material (Güler and Candemir 2014). The work of Taware et al (2010) also showed very low levels of polyphenols in five red vine leaves samples extracts from India, their contents average did not exceed 5 mg Eq in gallic acid / g dry plant material (Taware et al. 2010). Flavonoid contents were also variable from one region to another, the levels found in this work are close to those found by Güler and Candemir (2014), who reported total flavonoid values ranging from 5.08 to 7.22 mg catechin equivalent / g dry matter (Güler and Candemir 2014).

The same observation was found about condensed tannins with their variable distribution depending on samples. It was also noticed that all leave samples contained more flavonoids than condensed tannins. Generally, sub-humid and humid areas such as Oran, Mascara and, Boumerdes had high amounts of polyphenols, whereas semi-arid and Saharan areas (Ain Temouchent and El-Bayadh) had lower levels. Flavonoid and condensed tannin content variations do not necessarily depend on bioclimatic stage nature, since similar amounts for leave samples from humid and arid regions have been observed, so other factors besides the bioclimatic nature influence these variations.

It is important to remind that environmental factors have a major effect on polyphenol content, these factors can be pedoclimatic (soil nature, sun exposure, rainfall) or agronomic (cultivation in greenhouses. organic cultivation. hydroponic cultivation, fruit yield per tree, etc.)Light exposure has a considerable effect on flavonoid amounts. The degree of plant maturity significantly affects polyphenol concentrations. Generally, phenolic acid concentrations decrease during plant ripening, while anthocyanin concentrations increase. Many polyphenols, especially phenolic acids, are directly involved in plant response to different types of stress: they contribute to the healing process by the lignification of damaged areas, they possess antimicrobial properties and their concentration can increase after infection (Manach et al. 2004). Hydric stress is also one of the elements that can significantly influence polyphenolic composition as reported by (Król et al. 2014). With the current state of knowledge, it is extremely difficult to identify key factors causing the variability of phenolic type contents.

3.3. Emulsion control

• Organoleptic test

Both emulsions (control and active) presented a smooth and stringy consistency, a bright aspect with white color for the control and clear brown color for the active emulsion. The organoleptic evaluation was based on color change, odor, liquefaction, and phase separation during two months.

Color remained stable for both emulsions without any change during the whole 60 days period regardless of the storage temperature. Liquefaction was absent in both emulsions from D0 to D60 at 25°C and 40°C.

Phase separation was noticed after 40 days in the active emulsion stored at 40°C. There was no change in organoleptic characteristics of the control emulsion.

• Emulsions type

Both formulations were oil-in-water (O/W) emulsions, lipophilic dye (red Soudan III) colored only the oil phase giving a discontinuous aspect to the coloration whereas; hydrophilic dye (methylene blue) colored the continuous aqueous phase uniformly giving a homogeneous color, which was confirmed by emulsions water dilution (Figure 2).



Figure 2: Dye solubility method

• Microscopic examination

Microscopic observation confirmed the emulsions type (O/w), the observed drops had a relatively homogeneous size, with no flocculation and no coalescence phenomena (Figure 3).



Figure 3: Microscopic observation of the emulsions colored by methylene-blue (magnification 100)

• Centrifugation stability

There was no phase separation after centrifugation for both emulsions. (Figure 4).



Figure 4: Emulsions appearance after centrifugation

Topical emulsions find their applications in various dermatological affections and many of them are plant extract-based, for example, Huma, et al (2020) reported that beet leaf extract- based emulsion possessed antioxidant and anti-UV properties (Huma et al. 2020). Usually, the emulsion type is determined by the ratio of the oil phase and the aqueous phase.

In this work, the emulsion was prepared with 40.0% oil, and 60.0% water (a weight phase ratio of 2: 3), so the emulsion is oil/water type (O/W). The Microscopic analysis confirmed that both emulsions were oil/water type. There was no color change in both emulsions during the two months of the study period at different storage temperatures; this indicates the stability of the two formulations at different storage conditions.

Color conservation can be explained by different factors contributing to the emulsion stability; red vine leaves extract may contain antibacterial substances that protect the emulsion from possible microbial multiplication that could cause coloration change (Khan et al. 2013). Centrifugation test is a very useful method for assessing and predicting emulsion shelf life. No phase separation after centrifugation was observed in both emulsions. This suggests that the good speed homogenization used during the preparation, avoided its breakdown during the stress test as reported by (Colucci et al. 2020). After emulsion preparation, time and temperature-related factors could produce viscosity-altering processes, which result in emulsion liquefaction. No liquefaction was observed for both emulsions during 60 days In this study, the results were better than those of Khan, et al (2013), who observed a liquefaction process in Cassia fistula extractbased emulsion on D 21 kept at 40°C (Khan et al. 2013). As well as those of Sharif, et al (2014), who observed a liquefaction process in the grape-seed extract- based emulsion on D 21 kept at 40°C (Sharif et al. 2014). Creaming phenomenon is due to a density difference between two phases under gravity effects, which leads to their separation (Salager 2000). In this study, phase separation was observed for the active emulsion on D40 kept at 40°C. For Sharif, et al (2014) study, phase separation occurred earlier on D21 at 40°C, in contrast to Khan, et al (2013) work, where no phase separation was noticed (Khan et al. 2013; Sharif et al. 2014).

This delayed phase separation at high temperature, indicates good emulsion

stability based on creaming criteria, for a conservation period no longer than 40 days.

• Electrical conductivity

The electrical conductivity was measured for all samples kept at 25 oC and 40 o C, immediately after preparation and after 1, 7, 14, 21, 30, 40, and 60 days. Percentage changes in the conductivity values are presented in figure 5. The conductivity test showed a non-significant change in conductivity after two months for both emulsions kept at 25°C.

A significant conductivity decrease was observed in the control emulsion kept at

40°C and a less significant decrease (5.40%) of the active emulsion conductivity kept at temperature. Conductivity the same differences occur if there is an emulsion creaming, with the oil fraction increasing in the upper part and the aqueous fraction increasing in the lower part (Salager 2000). In this study, a significant difference in conductivity change was observed for both emulsions kept at 40°C, which is a sign of instability at this temperature. These results agree with those of Khan, et al (2013) who also observed a significant difference in electrical conductivity change for all emulsions kept at 40°C (Khan et al. 2013).



Figure 5: Means percentage changes in the conductivity of base emulsion and *Vitis vinifera L.* emulsion between day 0 and day 60 at 25°C and 40°C.

• pH test

Figure 6 showed that the pH of the control on D1 kept at 25°C and 40°C was 5.34 and

5.9 respectively, and that of the active emulsion was 4.77 and 4.74 respectively



Figure 6: Evolution of pH as a time function in base emulsion and *Vitis vinifera L.* emulsion stored at 25°C and 40°C from D1 to D60

Control emulsion pH kept at 25°C increased gradually during the first three weeks then decreased progressively and stabilized on D40 at the value of 6.66 while for the control emulsion kept at 40°C, the pH decreased during the first week, then increased gradually exceeding its initial value and reaching 7.09 on D60.

pH evolution profiles for the active emulsion kept at 25°C and 40°C, were similar to those of the base emulsion with a slight decrease during the first week then a progressive increase reaching the value of 5.84 for the sample kept at 25°C and 5.05 for that kept at 40°C.

pH is an important indicator of topical emulsions safety and efficacy (Brossard et al. 2016), human body skin has a pH that

varies from 4.5 to 6.0, a value of 5.5 is supposed to be the average skin pH, therefore, the formulations recommended for dermatological use should have a pH in this range (Lambers et al. 2006).

According to the results of this work, active emulsion pH was within dermatological pH standards, unlike to control emulsion pH, so it does not lead to any modification of factors regulating skin hydration (Lambers et al. 2006).

• DPPH radical-scavenging activity Table 4 summarizes the percentage inhibition of DPPH radical for Oran extract sample and the emulsions as well as their IC 50.

sample	Total polyphenol	IC 50	% inhibition		
	content				
Grapevine leaves extract	923±88	2.9±0.04	48.80 ±9.24		
Control emulsion	112±1.15	15.6±0.13	9,22 ±1.66		
Active emulsion	208±14	9.37±0.21	18,20 ±2.29		
IC50 : concentration of antioxidants needed to decrease the initial DPPH concentration by 50%					
expressed by μg/ml					
Means ± SD (n=3)					

Table 2. Extract and emulsions DPPH radical scavenging activity

The addition of grapevine leaves extracts to the emulsion increased its antioxidant activity twice, but it remained lower than that of the crude extract used in the formulation. The same observation can be made for the polyphenol content of the emulsion, which was lower than that of the crude extract. The active emulsion showed a good antioxidant activity but lower than that of red vine leaf extract. This can be explained by the extract quantity (5%) used in the formulation. Active emulsion antioxidant activity was more important compared to that of the control emulsion, which indicates that the extract is effectively the factor responsible for inhibition degree change (from 9% for the control to more than 18% for the active emulsion). The comparison between polyphenol contents of the control and the active emulsion allowed us to think that the 18% DPPH free radical inhibition percentage is probably due to this phenolic fraction provided by the extract.

4. Conclusion

The main objective of this work was to valorize Vitis vinifera L. leaf extract by the development and the characterization of an emulsion containing this extract as an active ingredient, a work that has not been carried out to date. The quantitative analysis of the main polyphenolic classes (total polyphenols, flavonoids and, condensed tannins) gave us an overview for their variation contents, we could establish that bioclimatic stages have a relative influence on phenolic fraction composition; the samples from regions characterized by a humid climate are richer in polyphenols than those from regions with a dry climate. Variations in flavonoid and condensed tannin contents are obviously influenced by other factors that have to be identified.

Following the results of emulsion formulation and characterization part of this work, it can be concluded that the oil/water emulsion *Vitis vinifera L.* leaf extract based showed good physical properties, satisfying stability, and good antioxidant activity. All these properties offer a new delivery system potentially applicable in dermo-cosmetology.

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Author Contribution

Concept and Design, Resources, Analysis, Interpretation and Writing were performed by Mohammed Adil SELKA and Amel CHENAFA –Supervision, Materials and Critical Reviews were directed by Mohammed Yacine ACHOURI, Data Collection and Literature Search were conducted by Nazim BELLIFA

Conflicts of Interest

The author declares no conflict of interest.

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