

Proceeding Paper

Physical-Chemical Properties of the Phenolic Compounds of *Humulus lupulus* and Aromatic Plant Terpenes: Potential for Use in a Cosmetic Formulation †

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Abstract: Hop (*Humulus lupulus* L.) are known worldwide as an essential flavor in the beer industry. Their compounds have shown health benefits in terms of phytochemical, pharmacological and biological profiles, due to their antimicrobial, antioxidant, anti-inflammatory and anti-cancer activities. This study intends to develop a gel formulation incorporating hydroalcoholic extracts of different varieties of Hop such as Cascade, Polaris and spontaneous, from the cones and the vegetative plant parts, in different percentages. The essential oil of *Thymus zygis* was used as a natural preservative and the analysis of their composition was performed by GC and GC-MS. Additionally, the evaluation of the physical–chemical parameters, stability and capacity to inhibit microbial growth was performed. The pH measurement results of the formulations tend to be adjusted to the natural skin pH. The relative density obtained values of 1 and 0.857. It was found that there were no changes in the phase separation in the centrifugation, vibration, stability and accelerated test. However, changes were observed in the UV-Visible spectra, in texture, consistency and viscosity, and in the color test. In the light test there was phase separation of the samples, which may be related to the manufacturing technique of the formulations. However, anti-aging formulations with phenols from Hop could be developed in order to obtain formulations with relevant properties for consumers and the cosmetic industry.

Keywords: *Humulus lupulus*; anti-aging formulation; cosmetics; essential oil; phenolic compounds



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1. Introduction

The Hop (*Humulus lupulus* L.) are known worldwide as an essential flavor in the beer industry, however in recent years, its compounds have been showing health benefits in terms of phytochemical, pharmacological and biological profiles, due to their antimicrobial, antioxidant, anti-inflammatory and anticancer activities [1].

In commercial and scientific terms, lately there has been a substantial increase in the interest in and development of cosmetic formulations and methodologies for maintaining a youthful appearance, reducing the signs of aging. Since very early in the history of mankind, extracts of plants and herbs have been used in this perspective since they are rich in bioactive compounds such as terpenes, alkaloids and phenols [2]. In addition to the importance of bioactive compounds present in the cosmetic formulation, it is also essential to use efficient preservatives, and there are aromatic and medicinal plants with antimicrobial activity already well described, as is the case with *Thymus zygis*. *T. zygis* is widespread throughout the world, and his flowers and leaves are used, which have

antimicrobial properties for gram positive and gram negative bacteria as well as antioxidant capacity [3]. The main constituent of the essential oil of thyme is thymol (with greater activity), [4], can provide health benefits, related to the control of the reactive species production and oxidative stress and also in the prevention of aging and cell damage [5]. It was with this aim of being antimicrobial that the essential oil of thyme was used as a preservative in the cosmetic formulation.

This study intends to develop a gel formulation incorporating different percentages of hydroalcoholic extracts obtained from the cones and the vegetative parts of spontaneous hop and from Cascade and Polaris varieties, and to analyze the physical-chemical and microbiological stability of the formulation developed with these extracts.

2. Experiments

2.1. Biological Samples

Plant material: The samples of the Hop plant of the Polaris and Cascade variety were collected in the Quinta do Polão experimental field of the Escola Superior de Agrária of the Polytechnic Institute of Bragança. The Spontaneous Hop sample was collected in Bragança away from cultivated reas of commercial varieties. The sample of the plant *Thymus zygis* L. (Thyme) subsp. *zygis*, collected in Rebordãos, Bragança, Portugal. After being identified botanically, (at the BRESA herbarium by a botanical expertise, Professor Carlos Aguiar, a voucher was preserved in the Herbarium) the samples were frozen at -20°C until use in the extraction process.

2.2. Development of Cosmetic Formulation

The cosmetic formulation was developed based on the following recipe: Methyl-cellulose (3.7%); 96% Alcohol (18.9%); Distilled water (78%); Glycerin (0.13%) Extract of *Humulus lupulus* L. (variable); and *Thymus zygis zygis* (variable). The amount of hop extract depends on the concentration under study in each sample (1.25%; 2.5% and 5%), and in the case of Thymus the tested formulation samples have 0.013%.

2.3. Essential Oils Extraction

The essential oil of aerial parts of *Thymus zygis*, used as a natural preservative of the cosmetic formulations, was obtained by destilation extraction from the female cones and leaves using a Likens-Nickerson system, and analyzed by GC and GC-MS [6].

2.4. Phenolic Compounds Extraction

Solid-liquid extraction with an 80% ethanolic solution was used to prepare the extracts from cones and vegetative parts of spontaneous hop and from Cascade and Polaris varieties. The phenolic compounds have been determined used the Folin-Ciocalteu method [7–9].

2.5. Stability Tests of the Cosmetic Formulation

Physical-chemical parameters and stability were determined used different methodologies:

- Analyses of the variations in pH
- Determination of density
- Assay in the centrifuge
- Assay in vortex
- Spectrophotometer determination
- Texture
- Light
- Tests of temperature

pH test: 3 g of the gel formulation was used and dissolved in distilled water for pH measurement with the help of a digital pH meter (pH 7310 wtw xylem brand inolab).

On day zero the pH of the gel was measured without the hydroalcoholic extracts and then with the spontaneous hop hydroalcoholic extracts and the Cascade and Polaris

varieties, and also the control (methylcellulose gel, without extracts). The pH was measured at three days, one week and fifteen days after production.

Density: The relative density was determined by the formula:

$$D_r = m/v$$

Relative density (D_r), mass in grams (m) and volume in millilitres (v).

It was used as a way to determine the relative density, the water displacement method.

Centrifuge assay: To see the stability of the formulation, in which there may be a tendency to phase separation, we centrifuged at 25 °C, 3000 RPM, for 30 min in an equipment (hettich mikro 24–48 r zentrifugen) with one gram of each sample in eppendorfs of 1.5 mL.

Assay in vortex (Mechanical vibration test): This test aims to replicate the transport conditions, indicating whether the vibration movements of the same can modify the sample. The procedure used was one gram of each sample in 1.5 mL eppendorfs submitted to 2000 RPM in vibration on a vortex shaker (Velp scientifica zx 3 advance vortex mixer) for 10 s.

Spectrophotometric test: The samples were diluted in distilled water and placed in quartz cuvettes and subsequently subjected to a spectrophotometric analysis in a spectrophotometer (VWR UV-1600pc Spectrophotometer), with the spectrum being traced, in the UV-Visible region, between 210 and 600 nm.

Texture: This test aims to demonstrate the cohesiveness, consistency, firmness and viscosity index of the samples and were performed on a texture analyzer (Stable Micro Systems TA-XT Plus) and equipped with a probe coupled with a 35 mm compression disk that prints a force on a 50 mm diameter cylindrical container. The equipment operated at a height of 100 mm, the gel column was 50 mm and occupied about 50% of the total volume of the container. After starting its movement, the compression disc exerts a force on the sample surface for a short period and descends to a depth of 20 mm, at a constant speed, using a return speed of 20 mm min⁻¹ until it returns the sample surface.

Light: In this test we placed fifteen grams of each type in sterile transparent plastic Petri dishes in which they were subjected to extreme lighting conditions, 16 h on and 8 h off, using a daylight lamp, for 15 days.

Test of temperature (Temperature and Humidity test), one gram of sample, was placed in 1.5 mL Eppendorf (with three repetitions each, in total 57 Eppendorf's) and stored in an incubator (Mettler) at a temperature of 25 °C ± 2 °C at 60% ± 5% relative humidity (RH) for two weeks. This process was repeated, for the same number of samples at a temperature of 40 ± 2 °C at 75% ± 5% RH during the same time. Organoleptic characteristics (color, smell, phase separation, texture and consistency) and the pH value every eight days were analyzed.

3. Results

3.1. Chemical Composition

3.1.1. Phenolic Composition of Hop Extracts

In general, cone hydroethanolic extracts are richest in phenolic compounds than extracts obtained from vegetative parts. In fact, higher amounts of phenolic compounds have been determined in cone extracts of Polaris and Cascade varieties (22.7 ± 2.4 and 10.4 ± 2.1 mg/GAE g of plant, respectively) comparing to the obtained for extracts of vegetative parts of the same plants (Table 1).

3.1.2. *Thymus zygis zygis* L. Essential Oil Composition

The yield of the essential oil of the aerial parts of *Thymus zygis zygis*, based on the dry mass of the plant, was as follows 1.14% (Table 2), in which carvacrol, terpinen-4-ol, p-cymene and *trans*-sabinene hydrate were the main compounds (43.60%, 25.80%, 24.10%, 15.8%, respectively).

Table 1. Yield of extraction (%) and total phenolic compounds (mg GAE/g plant) of a spontaneous and two varieties of hop.

Sample	Yield (%)	Total Phenolic Compounds (mg GAE/g Plant)
Spontaneous C	13.1 ¹	ND
Spontaneous VP	21.1	3.1 ± 0.5 ²
Cascade C	30.3	10.4 ± 2.1
Cascade VP	9.3	2.1 ± 0.7
Polaris C	25.8	22.7 ± 2.4
Polaris VP	11.8	1.1 ± 0.2

¹ Mean values; ² Mean values ± SD; C—Cones; VP—Vegetative Parts; ND—Not determined.

Table 2. Essential oil composition of aerial parts of *Thymus zygis zygis*.

Components	Amount
<i>cis</i> -linalool oxide	0.6
<i>cis</i> -sabinene hydrate	0.1
camphor	3.2
borneol	1.2
terpinen-4-ol	25.8
α-terpineol	11.8
thymol	0.3
carvacrol	43.6
α-thujene	1.6
α-pinene	0.8
camphene	1.0
3-octanol	1.0
β-myrcene	1.0
α-terpinene	1.4
p-cymene	24.1
limonene	0.2
<i>trans</i> -β-ocimen	1.1
<i>trans</i> -sabinene hydrate	15.8
Yield (% <i>v/w</i>)	1.14 ¹

¹ Mean values.

3.1.3. Stability Tests

The pH test: The control formulation (Gel) showed a slightly acidic pH value. However, after fifteen days, all formulations with varieties showed a pH trend between 7.5 and 6.5 (Figure 1).

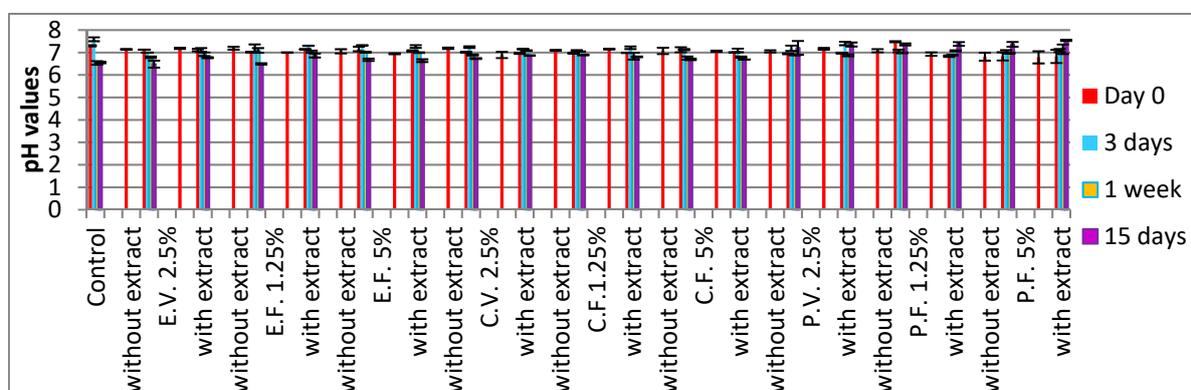


Figure 1. In the graph is possible to see that que pH is very stable and slightly acidic.

Density test: In the measurement of relative density, values of 1 and 0.857 were obtained (Table 3). The value of 0.857 was obtained, only for spontaneous Hop and the Polaris variety both in flower. So it can be concluded that the relative density of the formulation with different concentrations of vegetative extracts of all the plants and also Cascade flowers don't have any alteration compared the control (without any extract). Only in flowers extract (Polaris and spontaneous) the relative density have change, with lower's data compared to the gel and the other formulations, which can be associated to the different yield in Total Phenolic Compounds (different chemical composition) of the extract as is shown in the phenols table data (Table 1).

Table 3. Measurement of relative density.

	Relative Density			
	Weight (g)	Initial Volume (mL)	Final Volume (mL)	Relative Density
Gel (control)	3	15	18	1
Spontaneous vegetative				
1.25%	3	15	18	1
2.5%	3	15	18	1
5%	3	15	18	1
Spontaneous flower				
1.25%	3	15	18.5	0.857
2.5%	3	15	18.5	0.857
5%	3	15	18.5	0.857
Cascade vegetative				
1.25%	3	15	18	1
2.5%	3	15	18	1
5%	3	15	18	1
Cascade flower				
1.25%	3	15	18	1
2.5%	3	15	18	1
5%	3	15	18	1
Polaris vegetative				
1.25%	3	15	18	1
2.5%	3	15	18	1
5%	3	15	18	1
Polaris flower				
1.25%	3	15	18.5	0.857
2.5%	3	15	18.5	0.857
5%	3	15	18.5	0.875

Centrifuge assay: There was no phase separation in all the samples, even wend the samples have different extracts (liquid) that can modify gel base of the formulation. These results can be interpret as a good stability of the formulation.

Assay in vortex (Mechanical vibration test): There was no phase separation in all tests for all samples. With these results is possible to predict that the formation is stable to the transportations.

Spectrophotometric test: This test aims to see the behavior of the gel, if have any alteration when adding extracts of the varieties in different concentrations. The verification of the modification in the gel is based in the changes on the intensity of the bands: hyperchromic effects (increase in absorption intensity) or hypochromic (decrease in absorption intensity), or variations in the wavelength at which maximum absorption occurs: bathochromic effects (change in absorption for longer wavelengths, due to solvent replacement or effect), or hypsochromic (change in absorption for shorter wavelengths, due to substitution or effect of the solvent), indicate the occurrence of a change in its color or intensity, which may be an indication of instability of the formulation.

In Figure 2 (graph), we can see that in the nineteen spectra the absorption bands are in the same position, verifying the existence of a maximum absorption peak, at 300 nm and

350 nm. These peaks are due to the compounds in the formulation used, which possibly have the phenols functional groups, which have typical bands at these wavelengths. In the formulations there is a hyperchromic effect compared to the gel. This change is possibly due to the proportions of some compounds, which translates into a change in the intensity of the bands. In addition to the effectyperchromic, it is also possible to see a hypsochromic effect, due to the low wavelengths that are located closer to the blue.

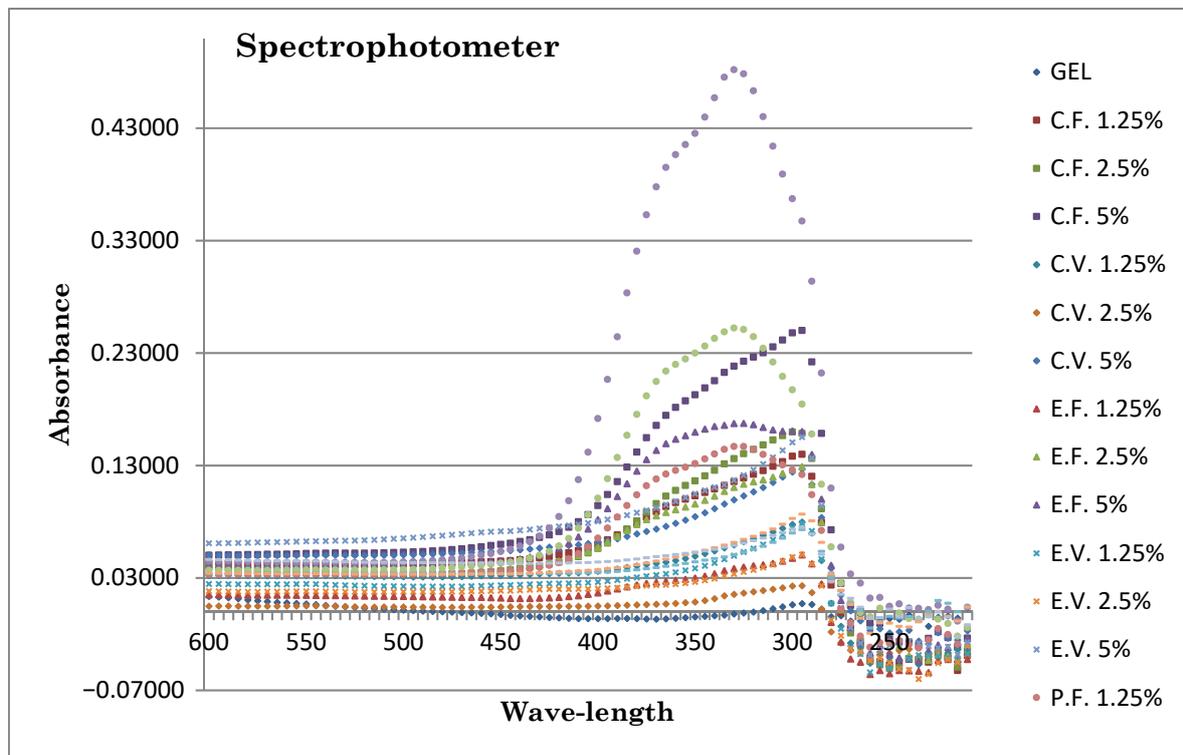


Figure 2. Grafic UV-VIS spectrum of samples in different concentrations; C.F.—Cascade flower; C.V.—Vegetative cascade; E.F.—Spontaneous flower; E.V.—Spontaneous vegetative; P.F.—Polaris flower; P.V.—Vegetative Polaris.

Temperature test: After the test period (2 weeks), it was verify whether the organoleptic characteristics of each sample (color, odor, phase separation, texture and consistency), in which they were compare with the samples in their natural/initial state and at the end and there was no change, in the organoleptic aspect or in the pH value.

Texture: The texture of the various gel samples was evaluated using a texturometer, through the performance of reverse extrusion tests (back extrusion), with recording of the parameters firmness, consistency, viscosity index, and cohesiveness. The graph below (Figure 3) show the results provided by the texturometer software for the samples, making it possible to identify the parameters recorded by the equipment. The results are consistent in all the samples with no significant variation, in viscosity and firmness, although the results of the extract from Polaris variety (flower and vegetative parts) are slightly different, with higher consistency values and lower viscosity values, which may be in agreement with the higher values of bioactive compounds, namely phenols obtained in this variety.

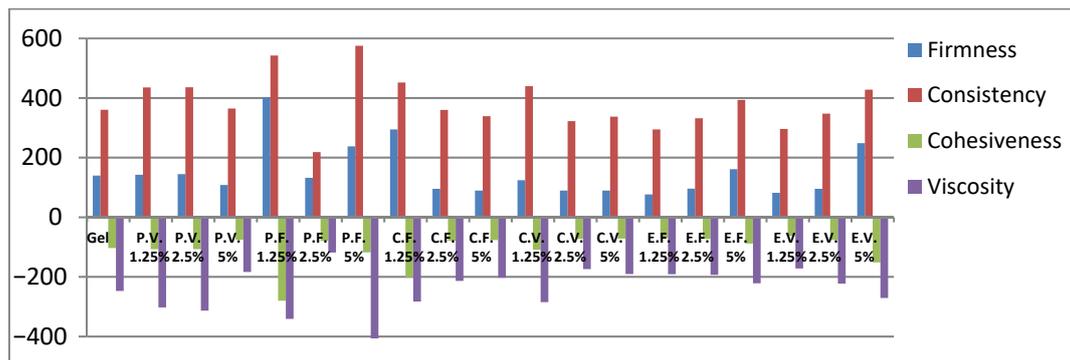


Figure 3. Gel—control; P.V.—Polaris vegetative parts; P.F.—Polaris Flower; C.F.—Cascade Flower; C.V.—Cascade Vegetative; E.V.—Spontaneous Vegetative; E.F.—Spontaneous Flower. In the graph, it is possible to observe the values of the parameters measured by the equipment, verifying that only the formulation with the extracts of the Polaris Variety deviates a little more from the remaining values. Featuring higher consistency values, and less viscosity.

4. Discussion

The results presented are still preliminary in the sense that several parameters have yet to be determined, including the effectiveness of the buoyant compounds on the skin and also the toxicity of the extracts, however the hop extracts are not referred to as having skin toxicity, so interesting perspectives are opened for the use of these extracts and formulations.

5. Conclusions

In conclusion, the gels are stable in time and resistant to the more basic tests so can be used as a main part of cosmetic formulation by further research. Concerning is bioactivity *Humulus lupulus* showed an optimistic stability physico-chemical and appropriate organoleptic characteristics, maintaining over time (more than 4 months) its microbiological stability which allows, together with microbiological data (data not shown) already developed in this study to ensure that stability. The continuity of this work involves developing more toxicity tests and other stability tests and testing on cell lines and sensory analysis panels, in order to analyze the possibility of this formulation, and others with Hop extracts, may have beneficial effects and effective in skin health.

Author Contributions: M.J.S., L.P. and O.R.P. conceived and designed the experiments; B.d.S. performed the experiments and analyzed the data. M.J.S. contributed reagents and materials; M.J.S., L.P. and O.R.P. reviewed and edited. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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