UTILIZATION OF BEE PRODUCTS AND TRÁS-OS-MONTES AROMATIC PLANTS ON THE DEVELOPMENT OF COSMETIC FORMULATIONS

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Abstract

The aim of this work is the development of a cosmetic cream formulation for the treatment and daily maintenance of the skin composed by bee products, plant material and vegetable oils from Trás-Os-Montes, a region located in the north of Portugal. The bee product intended for use is the beeswax, the plant material were two sage species (*Salvia elegans* and *Salvia officinalis*) and one thyme plant (*Thymus zygis zygis*). The vegetable oils used were olive oil and almond oil.

Figure 1: Elements used in the formulation of the cream

Objectives

The main objectives of this work are summarized in the figure below

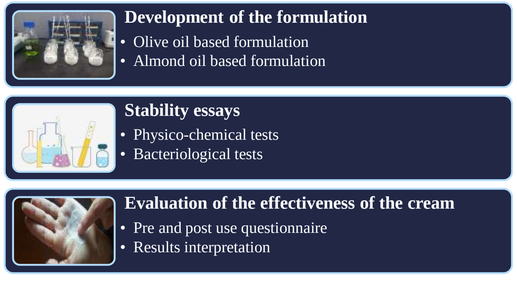


Figure 2 : Representation of the main objectives of the work

Methodology

The work was divided into six major parts

1. Extraction of *Thymus zygis zygis* essential oil by Clevenger apparatus [1]
   Solid-liquid hydroalcoholic extraction of *Salvia elegans* and *Salvia officinalis* [2]
2. Chemical analysis of *Thymus zygis zygis* essential oil by Gas chromatography-mass spectrometry (GC-MS) [3]
   Chemical analysis of *Salvia elegans* and *Salvia officinalis* hydroalcoholic extracts by (UHPLC –DAD ESI/MSn) [4]
3. Microbial stability of *Thymus zygis zygis* essential oil

Figure 3: Hydroalcoholic extraction

Figure 4: *S.elegans* et *S.officinalis* hydroalcoholic extracts

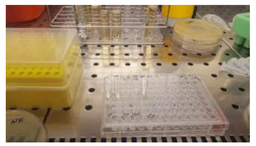


Figure 5: Tests of inhibition of microbial growth (microdilution technique)

Figure 6: Antimicrobial test (Agar diffusion technique)

4. Development of the cream formulations
   - Olive oil + 1.25% - 5% of *S. elegans* or 1.25% - 5% of *S. officinalis*
   - Almond oil + 1.25% - 5% of *S. elegans* or 1.25% - 5% of *S. officinalis*

Table 1 : Cream ingredients

Ingredients	Function
Methylcellulose gel	Excipient
Olive oil	Oily material
Almond oil	
Beeswax	
Salvia elegans hydroalcoholic extract	Source of natural active substances
Salvia officinalis hydroalcoholic extract	
Tween 80	Emulsifiyng agent
Starch	Thickening agent
Thymus zygis zygis oil	Preservative

5. Stability essays of the cream formulation

- Texture and consistency
- Centrifugation test
- Mechanical vibration test
- pH determination
- Spectrophotometric test

- Light test
- Viscosity test
- Relative density
- Extreme temperature
- Accelerated testing

6. Questionnaire pre and after use

Results

- **Chemical composition of *Thymus zygis zygis* essential oil**

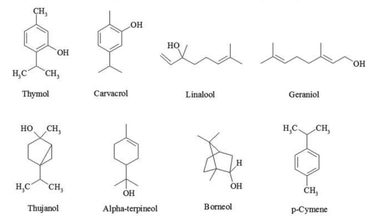


Figure 7 : Chemical structure of the main substances occurring in genus *Thymus* plant : thymol, carvacrol, linalool, geraniol, thujanol, α-terpineol, borneol, and *p*-cymene [5]

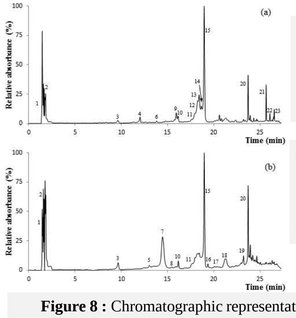
The chemical composition analysis also highlighted the presence of grouped components with a high percentage:

- Oxygen-containingmonoterpenes 49.8%
- Monoterpenes hydrocarbons 49.6 %
- Carvacrol 43 %
- Cymene 24.10 %
- Trans-sabinene 15.8 %

- **Microbial stability of *Thymus zygis* essential oil**

- **Bactericidal power** against *Escherichia coli*, *Pseudomonas sp* and *Streptococcus aureus*
- **Inhibitory power** of *Candida albicans*

- **Chemical analysis of *Salvia elegans* and *Salvia officinalis* hydroalcoholic extracts**



(a) *S. officinalis* hydroethanolic extract :
Rosmarinic acid (peak 15)
Apigenin-*O*-glucuronide (peak 13)
Scutellarein-*O*-glucuronide (peak 9)
Luteolin-7-*O*-glucuronide (peak 10)

(b) *S. elegans* hydroethanolic extract :
Rosmarinic acid (peak 15)
Salvianolic -acid K (peak 7 and 8)
Luteolin-7-*O*-glucuronide (peak 10)
Caffeic acid (peak 3)

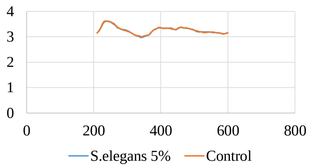
Figure 8 : Chromatographic representation of *S. officinalis* (a) and *S. elegans* (b) hydroethanolic extracts at 280 nm

- **Preparation of cream formulations (14 different formulations)**
- **Stability essays of the cream formulation**

- Smooth, fresh, good spreadability and smell of beeswax
- No phase separation
- Non significant variation of the colorimetric coordinates
- Non Newtonien behavior of the formulations
- pH domain close to natural skin pH

- Almost identical density values varying from 0.75 to 1 g/l
- No deterioration of organoleptic criteria or pH
- No deterioration of the organoleptic criteria

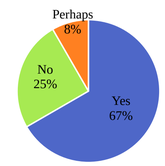
Olive oil formulation + *S. elegans* 5%



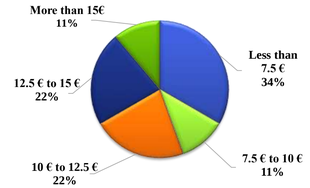
The olive oil formulations containing the extracts of *Salvia elegans* showed spectrophotometric profiles which are superimposed on those of control formulations, in particular that of 5%.

Figure 8 : Spectrophotometric profile of olive oil formulation + 5% *S.elegans* hydroalcoholic extract

- **Questionnaire after use**



Willingness to buy the product, in percentage of users



Willingness to pay for 50ml of product, in percentage of users

Conclusion

- Stability of the physico-chemical criteria of the different types of formulations
- Bactericidal power against *E.coli*, *Pseudomonas sp* and *S. aureus* and inhibitory power of *C. albicans* of Thyme oil
- Rich phenolic profiles of *S.elegans* and *S.officinalis* hydroalcoholic extracts
- Positive performance of the cream containing olive oil and 5% of *S. elegans* hydroalcoholic extract

References

[1] Lourenço, J. A. A. (2007). Destilação industrial de óleos essenciais. Curso TeóricoPrático, 3 edição(Faculdade de Ciências da Universidade de Lisboa, Centro de Biotecnologia Vegetal, Lisboa), 80–95.
[2] Pereira, O. R., Macias, I. R. R., Domingues, M. R. M., Marin, J. J. G., Cardoso, S. M. (2019). Hepatoprotection of Mentha aquatica L., Lavandula dentata L. and Leonurus cardiaca L.. Antioxidants, 8(8): 267.
[3] Dandlen, S. A., Miguel, M. G., Duarte, J., Faleiro, M. L., Sousa, M. J., Lima, A. S.,Figueiredo, A. C., Barroso, J. G., & Pedro, L. G. (2011). Acetylcholinesterase inhibition activity of portuguese thymus species essential oils. Journal of Essential Oil-Bearing Plants, 14(2), 140–150.
[4] Afonso, A. F., Pereira, O. R., Fernandes, Â. S. F., Calhelha, R. C., Silva, A. M. S.,Ferreira, I. C. F. R., & Cardoso, S. M. (2019). The health-benefits and phytochemical profile of salvia apiana and salvia farinacea var. Victoria blue decoctions.Antioxidants, 8(8).
[5] Nabavi, S. M., Marchese, A., Izadi, M., Curti, V., Daglia, M., & Nabavi, S. F. (2015).Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy. Food Chemistry, 173, 339–347.

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