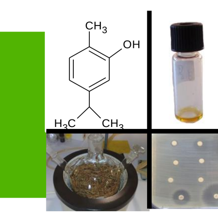
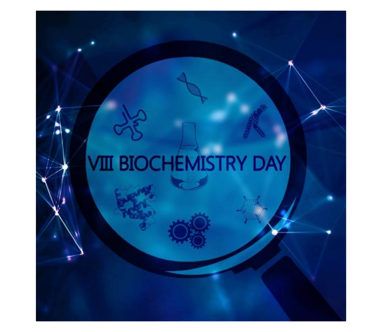
University of Aveiro
QOPNA

ResPoStA@UA
resposta.web.ua.pt



Chemical composition and antimicrobial potential of *Satureja montana agroindustrial* byproducts as possible ingredients in poultry feed

Jéssica Santos[a], Elisabete Coelho [a], Isabel Henriques[b], Cláudia P. Passos[a], Pedro Teixeira[b] , Manuel A. Coimbra[a]
(email: jessica.santos2604@ua.pt)
[a] QOPNA, Department of Chemistry, and [b] CESAM, Biology Department of University of Aveiro, 3810-193 Aveiro, Portugal.



Biochemistry Contest at UA 2018

Introduction

The livestock sector is one of the fastest growing parts of the agricultural economy as a consequence a greater demand for meat and derivatives[1]. To sustain high levels of productivity a wide variety of antibiotics are used in this sector. However, the use of dietary antibiotics have resulted in problems such as development of antibiotic resistant bacteria and drug residue in the final products [2].

Around the world, phytobiotics (herbs, botanicals, essential oils and oleoresins) have been investigated as natural alternatives to antibiotic growth promoters (AGPs) in poultry industry, being these plant-derived products recognized as GRAS and the beneficial effects of them attributed mainly to their antimicrobial properties [3].

The apical parts of *Satureja* showed great antimicrobial activity against several pathogenic strains, probably due to its great in carvacrol and thymol[4]. However, there are no studies evaluating the composition and antimicrobial properties of essential oils obtained from *Satureja montana* agroindustrial byproducts.

Objectives

The aim of this study was to obtain essential oils from the *Satureja montana agroindustrial* byproducts (stem shavings) by two different extraction techniques (hydrodistillation and hydrodistillation assisted by microwave), evaluating their chemical composition and antimicrobial properties *in vitro* against *Salmonella F2* and *E. coli* ATCC 25922.

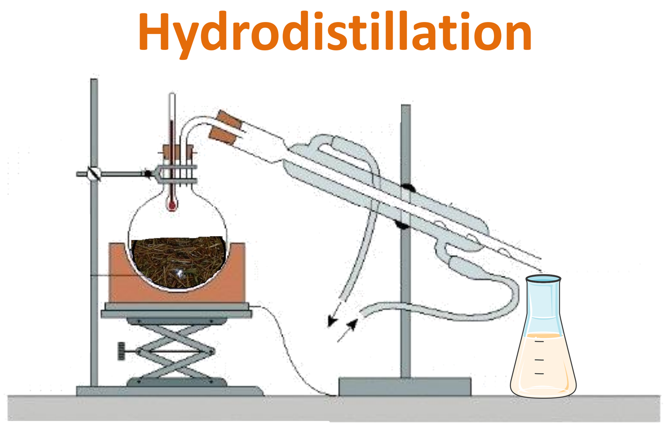
Methods

1. Samples

Stem shavings of *Satureja montana (agroindustrial* byproducts)

2. Extraction Methodologies

Hydrodistillation



MW-Assisted hydrodistillation

Liquid liquid extraction with dichloromethane

Concentration of the oil in a stream of nitrogen

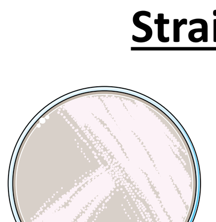
3. Chemical characterization of oils

Quantification and characterization of oil composition in terpenic compounds by GC-MS

For quantification, 2-undecanol was used as internal standard. Response factor of them in relation to Eugenol was determinated.

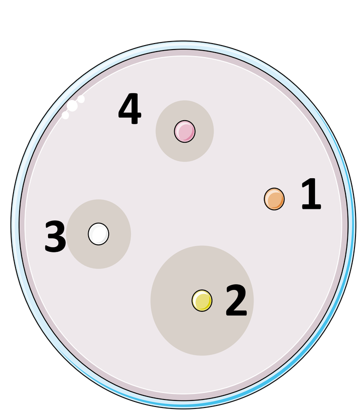
4. *In vitro* antimicrobial activity test

Strains tested:



Salmonella F2

***E. coli* ATCC 25922**



Disc diffusion method

1- Negative control (sterile water)
2- ciprofloxacin
3- gentamicin
4- *S. montana* essential oil

Results

Extraction yield

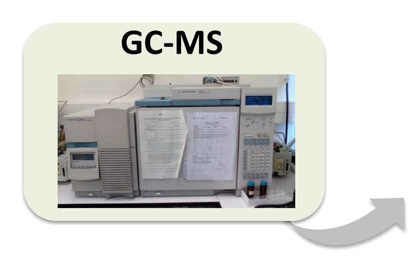
	Hidrodistillation		MW-Assisted hydrodistillation
	Harvest 2016 media (n=3)	Harvest 2017 media (n=3)	Harvest 2017 media (n=3)
Yield (mg/g)	0.42 ± 0.11	0.14 ± 0.07	0.10 ± 0.01

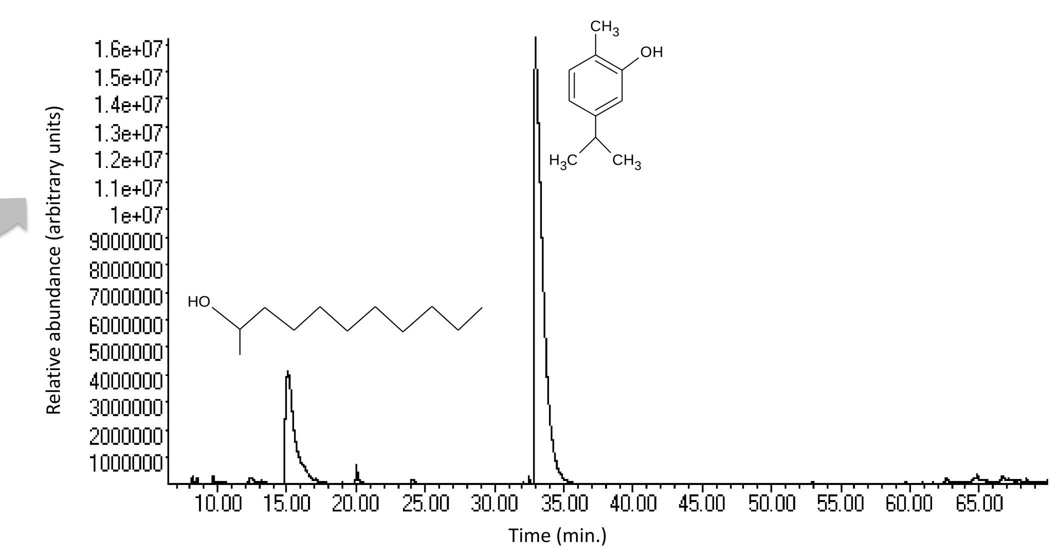
✓The yield of oil from the *Satureja montana* stems is about ninety times less than that obtained from the apical parts of the plant, however, the obtained oils promote the recovery of by-products discarded so far.

✓In addition, the apical parts (leaves) represent only 5% of the total mass of the plant, and therefore, recovering the essential oil from the stems may be profitable.

Chemical characterization of oils

GC-MS





Retention time	Compound	Hydrodistillation		MW-Assisted hydrodistillation
		Harvest 2016 media (n=3)	Harvest 2017 media (n=3)	Harvest 2017 media (n=3)
8.14	linalool	4.7 ± 0.9	3.3 ± 0.6	6.0 ± 3.5
9.64	terpineol-4	6.4 ± 0.0	3.8 ± 0.9	3.9 ± 2.3
12.4	endoborneol	4.7 ± 0.3	8.2 ± 3.2	8.8 ± 4.0
13.12	α-terpineol	5.9 ± 1.1	2.3 ± 0.7	1.7 ± 1.5
32.611	thymol	4.2 ± 0.0	3.0 ± 0.6	3.1 ± 1.0
33.1	*Carvacrol*	931.0 ± 19.8	847.5 ± 179.3	798.1 ± 209.0
	total:	956.9 ± 17.7	847.5 ± 185.3	821.6 ± 221.3

✓The composition of the oil was similar between the crop of 2016 and 2017 and between conventional hydrodistillation and MW-assisted hydrodistillation.

✓All compounds identified are monoterpenes, being carvacrol (≈ 97%) the main compound present in oils.

In vitro antimicrobial activity

Having a very similar chemical composition the obtained oils were analyzed as one.

	Inhibition zones media (mm) (n=3)	
	Salmonella F2	*E. coli* ATCC 25922
Oil (2μL ≈1.75 ±0.10 mg/disc)	12 ± 1	25 ± 3
Ciprofloxacina (5 μg/disc)	31 ± 1	34 ± 1
Gentamicina (10 μg/disc)	20 ± 1	21 ± 0
sterile water	0	0

✓The inhibition diameters in *E. coli* ATCC 25922 on the antibiotic disks are within the reference values reported in the literature [5].

✓The oils showed antimicrobial activity for both strains, however, the *E. coli* strain showed more susceptibility.

Conclusions

✓The essesntial oils obtained from *S. montana by-products* present antimicrobial activity and this activity may be due to the presence of carvacrol, since this compound has been reported as antimicrobial.

✓*S. montana* essential oils show in this first study potential to be used as ingredients in poultry feed, thus decreasing the dose of antibiotics to apply.

References

[1] FAO. (2014) Food and agriculture organization of the United Nations. *FAO's role Anim. Prod.*
[2] Barton, M. D. (2000) Antibiotic use in animal feed and its impact on human health. *Nutr. Res. Rev. 13*, 279–99.
[3] Kiczorowska, B., Samolińska, W., Al-Yasiry, A. R. M., Kiczorowski, P., and Winiarska-Mieczan, A. (2017) The natural feed additives as immunostimulants in monogastric animal nutrition - A review. *Ann. Anim. Sci. 17*, 605–625.
[4]Oussalah, M., Caillet, S., Saucier, L., and Lacroix, M. (2007) Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella Typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Control 18*, 414–420.
[5] EUCAST. European Committee on Antimicrobial Susceptibility Testing - Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. *Version 7.0* **2017**, 1–16.

Acknowledgements: Thanks are due to FCT/MEC for the financial support to the research unit QOPNA (FCT UID/QUI/00062/2013) and Waste to value project (PDR2020-101-031828, Partnership no. - 94 / Initiative n. - 189, 1.0.1 - Operational Groups - n. - 01 / Action 1.1 / 2016) through national funds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement. Elisabete Coelho thanks also FCT for the postdoctoral fellowship (SFRH / BPD / 70589/2010).

FCT
PDR 2020 PROGRAMA DE DESENVOLVIMENTO RURAL 2014-2020
PORTUGAL 2020
UNIÃO EUROPEIA