

Antimicrobial Activity of Aqueous Plant Extracts as Potential Natural Additives



Mariana C. Pedrosa, Jonata M. Ueda, Sandrina Heleno, Bruno Melgar, Marija Ivanov, Marina Soković, Marcio Carcho*, Isabel C.F.R. Ferreira and Lillian Barros^{1*}

1st International Electronic Conference on Food Science and Functional Foods,
10–25 November 2020;

Consumers

More aware of what they eat and the consequences of the food in their health

Preference of unprocessed food and for the use of natural food additives

Food preservation

The food has to endure the journey from harvest to the consumer's house

Ensure the freshness, quality and safety of foods and resist the passage of time

FOOD ADDITIVES

Industry

Overexposure of some synthetic additives have been related to health issue

Looking for **natural alternatives**, for example from plant sources

Plant extracts to replace artificial additives

More sustainable

Healthier

By-products

Positive perception by consumers

Introduction

• **Microorganisms**

Antifungal assay:

- *Aspergillus fumigatus*
- *Aspergillus niger*
- *Aspergillus versicolor*
- *Penicillium funiculosum*
- *Trichoderma viride*
- *Penicillium verrucosum* var. *cyclopium*

Antibacterial assay:

- *Staphylococcus aureus*
- *Bacillus cereus*
- *Listeria monocytogenes*
- *Escherichia coli*
- *Salmonella typhimurium*
- *Enterobacter cloacae*

• **Plant material**

Lemon Balm
(*Melissa officinalis* L.)



Basil
(*Ocimum basilicum* L.)



Oregano
(*Origanum vulgare* L.)



Salvia (*Salvia officinalis* L.)



Rosemary
(*Rosmarinus officinalis* L.)



Ultrasound-assisted Extraction (UAE)

Extraction of
bioactive
compounds

Analyzed by

**Ultrasonic
system**

Centrifugation

**Filtration
(filter
paper)**

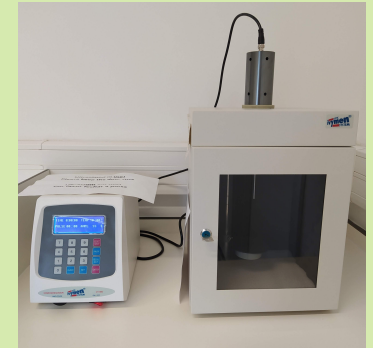
**Drying (vacuum
rotary evaporator at
40 °C)**

**Lyophilization of the
aqueous fraction**

Powdered extract

Ultrasound-assisted Extraction (UAE)

- **The design was built and randomized using Design expert 12.0.1 software**
- **The fixed variables:**
 - X1: time (7.5 and 12.5 min), X2: solvent (0 and 80% EtOH) and X3: UAE Power (275 and 450 W)
- **The dependent variable:**
 - Rosmarinic acid content (Y1)
- **The fractional factorial design**
 - Intercept = Intercept + ABC; A= A + BC; B= B + AC; and C= C + AB.
- **Secondary selection and evaluation of factors and levels**
 - Independent variables - X1: time (10-300 s) and X4: temperature (20-75 °C) at different levels.



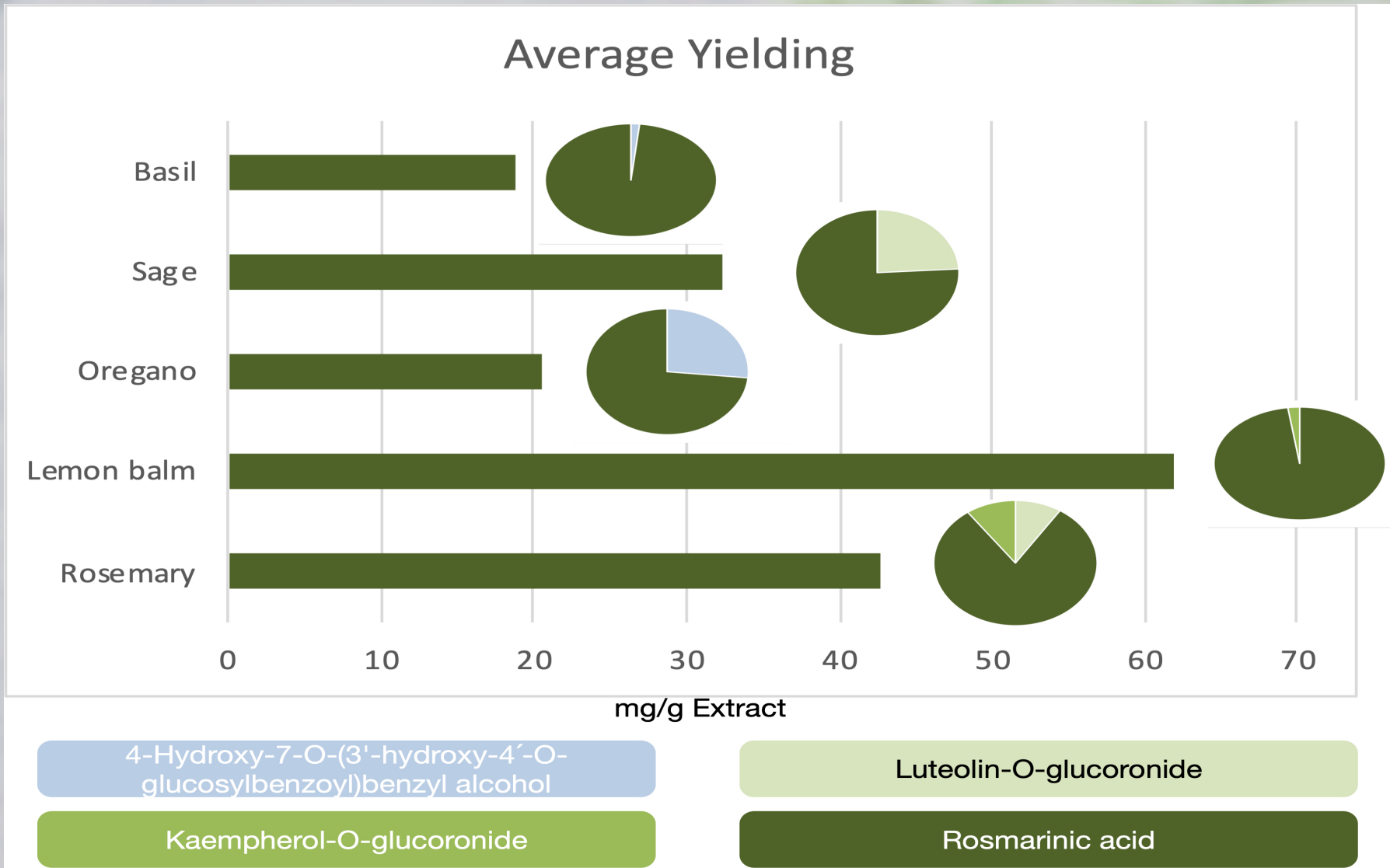
Phenolic Fingerprinting and Quantification, and Statistical Analysis

- UPLC, coupled to a diode array detector and an electrospray ionization mass detector, was used to acquire the chromatographic data;
- The compounds were identified considering the retention time, UV-Vis and mass spectra in comparison with available standards and with literature data;
- Fractional factorial design Pareto and analysis of variance (ANOVA) was carried out to determine the principal effects and their magnitudes.

Antimicrobial Activity

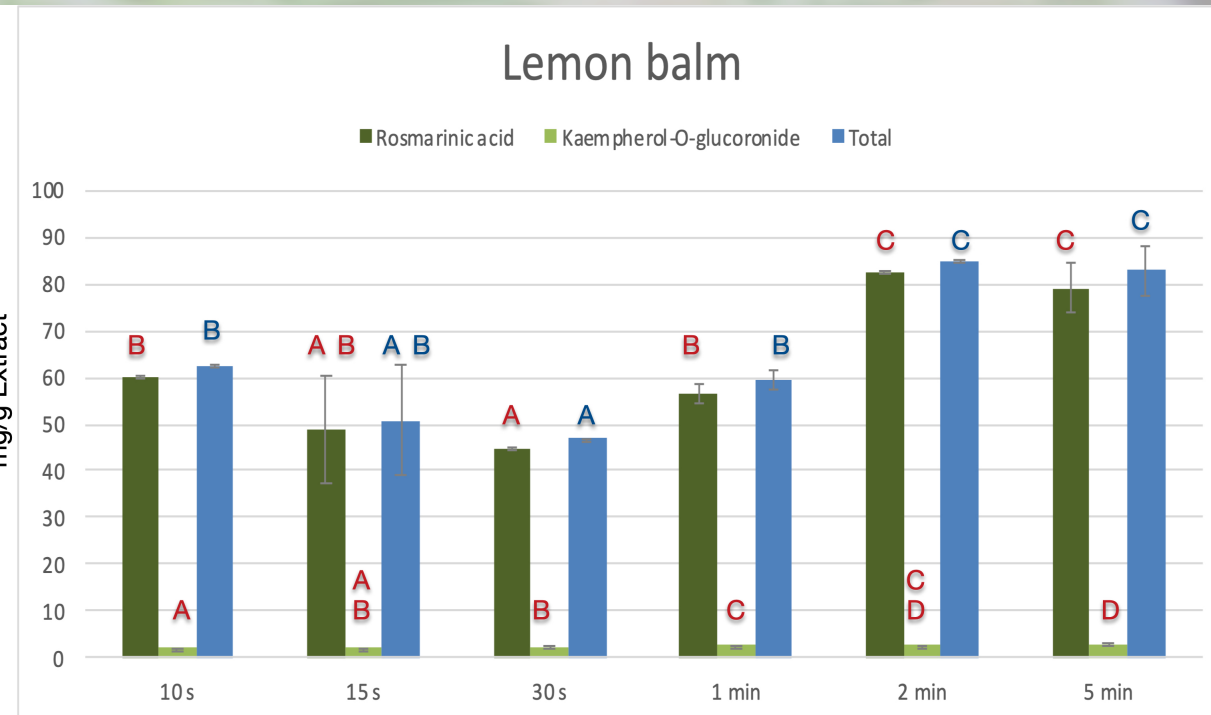
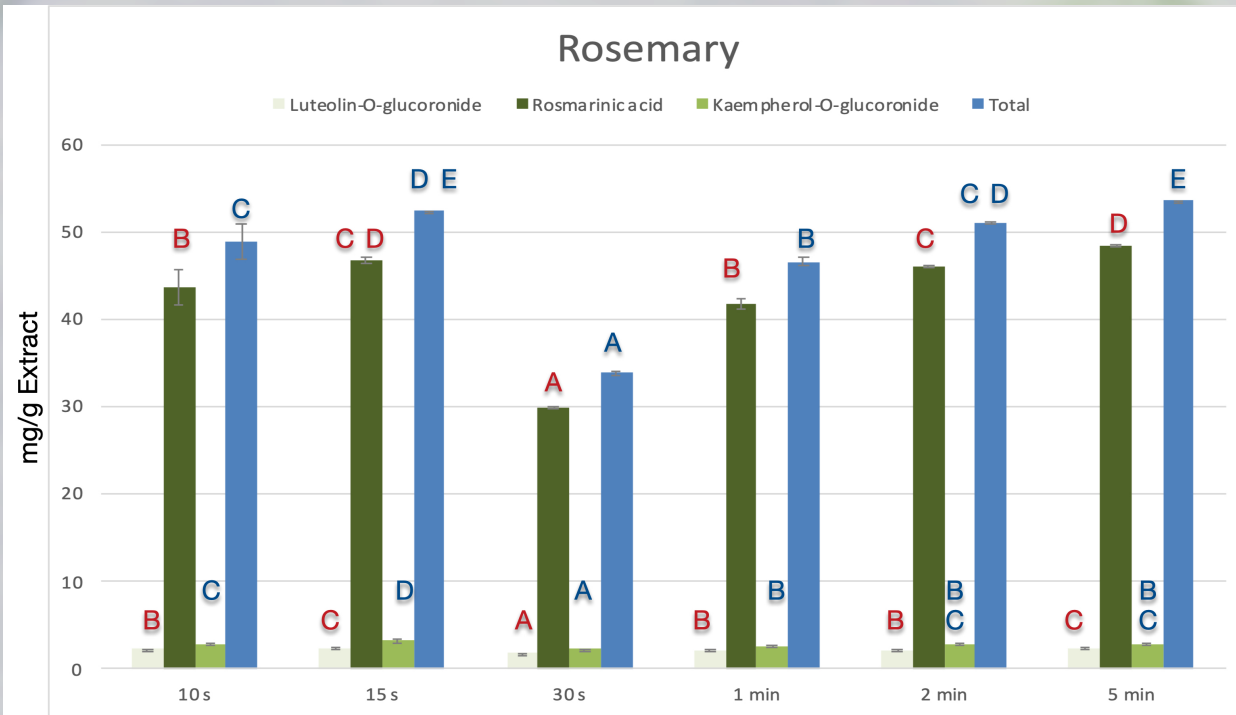
- Minimum inhibitory concentrations (MIC) were determined by:
 - Serial microdilution method and the rapid p-iodonitrotetrazolium violet (INT) colorimetric assay
- The results were expressed as MICs, MBCs and MFCs in mg/mL.

Extraction Optimization Studies



- 4 peaks were identified;
- The lowest UAE power was used in order to save resources.

Extraction Optimization Studies



- Rosemary and lemon balm - the two plants with the highest amount of total phenolic compounds.
 - The extraction time did not significantly increase the yield in phenolic compounds.

Extraction Optimization Studies

- Rosemary

- ↓ Phenolic content with ↑ heat;

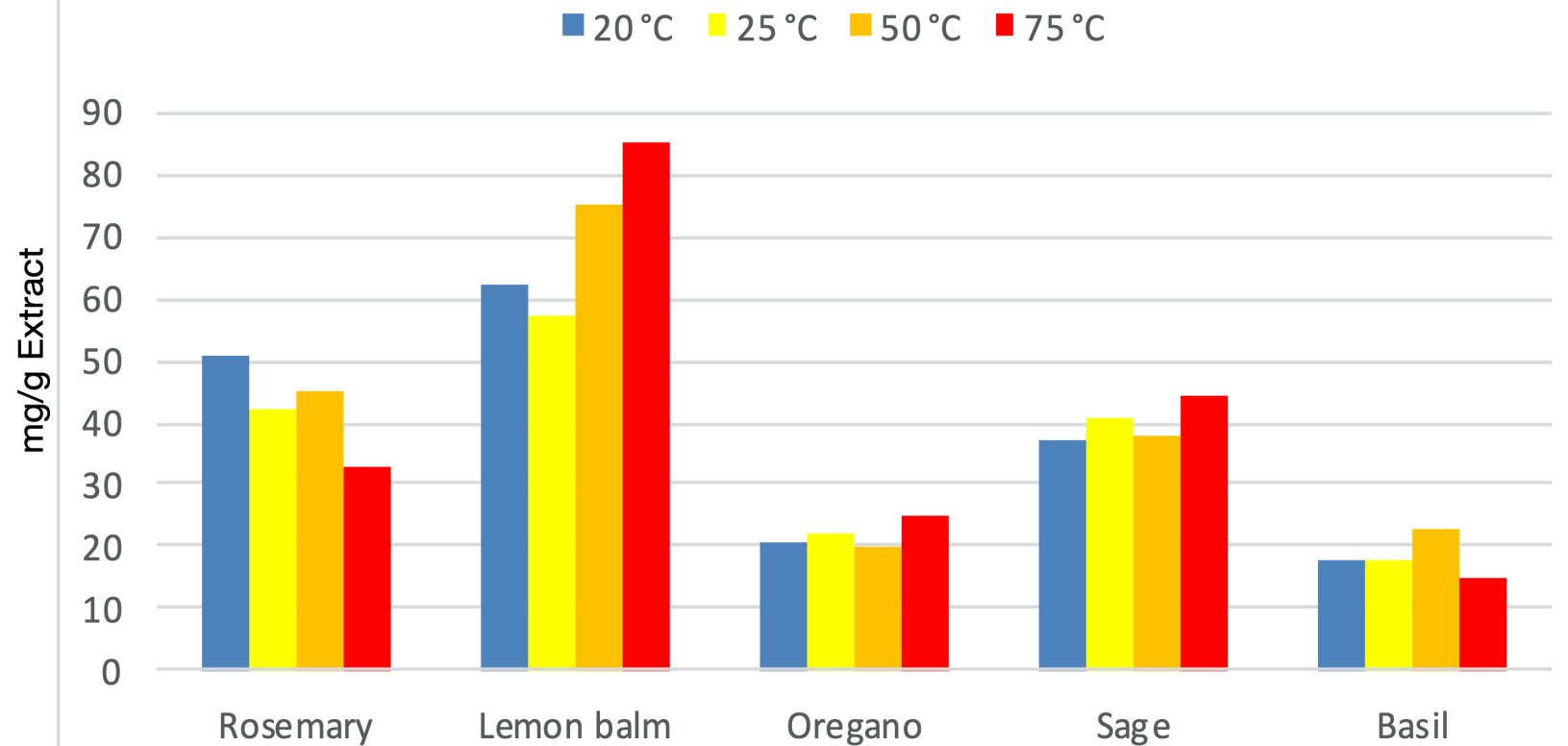
- Lemon balm

- ↑ Phenolic content with ↑ heat;

- Industrial level

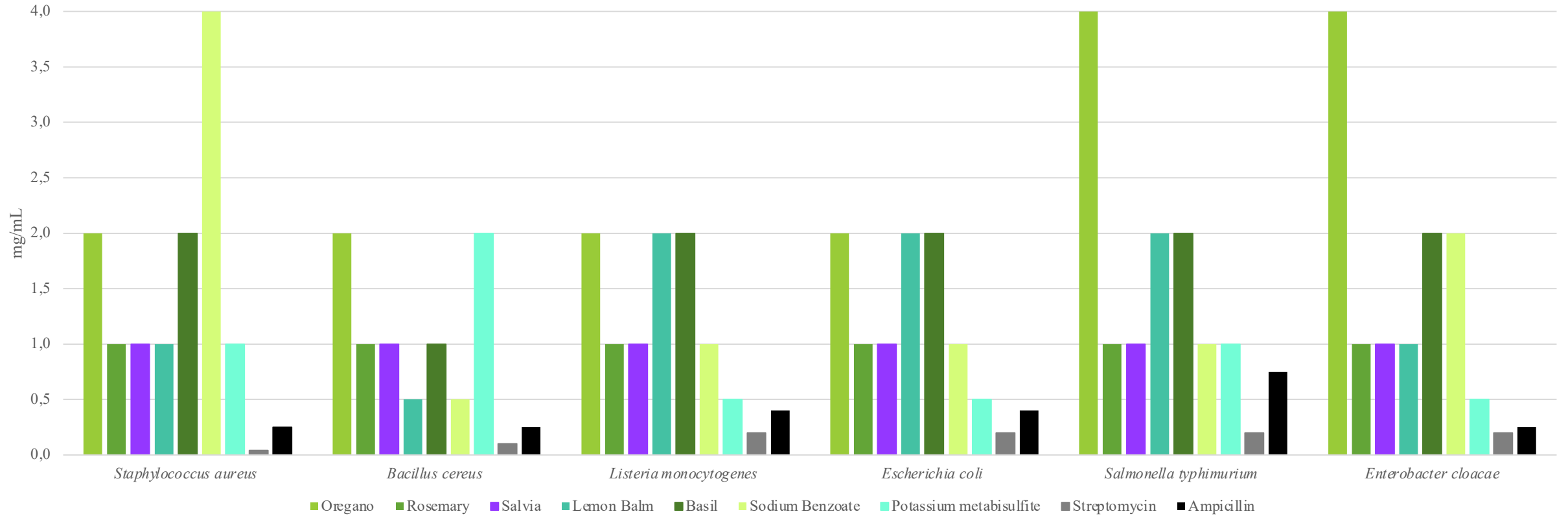
- Cost X Heating

Effect of Temperature



Antimicrobial Analysis

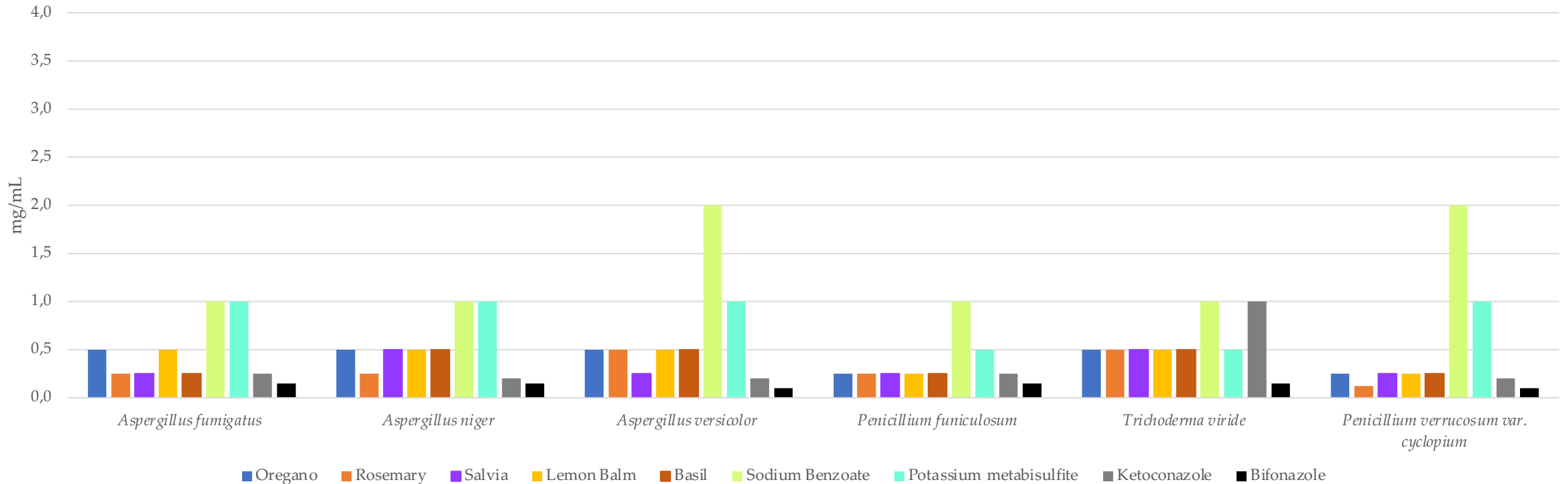
Minimum Inhibition Concentration (MIC) - Bacteria



- Rosemary and salvia
 - Better than or having equal effect as sodium benzoate for almost all bacteria.

Antimicrobial Analysis

Minimum Inhibition Concentration (MIC) - Fungi



- **Plant extracts** - higher effect against fungi than bacteria and better antimicrobial activity than both synthetic preservatives.

- **Rosemary and Salvia** - values under or equal to the positive controls - revealing an excellent capacity to be used in food to hinder fungi growth.



UAE seems to be a promising method to obtain polyphenolic extracts from *Lamiaceae* plants, namely rosemary, lemon balm, sage, oregano and basil.



UAE is a very easy and fast method to obtain these plant extracts and at any temperature



Rosemary and basil showed the highest amount of polyphenols of the studied plants.



The extracts could be used as natural antimicrobials for foods, due to their interesting antimicrobial activity, comparable in some instances to commercial synthetic counterparts.

Acknowledgments

- **Author Contributions:** conceptualization, M.C. and S.H.; methodology, M.C.P., J.M.U., and M.I.; software B.M.; validation, M.C.; investigation, M.C.P. and J.M.U.; data curation, B.M.; writing—original draft preparation, M.C.; writing—B.M.; supervision, M.S., L.B., and I.C.F.R.F.; project administration, L.B.
- **Funding:** This research was funded by the Portuguese Foundation for Science and Technology (FCT, Portugal) through national funds FCT/MCTES to CIMO (UIDB/00690/2020). S. Heleno and M. Carochó thank FCT for their individual research program-contract (CEECIND/00831/2018; CEECIND/03040/2017), while L. Barros thanks FCT for her institutional scientific employment program-contract.

Thank you!!