IECPS 2021 The 2nd International Electronic Conference on Plant Sciences
01–15 DECEMBER 2021 | ONLINE
10th Anniversary 2012 - 2021
Chaired by DR. ADRIANO SOFO
plants MDPI

Comparison between different extraction methods in the recovery of bioactive molecules from *Melissa officinalis* L. under sustainable cultivation: chemical and bioactive characterization

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INTRODUCTION

- *Melissa officinalis* L., from the Lamiaceae family, is one of the most important medicinal and aromatic plants with great potential in the market.
- Besides its medicinal effects, this plant can also be used in the form of infusion and decoction, as also an ingredient in several food preparations.

METHODOLOGY

- The objective of this work was to compare three different extraction methods: infusion (100% water), maceration (80:20 ethanol: water *v:v*) and ultrasound assisted extraction (UAE) under previously optimized extraction conditions (33.0 ± 3.2 min, 371.7 ± 19.3 W and 39.9 ± 1.4% ethanol), in terms of bioactive compounds profile and bioactive properties. Melissa plants were grown under sustainable cultivation with full irrigation (100% of water requirements).
- The studied parameters included:

Antioxidant activity	Thiobarbituric acid reactive substances - TBARS
Cytotoxicity	Sulforhodamine B
Anti-inflammatory activity	RAW cells
Profile of phenolic compounds	HPLC-DAD-ESI/MS
Profile of organic acids	HPLC-DAD

RESULTS

Table 1. Bioactive properties of *Melissa officinalis* samples in relation to the extraction method (µg/mL).

	Infusion	Maceration	UAE	Positive control (µg/mL)
Anti-inflammatory activity (GI_{50} µg/mL)				**Dexamethasone**
RAW cells	244 ± 11[a]	>400[c]	305 ± 9[b]	6.3 ± 0.4
Citotoxicity				**Ellipticine**
NCI-H460	226 ± 21[ab]	190 ± 7[a]	248 ± 26[c]	1.01 ± 0.01
MCF-7	139 ± 12[a]	169 ± 10[b]	175 ± 9[b]	1.02 ± 0.02
CaCo2	46 ± 4[a]	63 ± 4[b]	157 ± 10[c]	1.21 ± 0.02
AGS	34 ± 1[b]	46 ± 1[c]	24 ± 1[a]	1.23 ± 0.03
Antioxidant activity (EC_{50} µg/mL)				**Trolox**
TBARS	3.00 ± 0.14[a]	5.33 ± 0.30[b]	12 ± 0.15[c]	139 ± 5

Positive control ellipticine: GI_{50}= concentration inhibiting 50% of cell proliferation; EC_{50} = concentration exerting 50% of antioxidant activity;
NCI-H460: lung carcinoma; MCF-7: breast carcinoma; AGS: Caco2: colorectal adenocarcinoma; AGS: gastric adenocarcinoma.

The highest anti-inflammatory activity was recorded for the infusion, followed by UAE, whereas no activity was recorded for the maceration extract. The antitumour properties were evaluated in four tumor cell lines, being the best results recorded for the infusion, except for AGS where the UAE method gave best results. Moreover, the maceration extract was the most active against the NCI-H460 cell line.

Table 2. Phenolic compounds content (mg/g extract) of *Melissa officinalis* L. in relation to the extraction method.

Phenolic Compounds (mg/g of extract)			
Polyphenols	**Infusion**	**Maceration**	**Ultrasound**
Lithospermic acid A isomer	29.00 ± 0.32[c]	11.42 ± 0.01[a]	25.25 ± 0.06[b]
Caftaric acid hexoside	1.44 ± 0.04[c]	1.24 ± 0.02[a]	1.40 ± 0.03[b]
Hydroxylsalvianolic E	19.00 ± 0.01[c]	9.00 ± 1.00[a]	16.06 ± 0.03[b]
Sagerinic acid	2.14 ± 1.00[ab]	2.11 ± 0.03[a]	2.23 ± 1.00[c]
Rosmarinic acid	109.30 ± 0.40[a]	163.70 ± 0.40[b]	111.70 ± 2.20[a]
Lithospermic acid A isomer II	2.801± 0.002[b]	2.35 ± 0.06[a]	3.22 ± 0.10[c]
Lithospermic acid A isomer III	5.20 ± 0.06[b]	3.70 ± 0.03[a]	5.90 ± 0.03[c]
Salvianolic acid C derivative	2.54 ± 0.02[b]	1.62 ± 0.03[a]	2.45 ± 0.14[b]
Total phenolics	170.76 ± 0.61	194.98 ± 0.12	168.13 ± 1.87

Calibration curves for the calculation of phenolic compounds: Lithospermic acid A isomer I and Hydroxylsalvianolic E: y = 191291x – 652903, 280 nm; Caftaric acid hexoside, Sagerinic acid, Rosmarinic acid, Lithospermic acid A isomer II, Lithospermic acid A isomer III and Salvianolic acid C derivative: y = 325364x - 1E+06, 330 nm.

Rosmarinic acid was the main compound in all the tested extraction methods. The content of seven out of the eight polyphenols detected for maceration method was lower than that of the infusion and/or the UAE methods, except for the case of rosmarinic acid wher the highest content was recorded. In particular, Lithospermic acid A isomer (25.25 ± 0.01 mg/g) and Hydroxylsalvianolic E (111.70 ± 2.20 mg/g), and Caftaric acid hexoside were the highest in infusions, while Sagerinic acid, Lithospermic acid A isomer II and isomer III were the highest in maceration. Finally, no differences were recorded in Salvianolic acid C derivative content between infusion and maceration.
In terms of total phenolic compounds, maceration was the method that obtained the highest extractability due to the high recovery of rosmarinic acid.

CONCLUSION

- It is therefore concluded that the extraction method contributing to the highest extraction yield of phenolic compounds is the maceration, followed by infusion and UAE.
- Regarding the bioactive properties, infusion was the most efficient method, followed by maceration and UAE, showing the lowest concentrations capable of exerting 50% of inhibition.
- It is worth noting the high content in rosmarinic acid in maceration, and the bioactive properties recorded for all the tested mextraction methods, which makes these samples of great interest for increasing their production in order to obtain extracts enriched with this bioactive molecule that presents strong potential for industrial application.

ACKNOWLEDGEMENTS

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020). L. Barros and M.I. Dias thank the national funding by FCT through the institutional scientific employment program-contract for her contract, while M. Carocho and S. Heleno thank FCT through the individual scientific employment program-contracts (CEECIND/00831/2018 and CEECIND/03040/2017). I. Oliveira thanks FCT for her PhD grant (BD/06017/2020). To FEDER-Interreg España-Portugal programme for financial support through the project TRANSCoLAB 0612_TRANS_CO_LAB_2_P; to ERDF through the Regional Operational Program North 2020, within the scope of Project GreenHealth - Norte-01-0145-FEDER-000042.

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