Green analytical approach for extraction and chemical profiling of phenolic compounds from natural products: A case study of *Teucrium chamaedrys* L.

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INTRODUCTION

- One of the main principle of green analytical chemistry is to eliminate or reduce the use of organic solvents and reagents, and therefore use of Natural Deep Eutectic Solvents (NADES) as medium in sample extraction was recognized as green alternative to conventional one.
- NADES are a class of green solvents, which are a mixture of two or more compounds which one is hydrogen donor, second is hydrogen acceptor and both are associated by hydrogen bonding and have a melting point lower than its individual components. Due to the natural origin of the components, NADES are less toxic, cheap, available, and more eco-friendly solvents. Also, they have great physicochemical properties such as negligible volatility, a liquid state at temperatures below 0°C, adjustable viscosity, a broad range of polarities and ability to dissolve a wide range of compounds.
- **The aim of this study was to develop a green analytical procedure for extraction and chemical profiling of phenolic compounds from medicinal herbs using combination of extraction with NADES solvents and green HPTLC method for separation of metabolites in combination with chemometrics. Greenness of proposed methodology was confirmed by NEMI pictogram and analytical eco-scale.**

METHODS

Preparation of NADES and extraction of phenolic compounds

- The 19 different NADES mixtures were prepared by reflux method. Selected compounds (**Table 1.**) were heated to 80°C and stirred for 30 minutes, after adding water, stirring was continued for another 30 minutes
- Extraction was performed by reflux method. 500 mg of ground plant sample was mixed with 5 mL of previously prepared NADES mixtures and stirred for 45 minutes at a temperature of 50°C. The resulting mixture was centrifuged and purified using solid-phase extraction (SPE).

HPTLC analysis

- Stationary phase: HPTLC silica gel 60 F254
- Mobile phase: ethyl acetate-water-formic acid (17:2:2 v/v/v)
- Derivatization: 0.5% solution of NTS and 0.5% solution of PEG-600 in methanol.
- Documentation at 366 nm.

Image processing and multivariate analysis

- Image of the HPTLC chromatogram was processed using the Image J.
- Principal component analysis (PCA) was carried out by means of PLS ToolBox, v.6.2.1, for MATLAB 7.12.0 (R2011a).

RESULTS

- HPTLC profile showed differences in the metabolite profile of NADES extracts and pattern dominated by green- and orange-colored bands. (**Fig. 1**).
- The line profiles of selected NADES: E2, E5, E6, E10, E15 and E16 (**Fig. 2**) showed more intensive profiles compared to methanol. In common for E2, E10, E15 and E16 solvents is that one of NADES component is in liquid state (lactic acid or glycerol), which can contribute to reducing the viscosity and improving the extraction efficiency.
- Score plots of PCA model (**Fig. 3**) suggested the existence of two groups of NADES extracts which separate along the PC1 direction: first compact cluster consists of urea-based and second cluster is composed of acid-based. NADES with a lower percentage of water had positive score on PC2, while those with higher precent have negative score.
- The analytical eco-Scale for the proposed method was calculated and reported method suffered from a 16 PP due to the use of several non-green solvents such as formic acid and ethyl acetate and the waste produced without treatment and recycling. NEMI (National Environmental Methods Index) pictogram (**Fig. 4.**) shows that proposed HPTLC method is considered an ecofriendly green method.

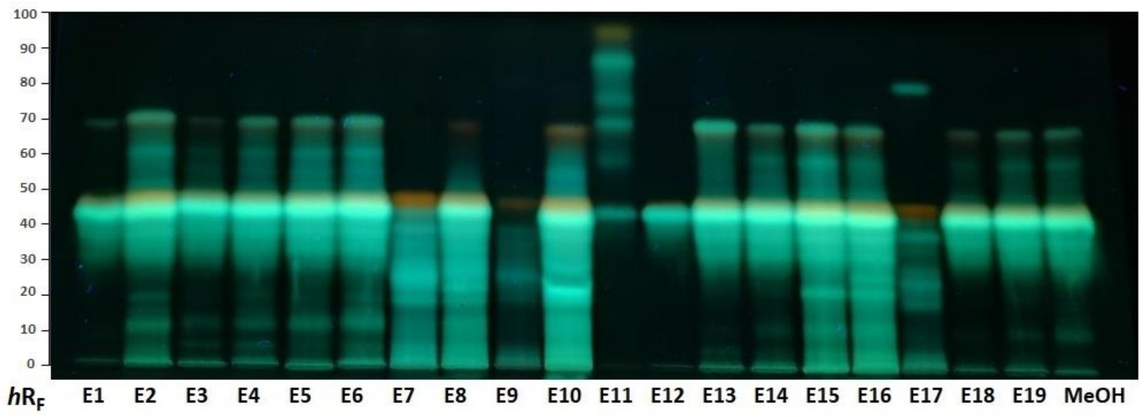

Fig. 1. HPTLC chromatograms for NADES extracts (E1-E19) and methanol extract (MeOH)

Table 1. The composition of the studied NADESs

	Eutectic mixture		H2O%
E1	L-proline: Maleic acid	1:1	20
E2	Lactic acid: Choline chloride	1:1	30
E3	Choline chloride: Tartaric acid	1:1	20
E4	Choline chloride: Tartaric acid	1:1	30
E5	Choline chloride: Tartaric acid	1:1	40
E6	Choline chloride: Tartaric acid	1:1	50
E7	Choline chloride: Urea	1:2	20
E8	Choline chloride: Urea	1:2	30
E9	Choline chloride: Urea	1:2	40
E10	Choline chloride: Urea	1:2	50
E11	Choline chloride: Succinic acid	1:1	20
E12	Choline chloride: Succinic acid	1:1	30
E13	Choline chloride: Succinic acid	1:1	40
E14	Choline chloride: Succinic acid	1:1	50
E15	Choline chloride: Glycerol	1:1	20
E16	Glycerol: Urea	1:1	20
E17	Glycerol: Urea	2:1	20
E18	Glycerol: Lactic acid	1:1	20
E19	Glycerol: L-Ascorbic acid	1:1	20

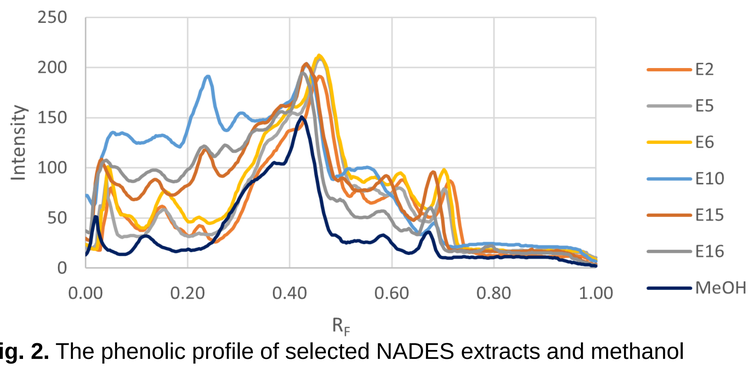

Fig. 2. The phenolic profile of selected NADES extracts and methanol

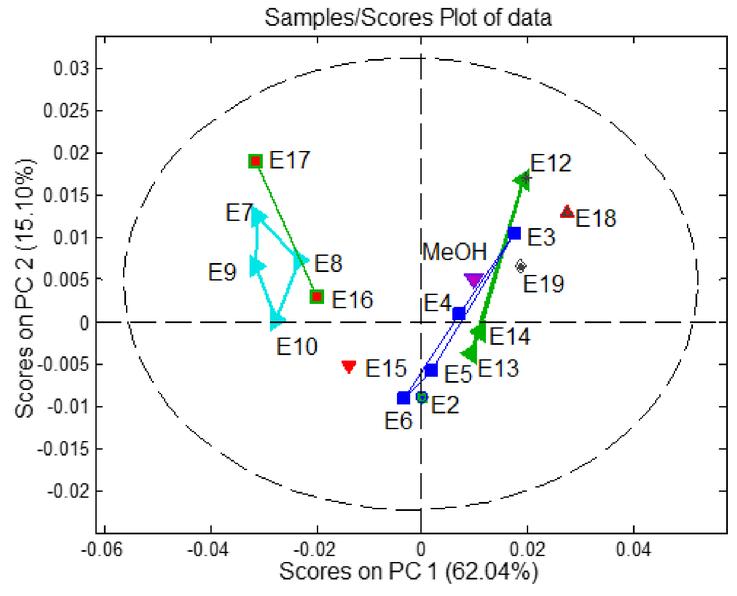

Fig. 3. Principal component analysis – Score Plot

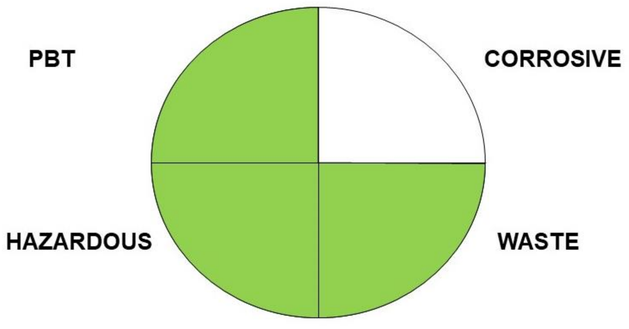

Fig. 4. The NEMI pictograms for assessment of greenness of selected analytical procedure

CONCLUSION

- The six different NADES have proven to be more efficient medium for extraction than methanol.
- Obtained results showed that both different chemical compositions of NADES and water content have influence on extraction of phenolic compounds.
- The NEMI and analytical eco- scale was excellent for the proposed approach.

Acknowledgments
This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract numbers: 451-03-68/2022-14/200168 and 451-03-68/2022-14/200288.