



Banat's University of Agricultural Sciences and Veterinary Medicine
"King Michael I of Romania", Timișoara
ROMANIA



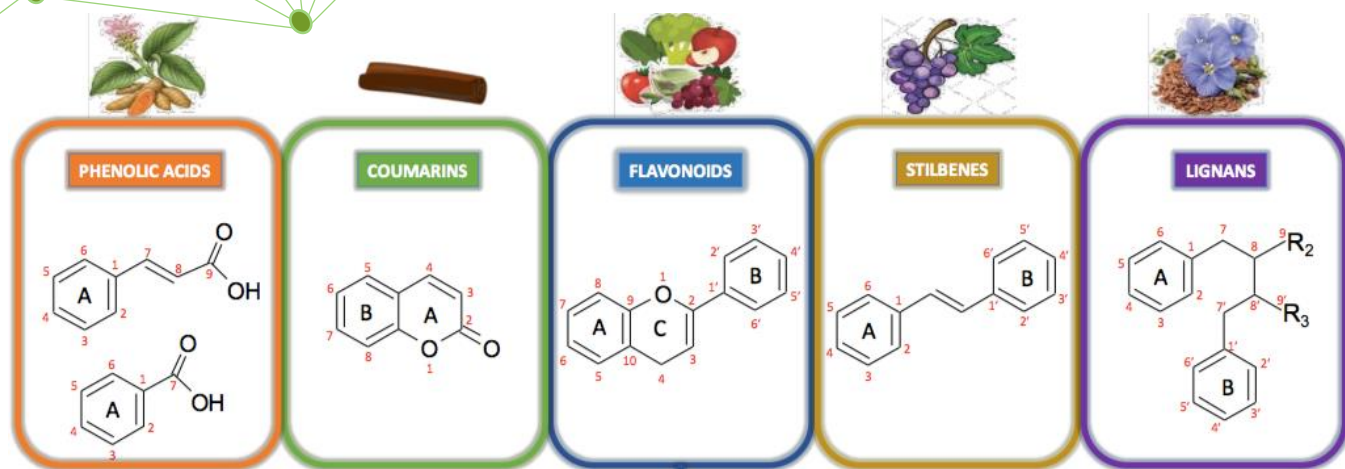
The Influence of Alkaline Electrolyzed Water on Plant Secondary Metabolites and their Implication in the Oxidative Stress Reduction Process

Tulcan Camelia, Tripon Maria Roberta, Gaspar Cristina, Boldura Oana Maria, Marc
Simona, Radulov Isidora, Alexa Ersilia, Negrea Monica,
Hutu Ioan and Mircu Calin

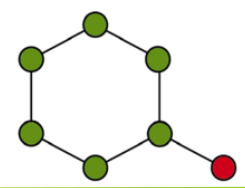
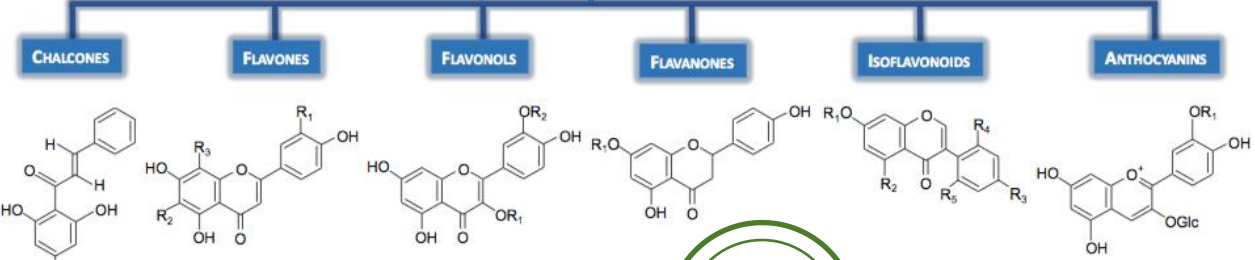
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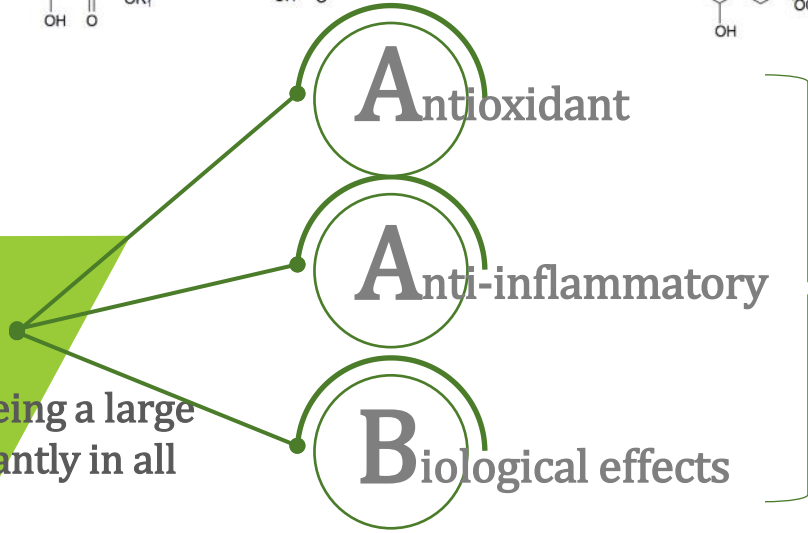


Hano C. and Tungmannithum (2020)



Polyphenols

are secondary metabolites in vegetal matrices, being a large and diverse group of substances present abundantly in all medicinal plants.



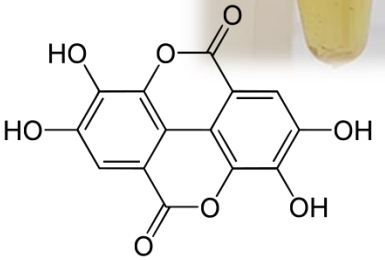
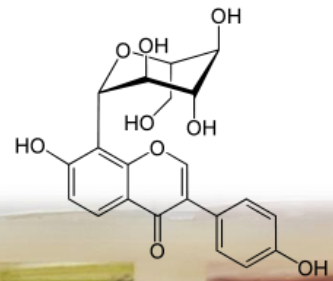
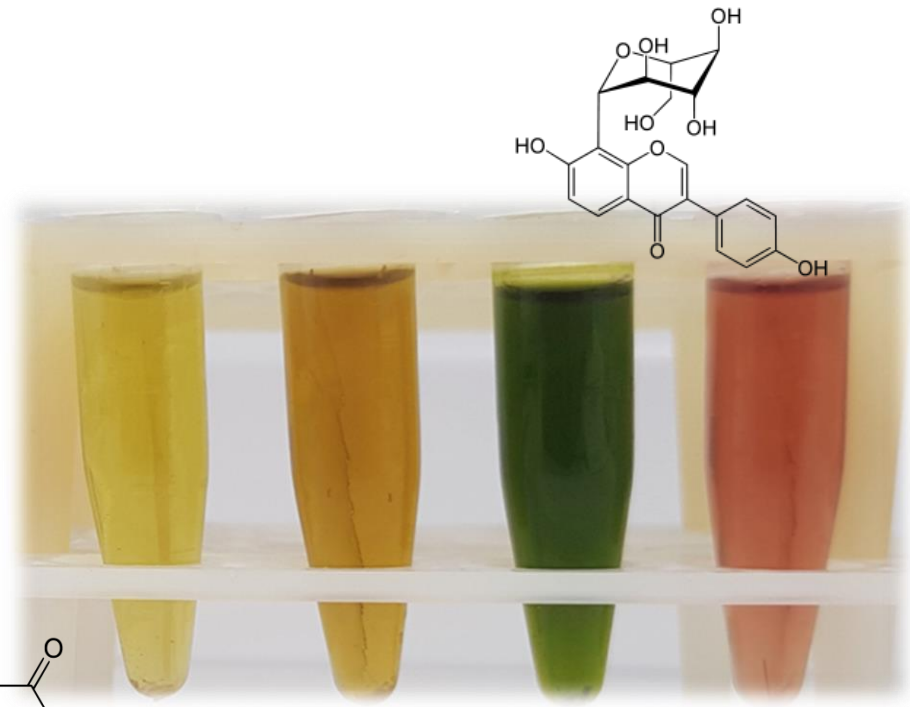
prevention of pathological conditions



Hyssopus officinalis



Chelidonium majus



Allium ursinum



Thymus serpyllum

EXTRACTION TECHNIQUE ?!

POLYPHENOLS: METHODS OF EXTRACTION

KARISHMA RAJBHAR*, HIMANSHU DAWDA and USHA MUKUNDAN

Plant Biotechnology Laboratory, Ramniranjan Jhunjhunwala College, Ghatkopar (West),
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(Received : 04.11.2014; Revised : 17.11.2014; Accepted : 20.11.2014)

The hydrophilic polyphenols including aglycones, glycosides, and oligomers, are extracted using water, polar organic solvents such as methanol, ethanol, acetonitrile and acetone, or their mixture of water. The liquid extracts are sometimes partitioned with solvents such as ethyl acetate, depending on the solubility



Original Articles

Extraction of Polyphenolics from Plant Material for Functional Foods—Engineering and Technology

John Shi, Haseeb Nawaz, Joseph Pohorly, Gauri Mittal, Yukio Kakuda & Yueming Jiang

Pages 139-166 | Published online: 06 Feb 2007

Download citation | <https://doi.org/10.1081/FRI-200040606>

Some polyphenolics are water soluble and some are lipid soluble. In general, catechins are lipid soluble and procyanidins are water soluble;

Because procyanidins are water soluble, they can be extracted by water without introducing any organic solvents.

Food Science and Technology Research, 25 (1), 123–129, 2019

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doi: 10.3136/fstr.25.123

Original paper

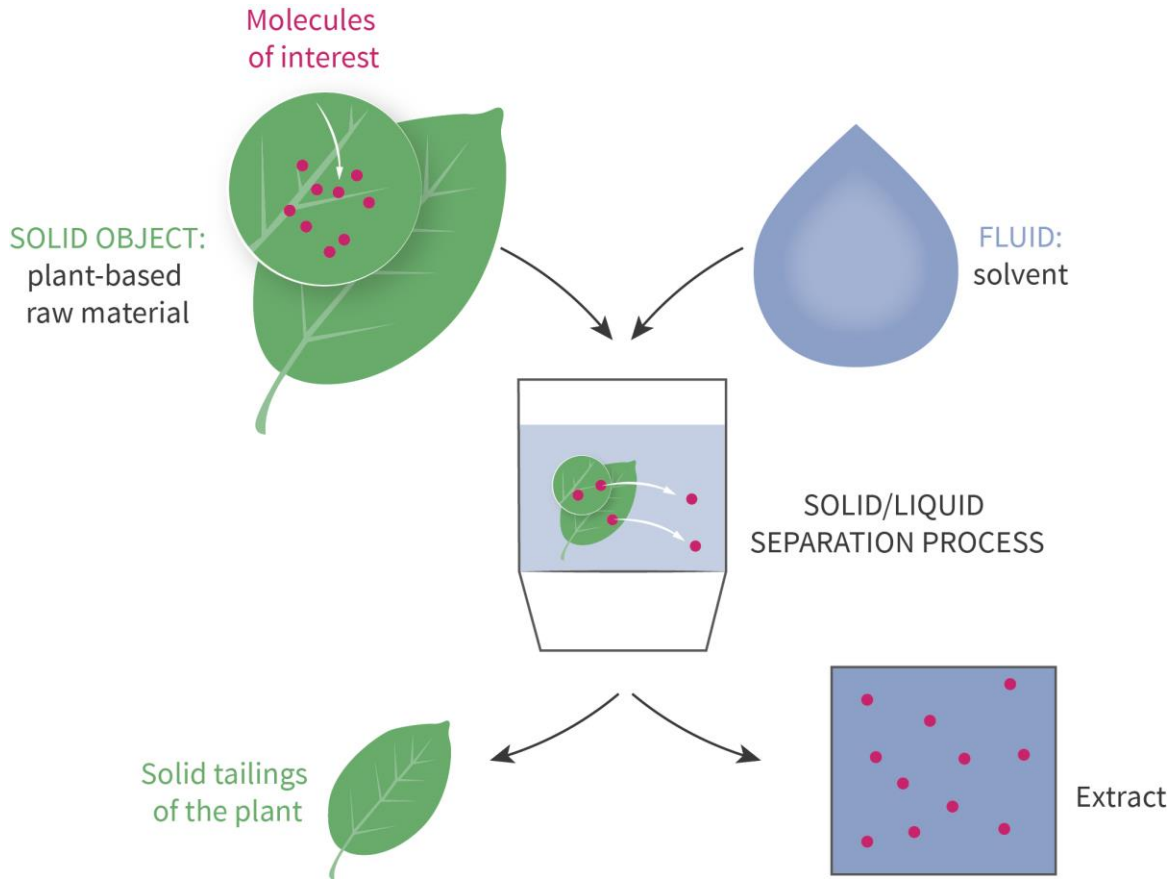
Stability of Polyphenols under Alkaline Conditions and the Formation of a Xanthine Oxidase Inhibitor from Gallic Acid in a Solution at pH 7.4

Sari HONDA*, Rika ISHIDA, Kayo HIDAKA and Toshiya MASUDA

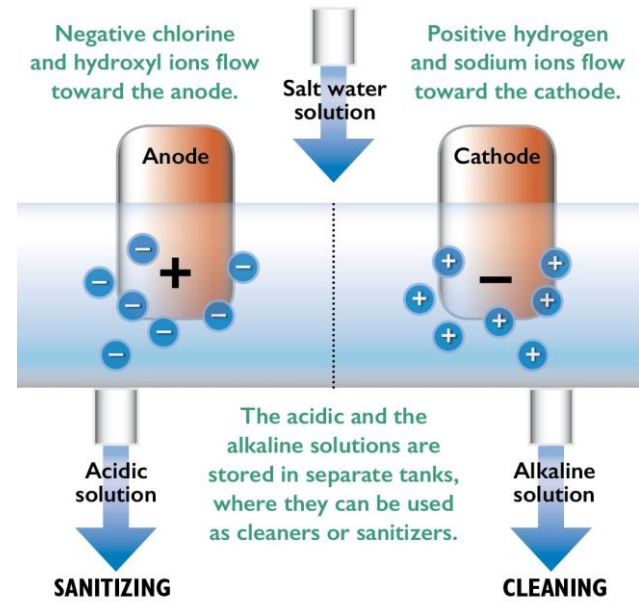
Polyphenols exhibit **strong antioxidant activity** that is closely linked to their high reactivity towards reactive oxygen species. Interestingly, **their reactivity is enhanced under alkaline conditions.**



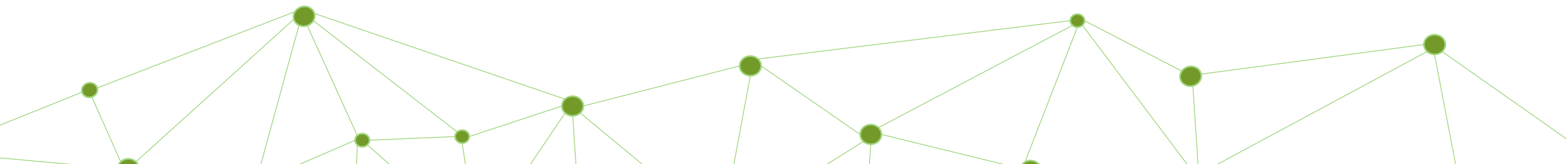
<http://www.jsfstr.org>



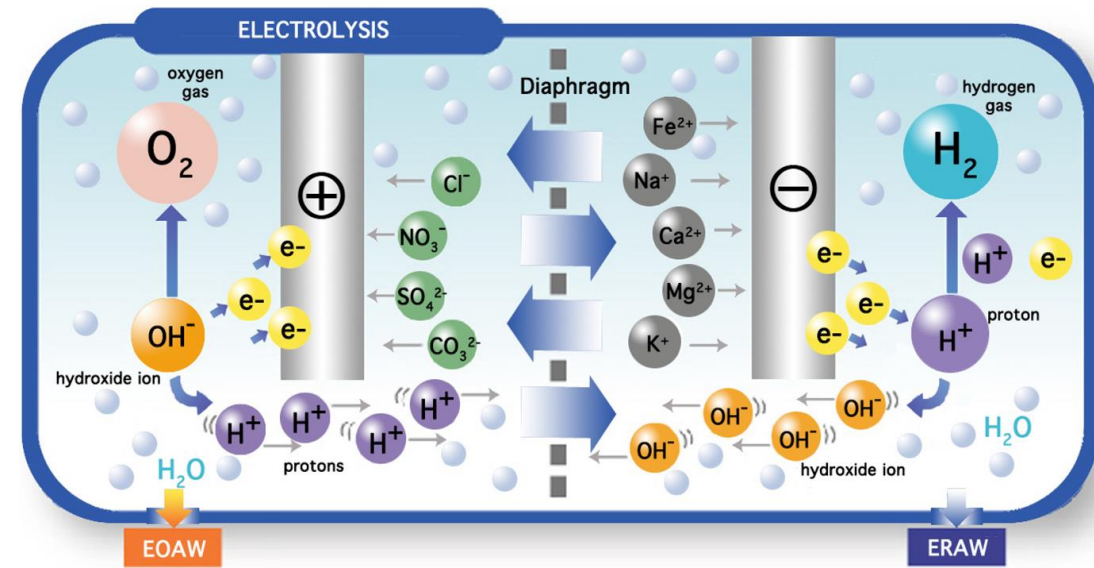
Making electrolyzed water



The **alkaline electrolyzed water** is recognized for its remarkable solvent properties, providing a very good extraction yield in particular on vegetal matrices.



How is AEW obtained?



AIM OF THE STUDY

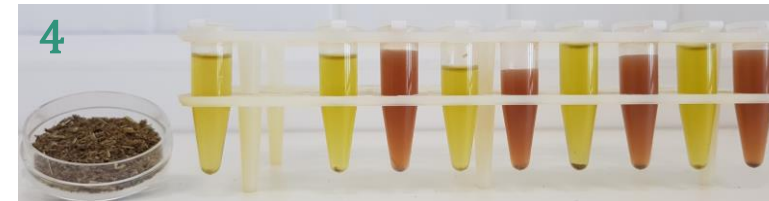
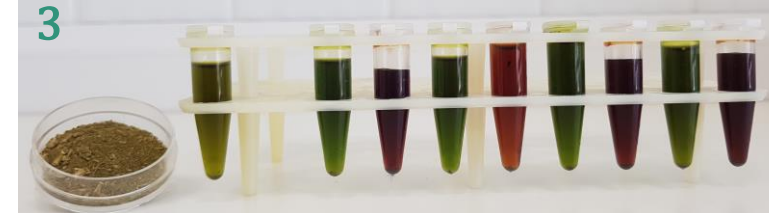
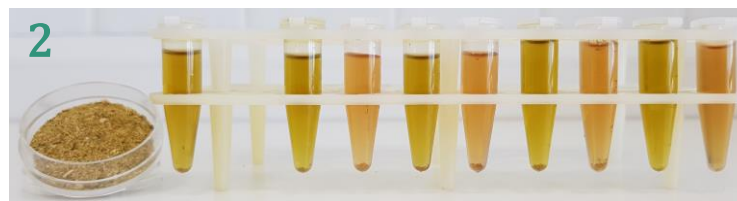
Preparation of alkaline electrolyzed water plant extracts (different pH)

Determination of total polyphenols and flavonoids from the extracts

Interpretation of the obtained values regarding the influence of AEW



Preparation of the extracts



the plants were harvested from the local area and dried (ISO 1573:1980)

each plant volume was reduced and a total amount of 15 g was used for the sample preparation

the chosen solvent was alkaline electrolyzed water (1:10), at different pH values and EtOH 70% (1:10)

the solvent-plant complex was fully mixed via stirring and ultrasonication

extracts were filtered (90 mm FP) and freeze dried

1. *Hyssopus officinalis*
(*Lamiaceae*)

2. *Allium ursinum*
(*Amaryllidaceae*)

3. *Chelidonium majus*
(*Papaveraceae*)

4. *Thymus serpyllum*
(*Lamiaceae*)



8,5

9,5

pH

9

11,5



Determination of total polyphenols

Performance characteristic evaluation of Folin-Ciocalteu micro-method for total polyphenols determination from plant extracts.

Author(s) : [Tripon, R.](#) ; [Repone, C.](#) ; [Urai, M.](#) ; [Chis, C.](#) ; [Oiegas, S.](#) ; [Boldura, O. M.](#) ; [Tulcan, C.](#)

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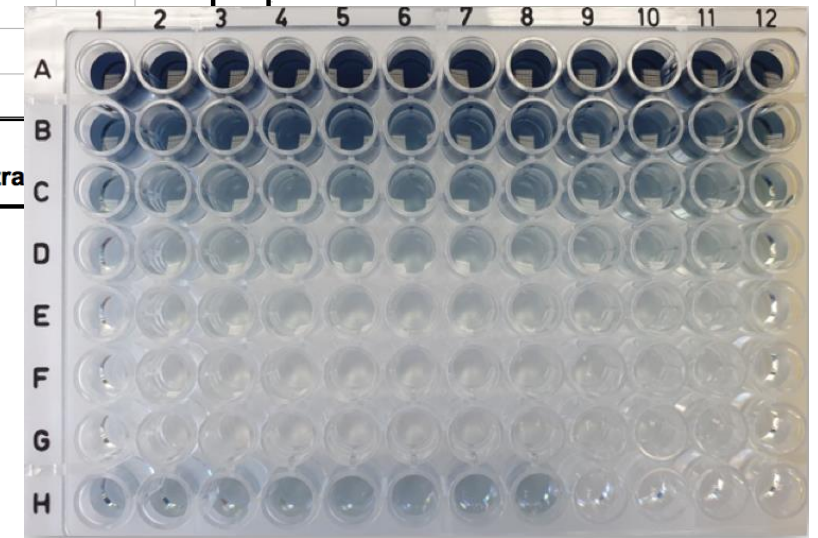
Journal article : [Lucrari Stiintifice - Universitatea de Stiinte Agricole a Banatului Timisoara, Medicina Veterinara 2020 Vol.53 No.1 pp.94-100 ref.11](#)

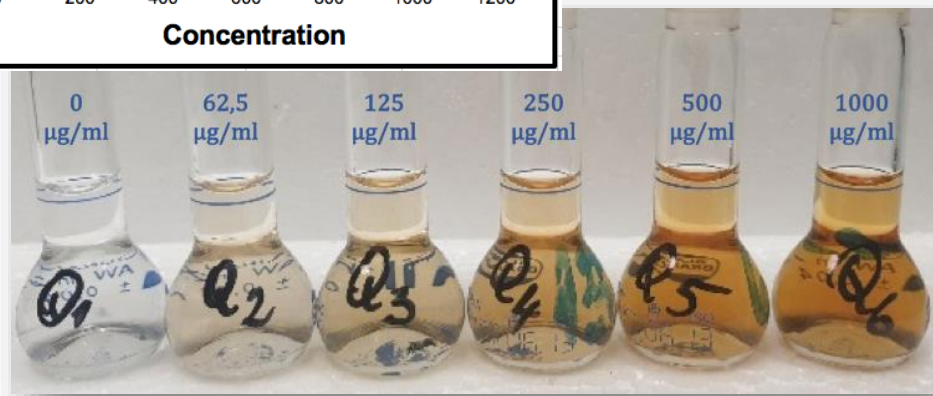
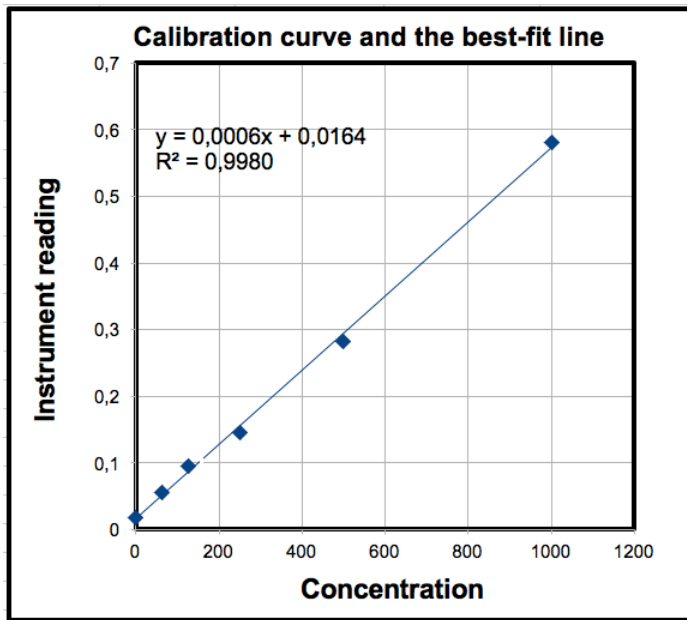


25 μ L sample
125 μ L Folin-Cicalteu Reagent
100 μ L Na₂CO₃

The mixture was kept at room temperature for 30' and the OD was read at 760 nm with a *Tecan Infinite M1000 Pro* spectrofotometer.

The calibration curve was made with gallic acid at the following concentrations: 3,9, 7,8, 15,62, 31,25, 62,5, 125 and 250 ug/ml.





For the calibration curve rutin (1mg/ml) was used and 5 standards were prepared (62,5, 125, 250, 500 and 1000 ug/ml)

 Springer Link

Open Access | Published: 11 February 2014

Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay

Anna Pękał & Krystyna Pyrzynska 

Food Analytical Methods **7**, 1776–1782 (2014) | [Cite this article](#)

36k Accesses | 233 Citations | 3 Altmetric | [Metrics](#)



The preparation of the samples was made in 5 ml volumetric flasks 500 µL of each sample and 150 µL NaNO₂ (5%) were mixed and kept at room temperature for 5';
250 µL AlCl₃ (2%) were added and kept for another 6';
250 µL NaOH were added on the resulted mixture and kept at room temperature for 10';
The OD of the samples was read at 510 nm (Perkin Elmer Lambda 25 spectrofotometer).



RESULTS

Total POLYPHENOLS *stirring*

Total POLYPHENOLS *ultrasonication*

	EtOH mg GAE/g	pH 8,5 mg GAE/g	pH 9 mg GAE/g	pH 9,5 mg GAE/g	pH 11,5 mg GAE/g
<i>H. officinalis</i>	75,14 ±0,32	74,82 ± 0,29	78,31 ± 0,11	74,93 ± 0,22	73,36 ± 0,14
<i>A. ursinum</i>	36,9 ± 0,18	34,11 ± 0,20	38,03 ± 0,25	35,01 ± 0,27	31,12 ± 0,32
<i>Ch. majus</i>	20,11 ±0,07	19,98 ± 0,13	22,71 ± 0,25	21,03 ± 0,29	19,34 ± 0,14
<i>T. serpyllum</i>	97,71 ± 0,93	97,03 ± 0,57	99,97 ± 0,95	98,71 ± 0,74	97,98 ± 0,42

	EtOH mg GAE/g	pH 8,5 mg GAE/g	pH 9 mg GAE/g	pH 9,5 mg GAE/g	pH 11,5 mg GAE/g
<i>H. officinalis</i>	83,81 ± 0,11	85,13 ± 0,21	90,03 ± 0,34	85,41 ± 0,63	84,32 ± 0,56
<i>A. ursinum</i>	43,14 ±0,12	45,21 ± 0,22	48,74 ± 0,14	45,91 ± 0,33	43,91 ±0,44
<i>Ch. majus</i>	29,45 ± 0,23	31,51 ± 0,53	34,31 ± 0,25	32,13 ± 0,11	39,97 ±0,34
<i>T. serpyllum</i>	117,03 ±0,98	119,14 ±0,82	123,11 ±0,74	121,91 ±0,92	120,06 ±0,88



RESULTS



Total FLAVONOIDS *stirring*

Total FLAVONOIDS *ultrasonication*

	EtOH mg RE/g	pH 8,5 mg RE/g	pH 9 mg RE/g	pH 9,5 mg RE/g	pH 11,5 mg RE/g
<i>H. officinalis</i>	1,34 ± 0,02	1,42 ± 0,09	1,98 ± 0,04	1,47 ± 0,03	1,36 ± 0,08
<i>A. ursinum</i>	2,11 ± 0,03	2,97 ± 0,01	3,87 ± 0,06	3,07 ± 0,02	3,19 ± 0,05
<i>Ch. majus</i>	9,71 ± 0,02	10,12 ±0,05	13,01 ±0,02	12,03 ± 0,04	11,07 ±0,06
<i>T. serpyllum</i>	18,03 ± 0,04	20,91 ±0,05	23,03 ±0,03	21,97 ± 0,08	21,01 ±0,05

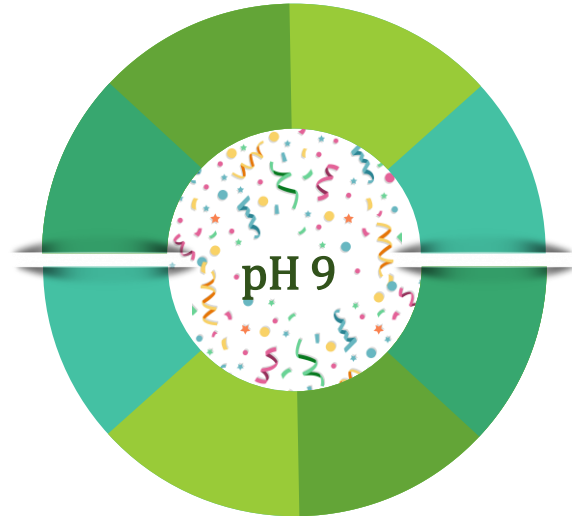
	EtOH mg RE/g	pH 8,5 mg RE/g	pH 9 mg RE/g	pH 9,5 mg RE/g	pH 11,5 mg RE/g
<i>H. officinalis</i>	1245 ± 0,01	2,51 ± 0,04	3,11 ± 0,08	2,56 ± 0,07	2,48 ± 0,02
<i>A. ursinum</i>	3,91 ± 0,03	4,34 ± 0,05	5,04 ± 0,09	4,45 ± 0,05	4,03 ± 0,08
<i>Ch. majus</i>	11,44 ±0,05	12,03 ±0,07	14,43 ±0,11	13,97 ± 0,09	12,43 ±0,11
<i>T. serpyllum</i>	20,98 ±0,12	32,71 ±0,22	33,55 ±0,16	32,56 ± 0,18	31,14 ±0,23

Conclusion

Alkaline electrolyzed water facilitates the extraction process of phytoconstituents, preventing their degradation.

The basic pH increases the reactivity of the compounds, being a particularly important aspect in the case of polyphenols, due to their strong antioxidant activity that is closely linked to the high reactivity towards reactive oxygen species;

The highest values of total polyphenols and flavonoids were obtained in the case of plants extracted with alkaline electrolyzed water at pH 9, by using the ultrasonication technique.



Future plans

Characterization of polyphenol composition
LC MS QTOF

Total antioxidant capacity

IC50

Antimicrobial activity



SPECIAL THANKS FOR YOUR ATTENTION!

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