

# Voltametric analysis of the antioxidative potential of medicinal plants traditionally used in North Macedonia

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CURRENT  
RESEARCH TOPICS  
IN PHARMACY:  
Traditional  
Medicine Talks



# Flora of North Macedonia

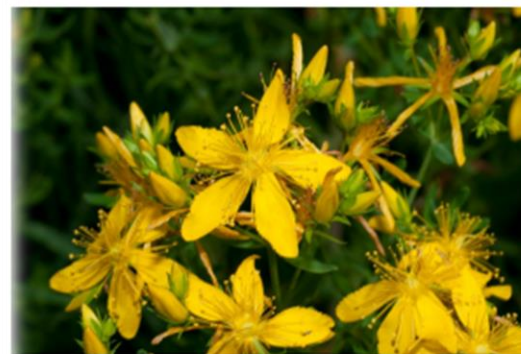
- Medicinal plants in North Macedonia
- Flora in North Macedonia – above 3200 plant species
- Endemic species *Pinus peuce*
- Around 200-300 used in traditional and folk medicine



# Popular plants used in traditional and folk medicine in North Macedonia



*Origanum vulgare* L. (Mountain tea)



*Hypericum perforatum* L. (St. John's wort)



*Gentiana lutea* L. (cowslip)



*Melissa officinalis* L. Lemon balm



*Thymus serpyllum* L. Wild thyme



*Mentha piperita* L. (Mint tea)



*Chamomilla recutita* L. (chamomile)



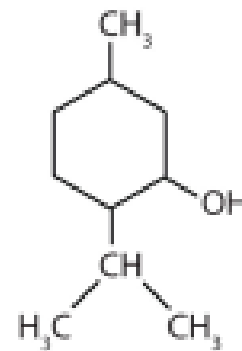
*Primula veris* L. (cowslip)



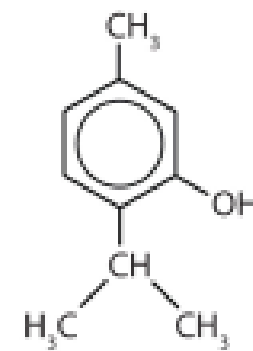
*Orchis morio* L. (orchid)

# Neutralization of free radicals

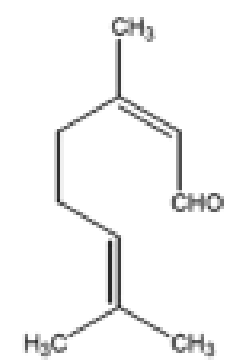
Antioxidant effect as basic property for further pharmacological activities.



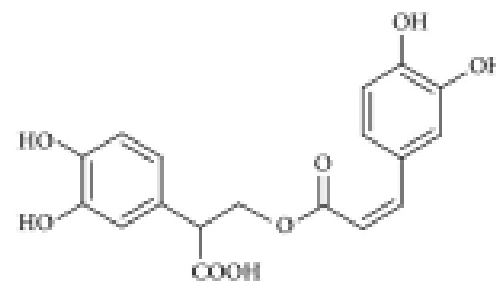
Menthol



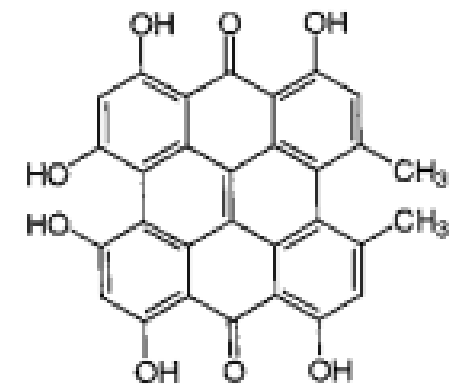
Thymol



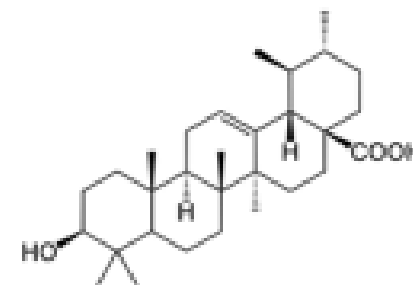
Citral



Rosmarinic acid



Hypericin



Ursolic acid

# What's the benefit from exploring plant extracts and their antioxidative potential?

## “Bright side” of using plant extracts as antioxidant

Natural origin

Many new still unexplored bioactive compounds

**Synergistic effects**

## “Challenges” of using plant extracts as antioxidants

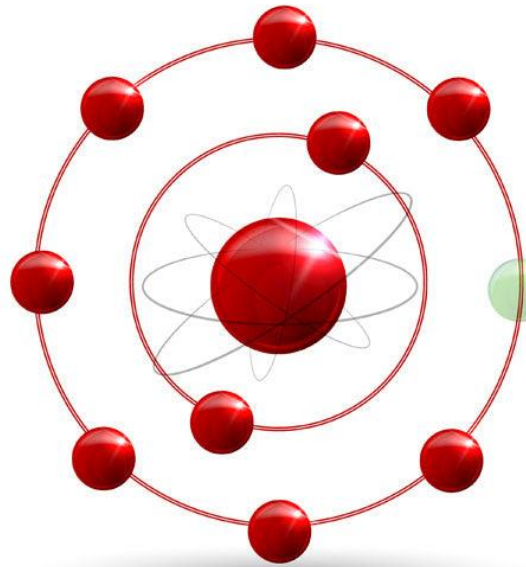
Bioactive molecules found in very small concentration

**Pharmacokinetics**  
(ADME effects)

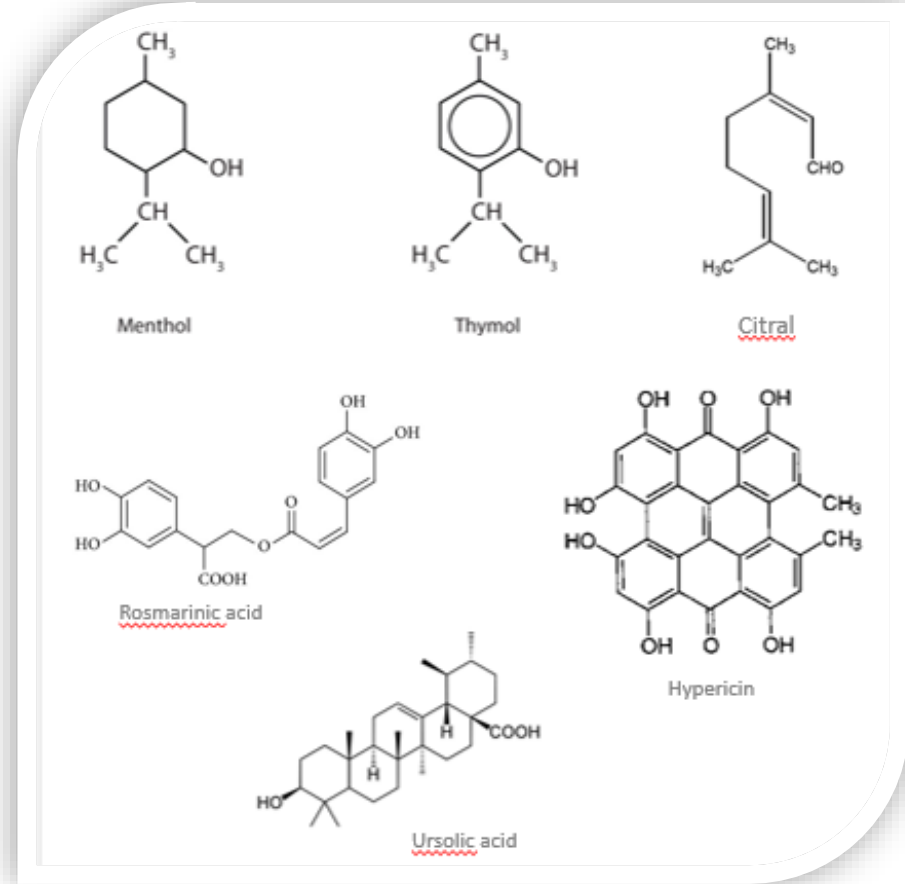
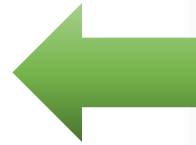
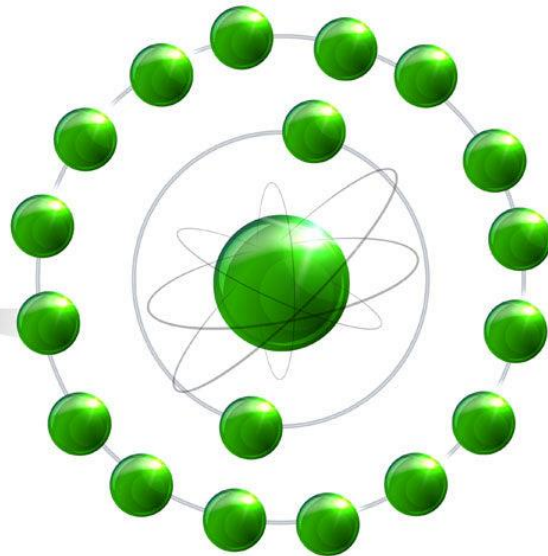
Different cell factors influencing their antioxidative ability (pH value in cell, physiological pathways activated by free radicals....)

# Mechanism of antioxidative effect

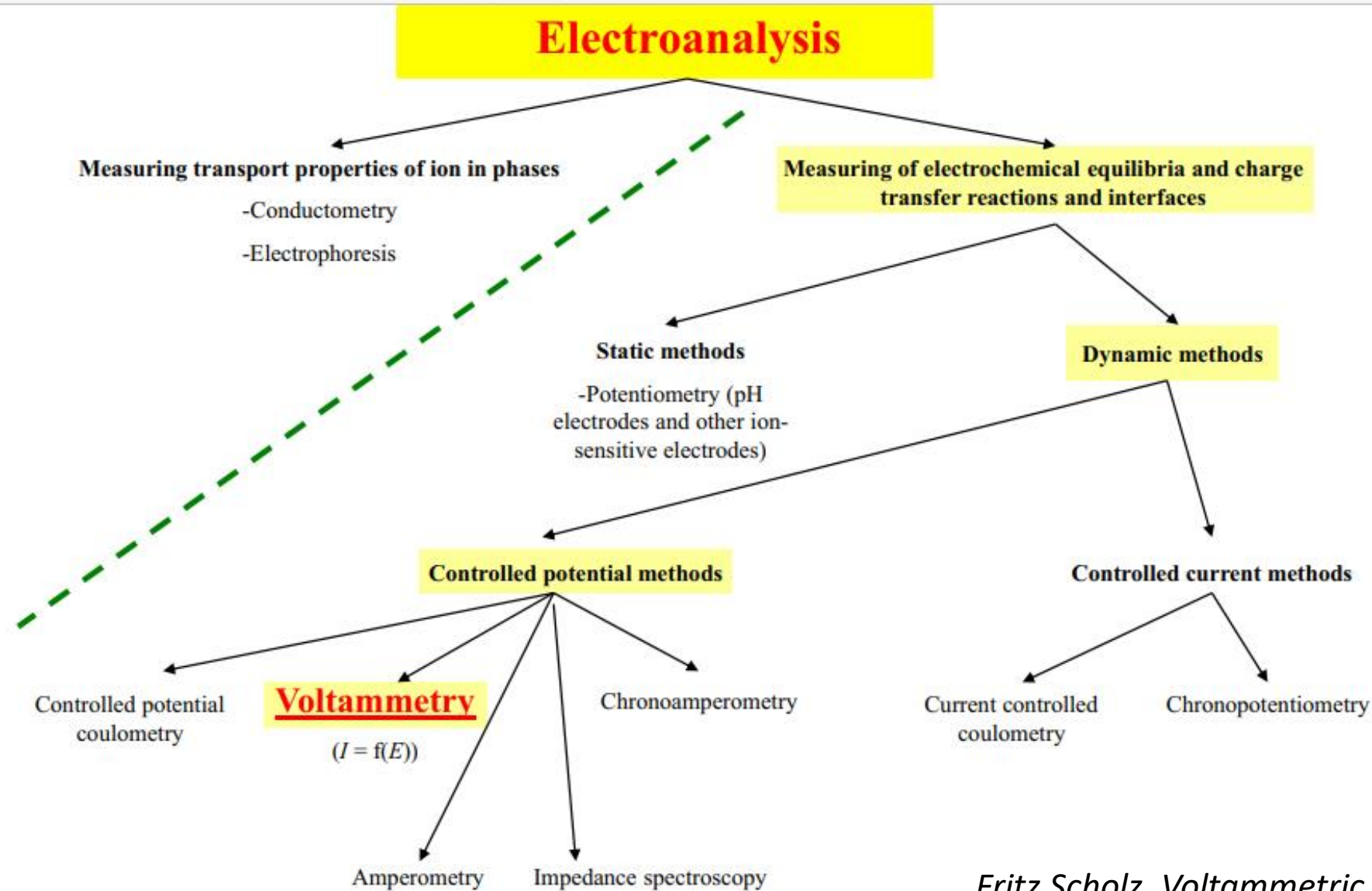
FREE RADICAL WITH MISSING ELECTRON



ANTIOXIDANT DONATES ELECTRON

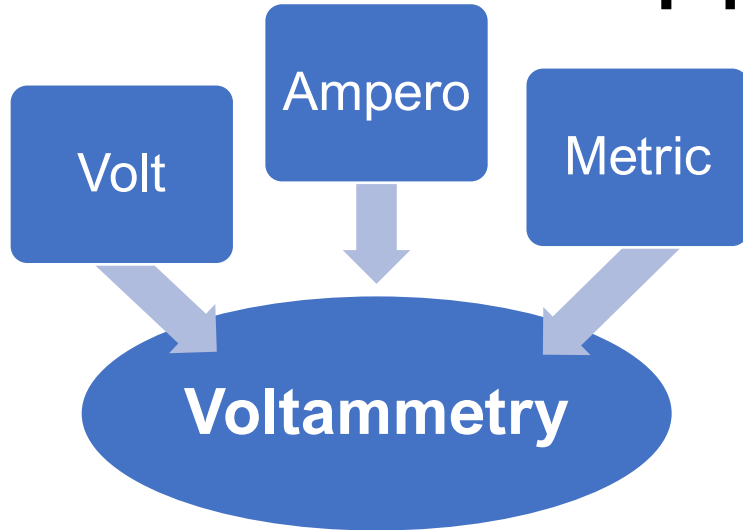


# Wide overview of electrochemical techniques



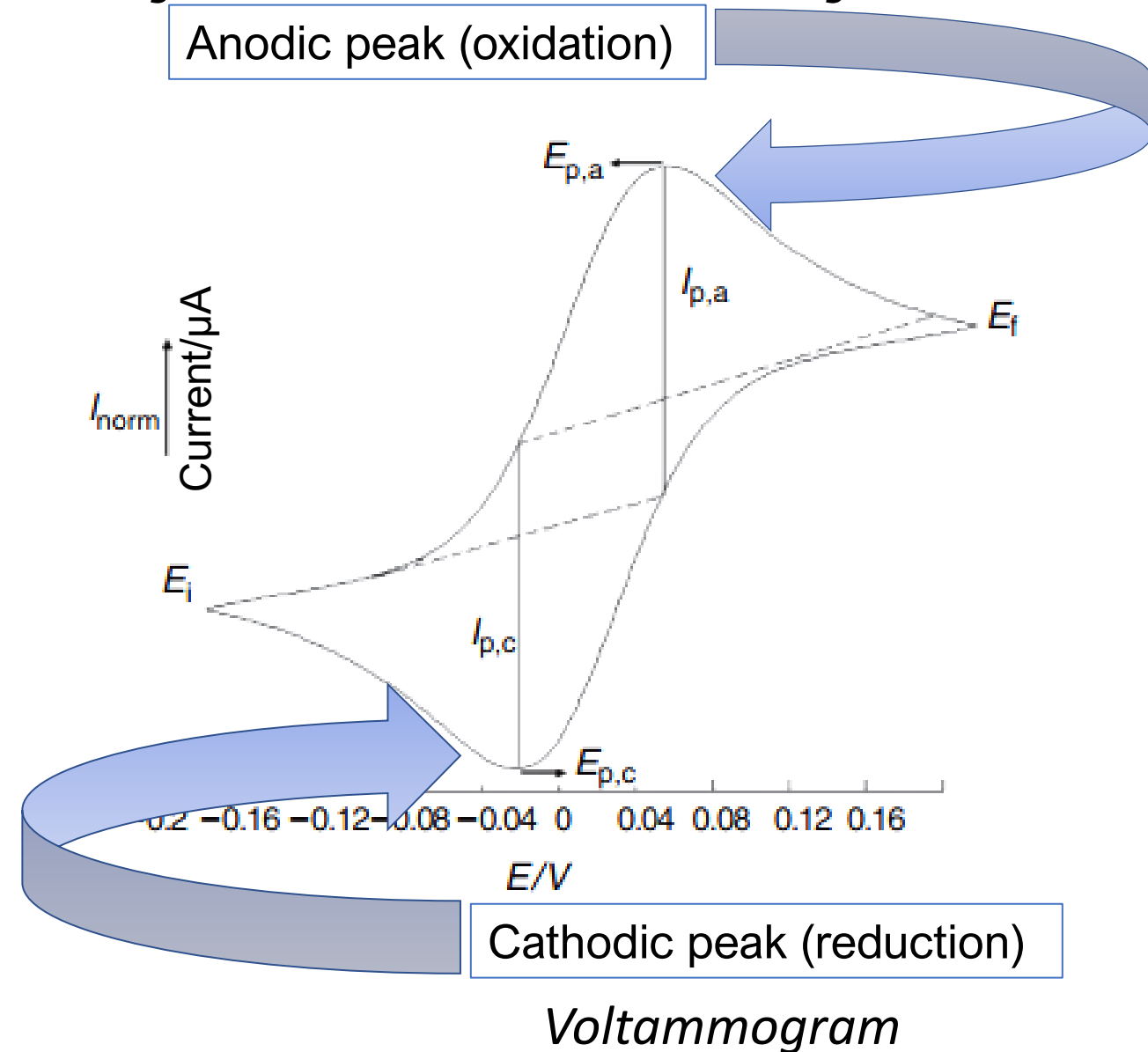
*Fritz Scholz, Voltammetric techniques of analysis: the essentials. ChemTexts (2015) 1:17*

# Principles of cyclic voltammetry



- Measuring the current as a function of voltage (electrode potential)

- rate of oxidation/ reduction reaction
- anodic/cathodic peak potential



Measuring the potential of oxidation/reduction of a substances in a solution



# Electrochemistry as a tool for measuring the oxidation/reduction rate

## Cyclic voltammetry (electrocatalytic reactions)

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The system is composed of:

- electrochemical cell
- 3 electrodes are immersed in electrolyte solution:
  - Working electrode,
  - Reference electrode and
  - Helping electrode

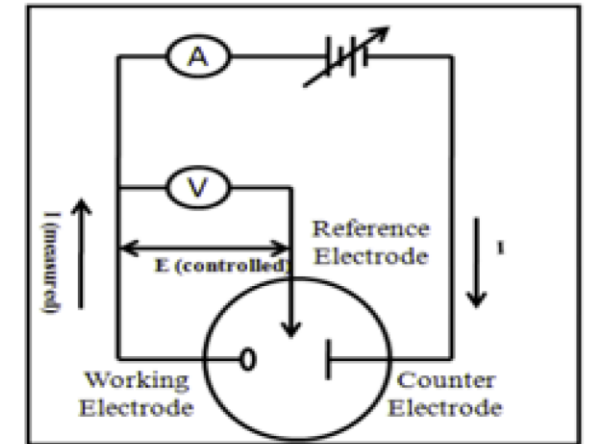
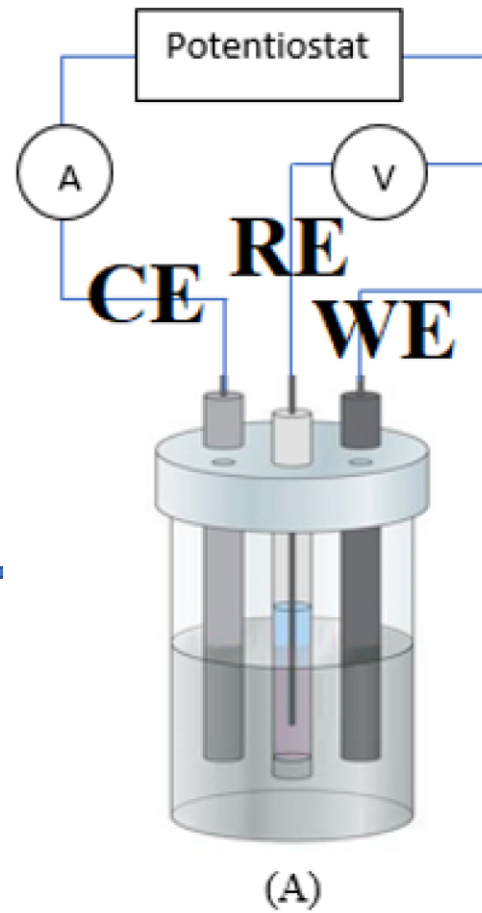


# Electrochemistry as a tool for measuring the oxidation/reduction rate

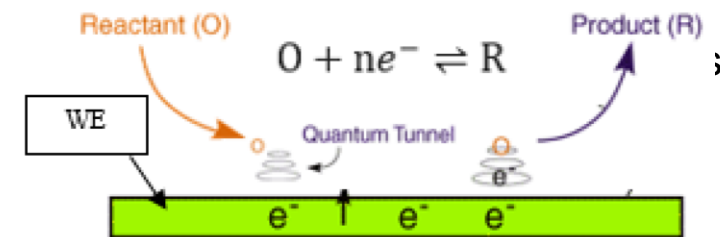
Cyclic voltammetry (electrocatalytic reactions)



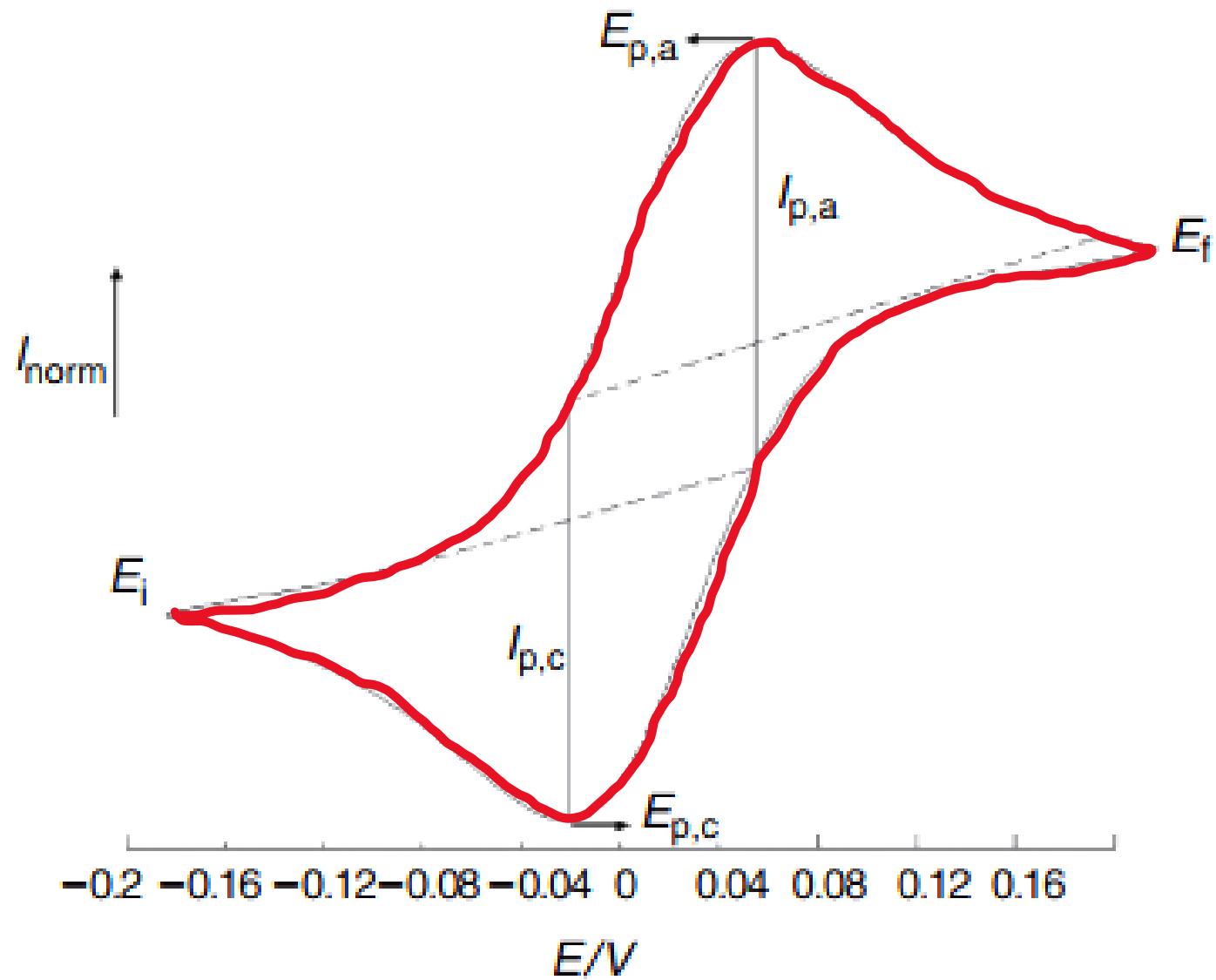
Autolab potentiostat



(B)



(C)



Adapted from: Gulaboski R., and Pereira C., 2009. Electroanalytical Techniques and Instrumentation in Food Analysis, 379- 402.

**Application  
of  
voltammetry**

Identification  
of different  
plant  
bioactive  
substances

Quantification  
on different  
plant bioactive  
substances

Mechanism  
of  
interaction  
with other  
reductive  
substances

Antioxidative  
potential of  
plant  
bioactive  
molecules,  
plant  
extracts, or  
antioxidants  
in plasma

# Experimental work

○ PalmSense Potentiostat connected to a PS Trace system 3.0.

| Name      | Value | Unit |
|-----------|-------|------|
| E vtx1    | 1     | V    |
| E vtx2    | -0.2  | V    |
| E step    | 0.001 | V    |
| E start   | -0.2  | V    |
| E cond    | 0     | V    |
| E dep     | 0     | V    |
| scan rate | 0.010 | V/s  |
| n scans   | 3     |      |



○ One compartment 5 ml electrochemical cell in which are immersed three electrodes:

➤ Glassy carbon electrode GCE ( $d=1,5\text{mm}$ ) as working electrode,

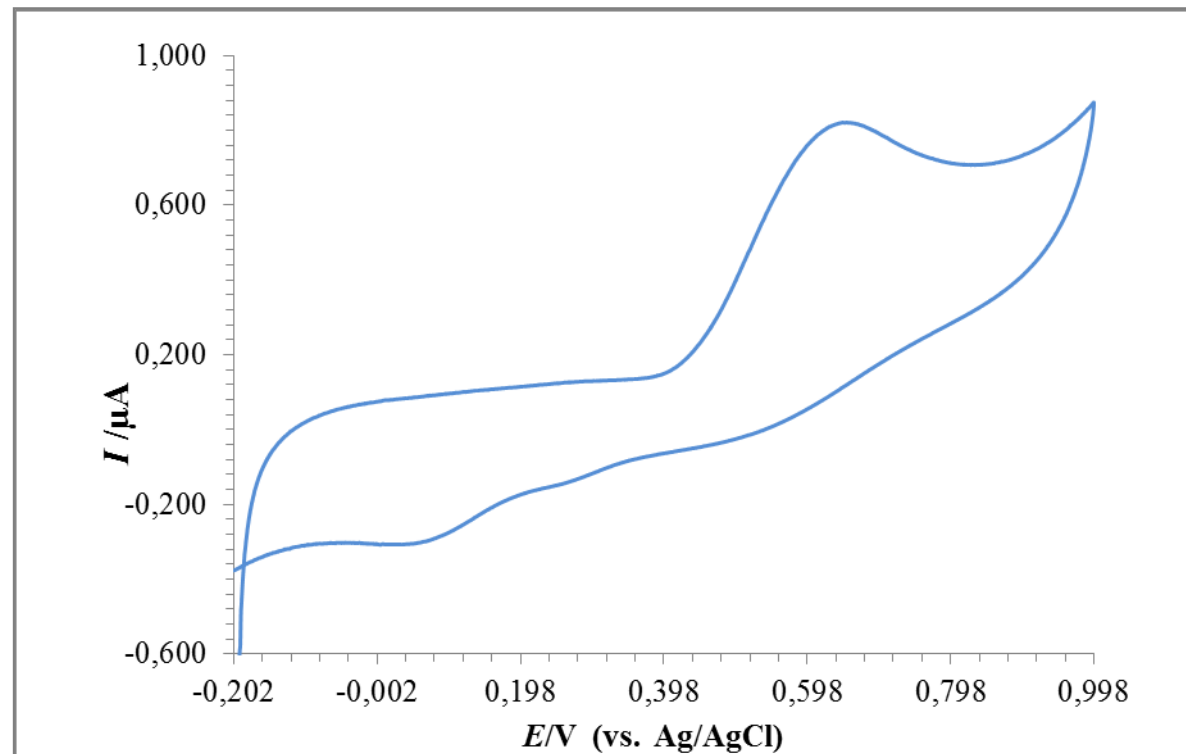
➤ Ag/AgCl (KCl 3mol/L) reference electrode,

➤ Pt wire as helping electrode.

○ Capsaicin (<99, 8%) Sigma- Aldrich, 96% (V/V) ethanol

○ Solution of KCl (0,01mol/L) was used as an electrolyte in the electrochemical cell.

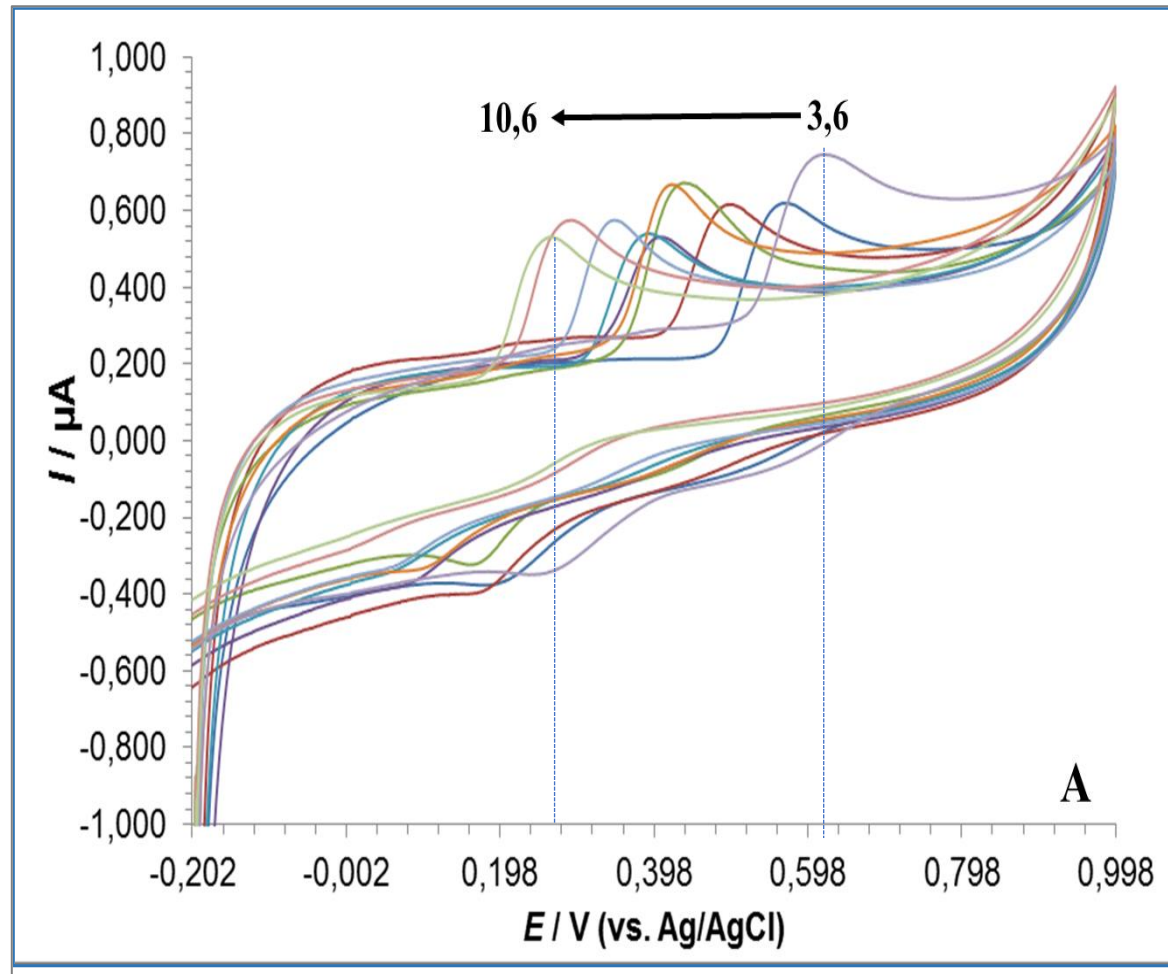
# Redox behavior of capsaicin



Cyclic voltammogram of capsaicin 100  $\mu\text{mol/L}$ , on GCE, scan rate  $\nu = 10 \text{ mV/s}$ , KCl as electrolyte solution  $c = 0,010 \text{ mol/L}$ .

# Redox behavior of capsaicin

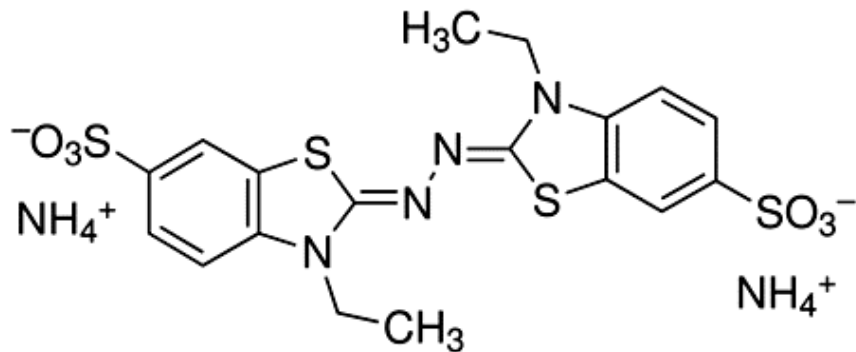
| pH   | buffer solutions  |
|------|---|
| 3.6  | HAcO + NaAcO  |
| 5.5  | HAcO + NaAcO  |
| 6.6  | NaH <sub>2</sub> PO <sub>4</sub> + Na <sub>2</sub> HPO <sub>4</sub> |
| 7.1  | NaH <sub>2</sub> PO <sub>4</sub> + Na <sub>2</sub> HPO <sub>4</sub> |
| 7.6  | NaH <sub>2</sub> PO <sub>4</sub> + Na <sub>2</sub> HPO <sub>4</sub> |
| 8.0  | NaH <sub>2</sub> PO <sub>4</sub> + Na <sub>2</sub> HPO <sub>4</sub> |
| 9.4  | NaHCO <sub>3</sub> + Na <sub>2</sub> CO <sub>3</sub>                |
| 10.6 | NaHCO <sub>3</sub> + Na <sub>2</sub> CO <sub>3</sub>                |



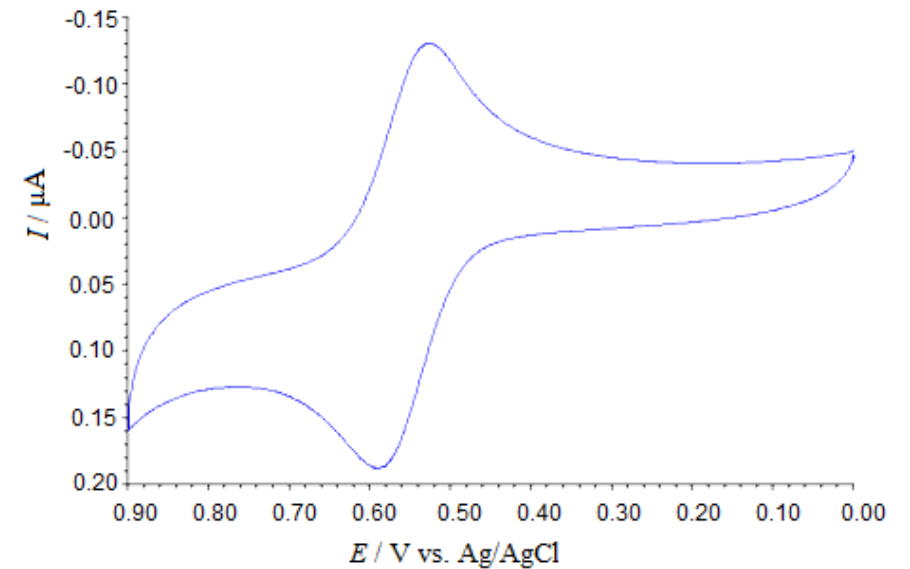
Cyclic voltammogram of capsaicin 50  $\mu\text{M}$ , in different buffers (pH =3.6-10.4) and KCl as electrolyte

## Cyclic voltammetry in measuring antioxidant potential of medicinal plants extracts by using a redox reaction of ABTS

- The total antioxidant capacity of these medicinal plants has also been studied by measuring the rate of the homogeneous redox reaction with ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)).

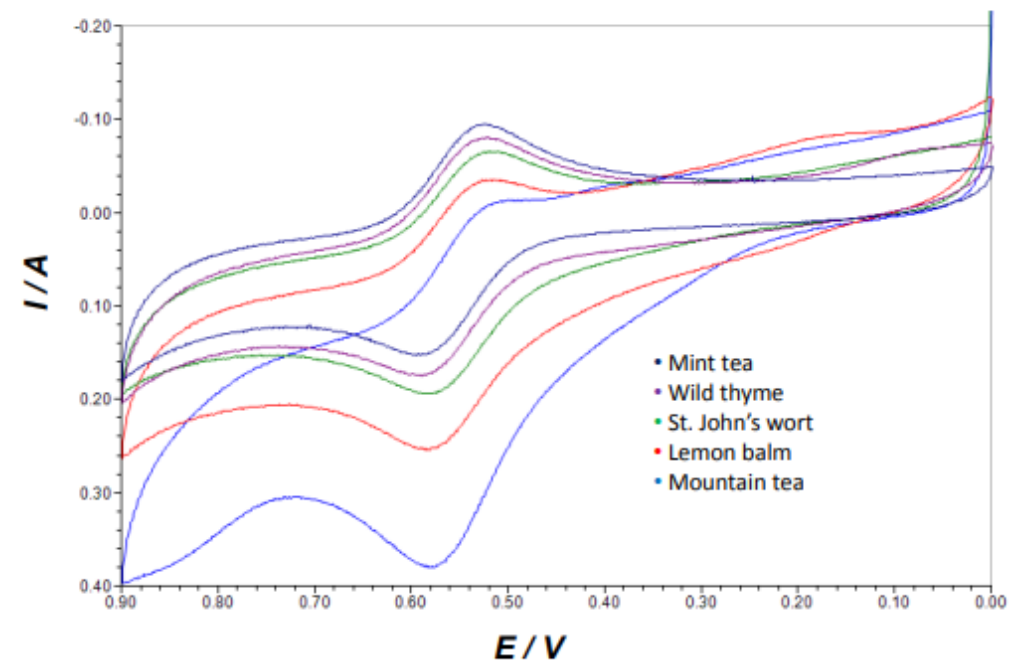


- ABTS<sup>•+</sup> radical was electrochemically *in situ* generated at the surface of glassy carbon electrode by electrochemical oxidation of ABTS in ethanol electrolyte solution. The method is based on the well-known regenerative catalytic EC' mechanism, where the ABTS<sup>•+</sup> radical serves as a redox mediator for catalytic oxidation of antioxidants present in the plant infusion.



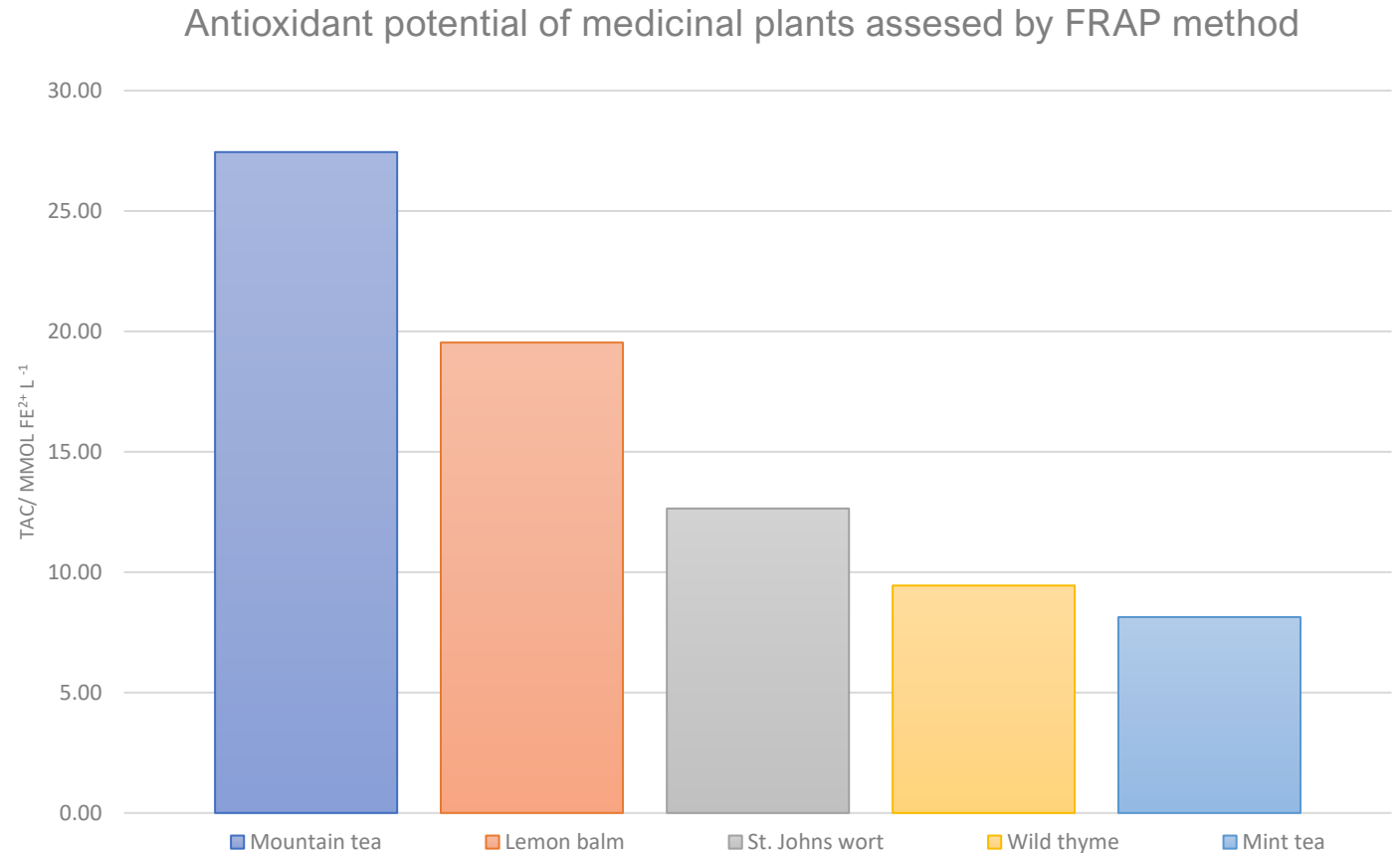


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- Cyclic voltammetry performed with plant infusion for investigation of the antioxidant capacity



**Figure 1.** Cyclic voltammograms of Mountain tea (*Origanum vulgare* L.), Lemon balm (*Melissa officinalis* L.), St. John's wort (*Hypericum perforatum* L.), Wild thyme (*Thymus serpyllum* L.) and Mint tea (*Mentha piperita* L.). Extract recorded in 0.1 mmol/L ethanol solution of ABTS and solution of  $\text{LiClO}_4$ , at  $v = 10$  mV/s.

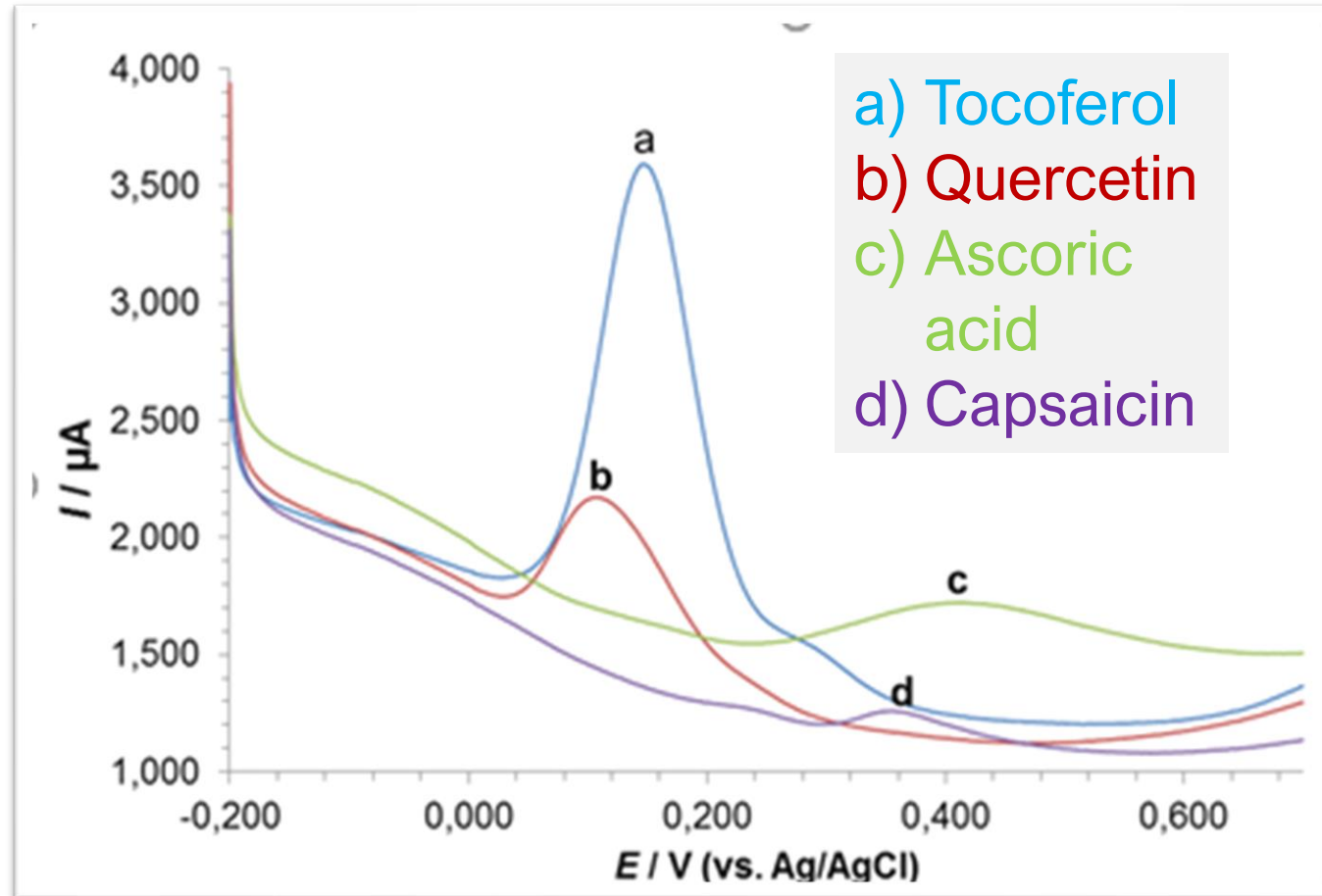
In order to confirm cyclic voltammetry results, FRAP assay was also conducted



This method confirmed that mountain tea has the highest total antioxidant capacity

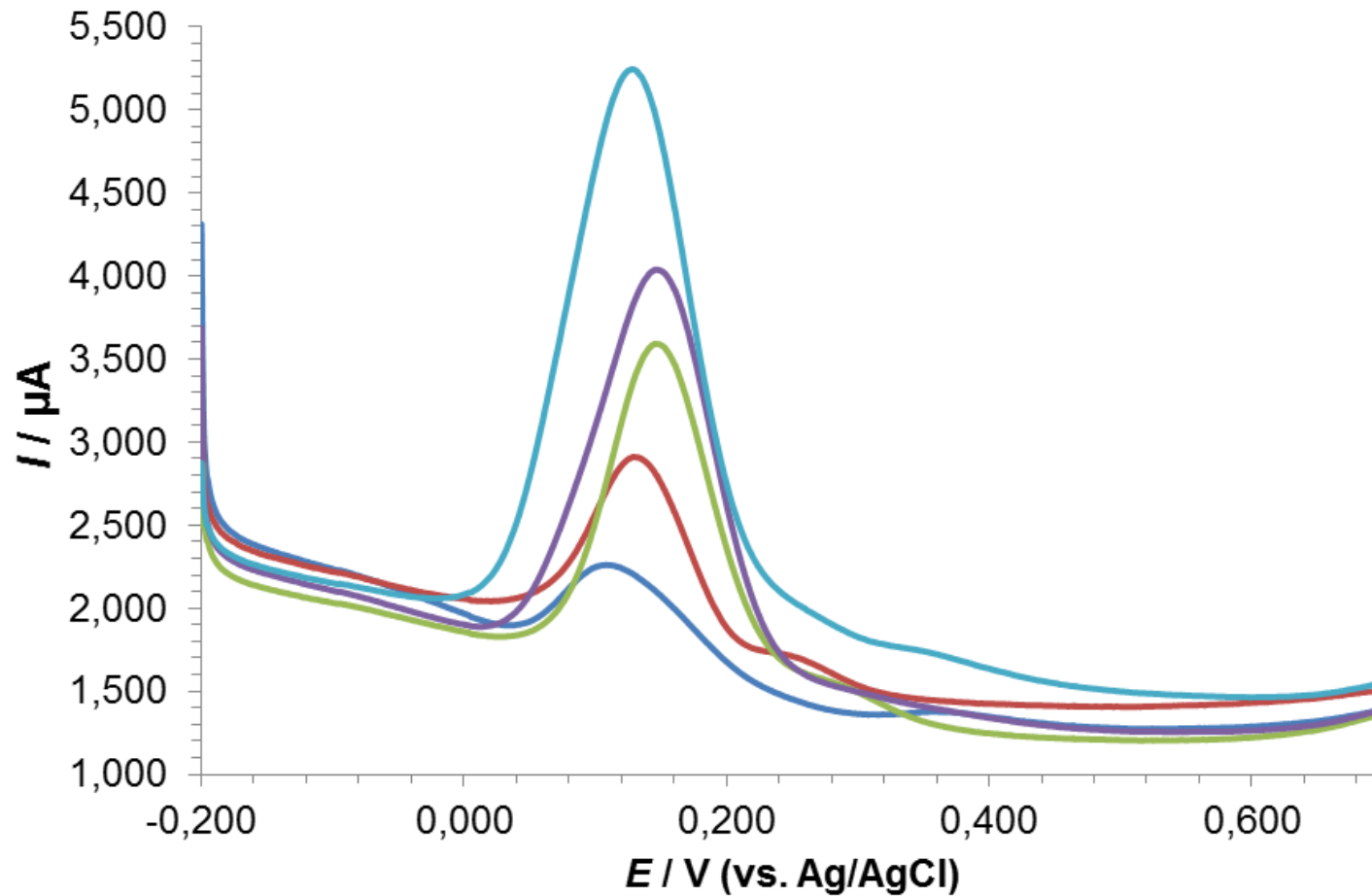
FRAP results have shown no significant differences v.s results from CV

# Results obtained from Square Wave Voltammetry (SWV)



**SWV of capsaicin, quercetin, ascorbic acid and  $\alpha$ -Tocopherol (10  $\mu\text{mol/L}$ )**  
measured at GCE, pH= 7, potential step  $dE = 0,001$  V, amplitude  $E_{sw} = 0,05$  V and  
frequency of 10 Hz.

# Determination of synergistic antioxidant effect of capsaicin



| Compound (10 μmol/L)                     | $I_{\text{net}}/\mu\text{A}$ |
|--|------------------------------|
| α - tocopherol                           | 1.615                        |
| Ascorbic acid                            | 0.098                        |
| Quercetin                                | 0.580                        |
| Capsaicin                                | 0.106                        |
| Equimolar mixture of all these compounds | 3.313                        |

SW voltammograms recorded for the mixture of standard solutions (vitamin E, quercetin, ascorbic acid and capsaicin) in equimolar concentrations (1 до 10 μmol/L) at phosphic buffer, pH = 7,1. Instrumental parameters were:  $dE = 0.001\text{V}$ ,  $E_{\text{sw}} = 0.050\text{ V}$  and frequency of 10 Hz.

# Conclusions remarks:

Cyclic voltammetry is a simple and rapid tool for effective estimation of the total antioxidant capacity (TOC) in medicinal plants extracts.

- Infusions prepared from medicinal plants originated from our country exhibit strong antioxidant potential and this fact justify their use as potent natural antioxidant agents.

- There were strong correlations between the results obtained with FRAP method and Cyclic voltammetry and both methods can be used for evaluation of total antioxidant capacity in medicinal plants infusions, but cyclic voltammetry has few adavtages.

Thank you for  
your attention!

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