

TLC WITH BIODETECTION TO SCREEN PLANT EXTRACTS FOR THE PRESENCE OF POTENTIAL DRUGS TO BE USED IN NEURODEGENERATIVE DISEASES

Session 5 – Food supplements and phytochemistry

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Performance Thin-Layer Chromatography
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Basel 06th – 08th July 2011

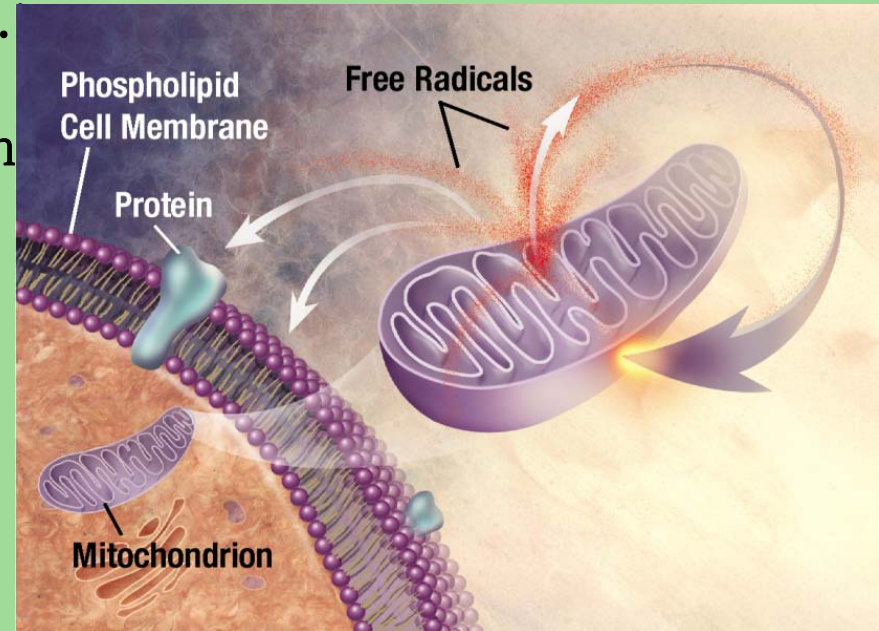
FACTS ABOUT ALZHEIMER'S DISEASE

- * the cause of Alzheimer's disease remains still unknown;
- * the therapy is hypothesis driven not causal;
- * the changes observed in the brains of AD patients: neuritic plaques, neurofibrillary tangles, decrease of acetylcholine level;
- * the current hypotheses: oxidative stress theory of neurodegeneration most frequently combined with the theory of A β plaques formation; cholinergic theory of AD dementia;
- * **limited amount of approved drugs; a need to look for new ones;**
- * **plant species most commonly mentioned in ethnobotanical literature to alleviate the symptoms of dementia: *Salvia spp.*, *Melissa offic.*, *Lavandula offic.*, *Rosmarinus offic.* and still others.**

Reactive oxygen species (ROS) oxidative stress theory of neurodegenerative

Reactive oxygen species (ROS) are involved in pathogenesis of many diseases, e.g.

- reperfusion injury and inflammation
- atherosclerosis;
- cancer;
- dementia;
- diabetic vascular disease;



Antioxidants as therapeutic agents – one of the hottest areas in biomedicine!

EFFECT-DIRECTED ANALYSIS VS COMPOUND DIRECTED DETECTION

BIOMONITORING

DETECTION MECHANISM
BASED ON
A BIOLOGICAL RESPONSE

DIRECTING THE ANALYTICAL
PROTOCOL FOR THE
ISOLATION AND
IDENTIFICATION OF
COMPOUNDS WITH
SPECIFIC PROPERTIES

NO PRESUMPTIONS OF COMPOUND
IDENTITY ARE REQUIRED;

CONVENTIONAL DETECTION TECHNIQUES

DETECTION BASED ON A
PHYSICAL
INTERACTION OR CHEMICAL
REACTION

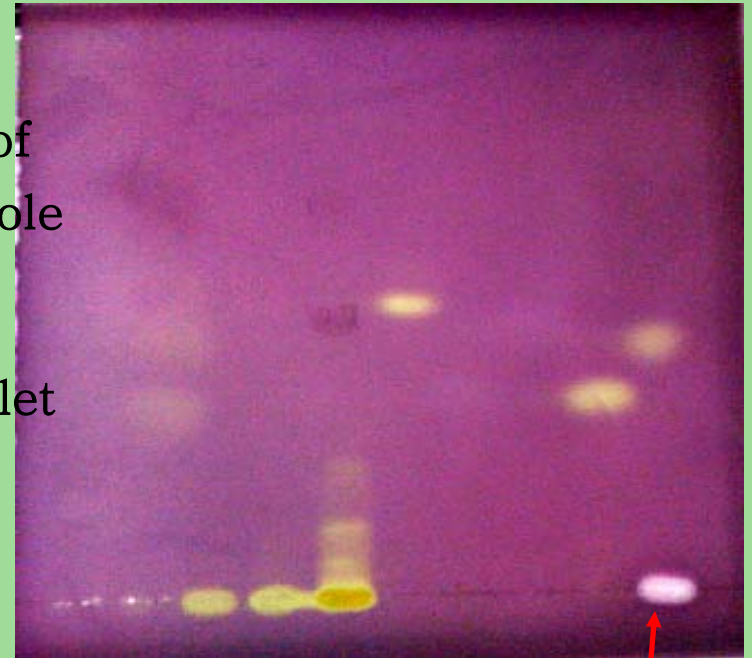
TARGET COMPOUND ANALYSIS –
EVALUATING THE PRESENCE OF
A KNOWN COMPOUND IN A
MIXTURE

IT IS CRUCIAL TO KNOW IN
ADVANCE
WHAT COMPOUNDS TO LOOK
FOR

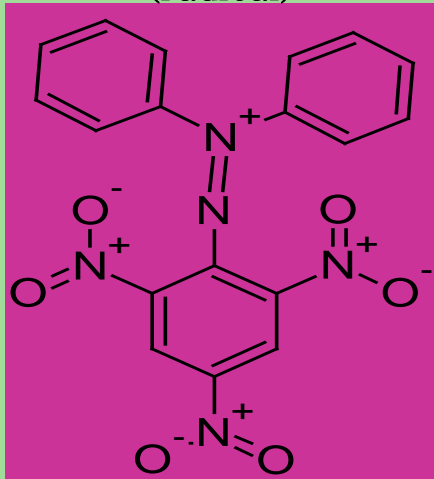
TLC FOR SCREENING NATURAL SAMPLES FOR THE PRESENCE OF ANTIOXIDANTS

DPPH – 1,1-diphenyl-2-picrylhydrazyl:

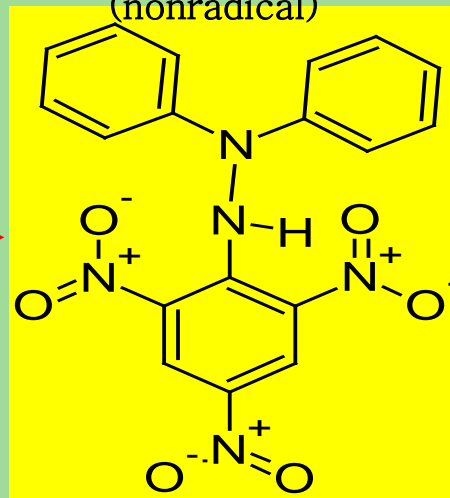
- stable free radical due to the delocalisation of the spare electron, over the molecule as a whole (the molecules do not dimerise);
- the delocalisation gives rise to the deep violet colour characterized by an absorption band maximum at 517 nm.



1,1-diphenyl-2-picrylhydrazyl
(radical)



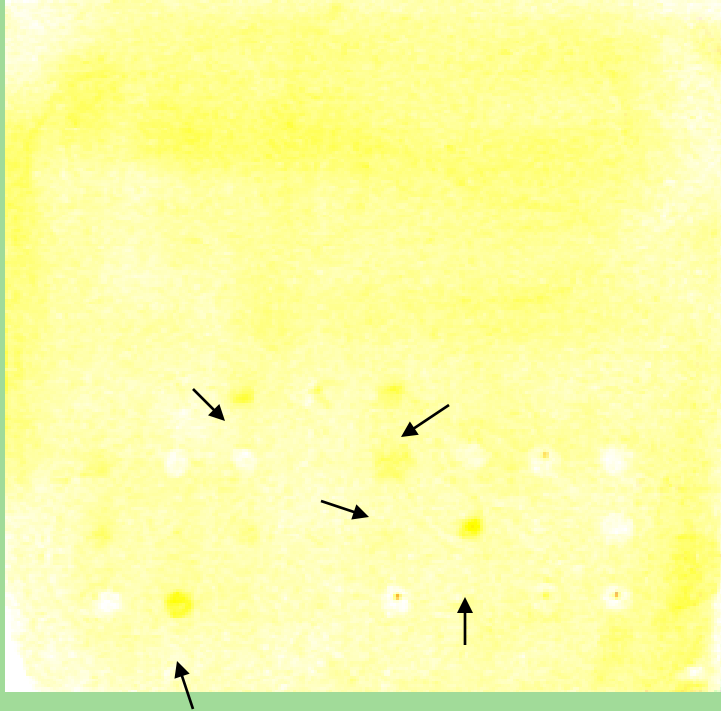
1,1-diphenyl-2-picrylhydrazine
(nonradical)



False-positive
result!!!

OTHER TLC-BASED ASSAYS FOR THE SEARCH OF ANTIOXIDANT

β -CAROTENE – TLC ASSAYS



1. Inhibition of bleaching orange color of β -carotene

- * a plate is sprayed with solution of β -carotene in chloroform
- * after exposition to sunlight orange spots remain on a white background

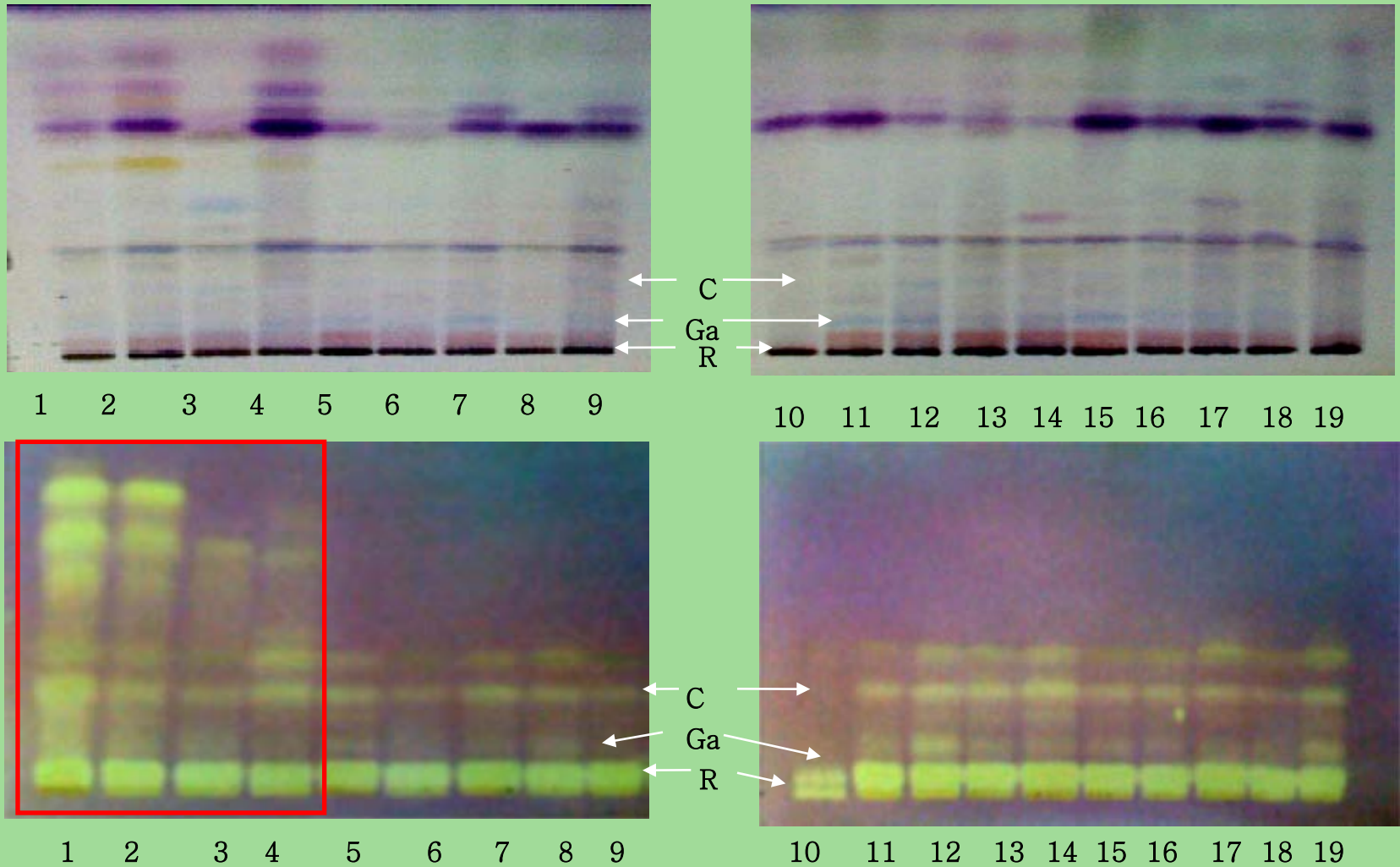
2. β -carotene – lipid peroxidation TLC assay

- * a plate is sprayed with linoleic acid in ethanol and subsequently with β -carotene in chloroform
- * substances exhibiting antioxidant properties appear as orange spots on yellowish background

* the major problem – only a slight difference between the color of the spots exhibiting positive results and the background

TLC FOR SCREENING NATURAL SAMPLES FOR THE PRESENCE OF ANTIOXIDANTS (CHEMICAL AND BIOLOGICAL FINGERPRINTING)

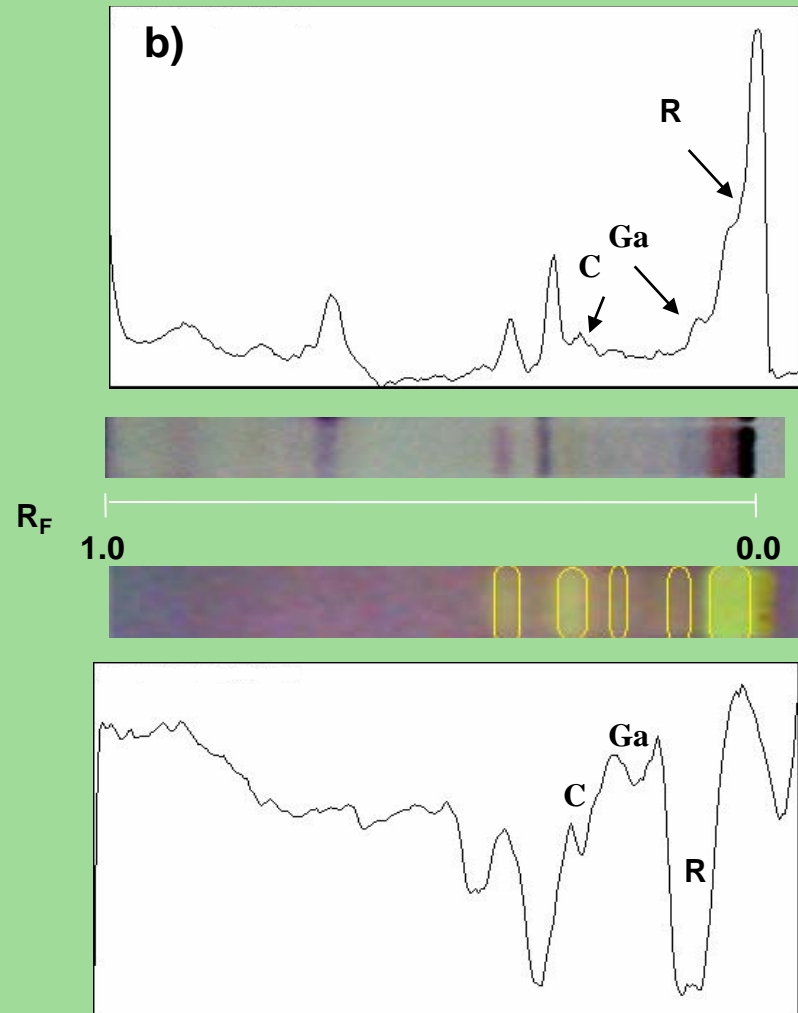
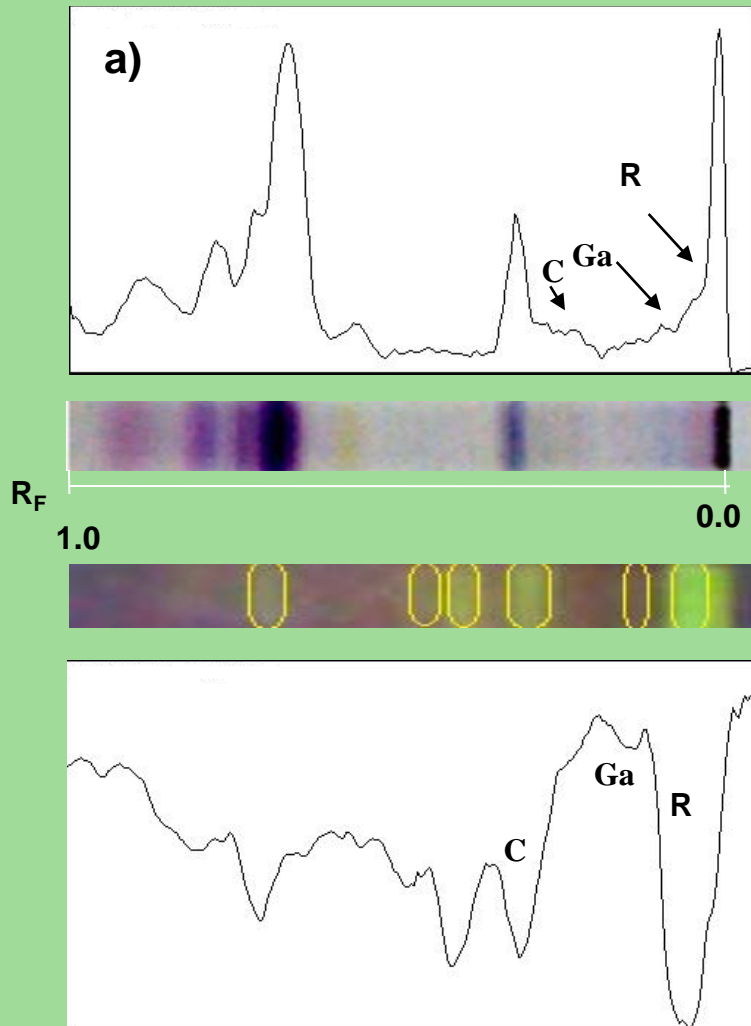
Less polar fraction (toluen-ethyl acetate-formic acid(60:40:1))



TLC FOR SCREENING NATURAL SAMPLES FOR THE PRESENCE OF ANTIOXIDANTS

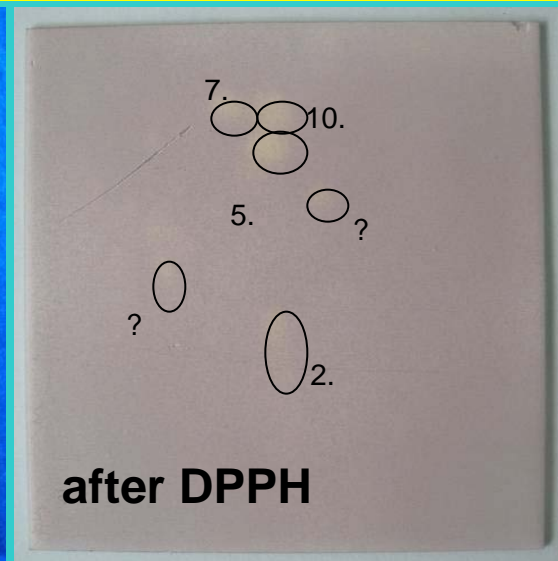
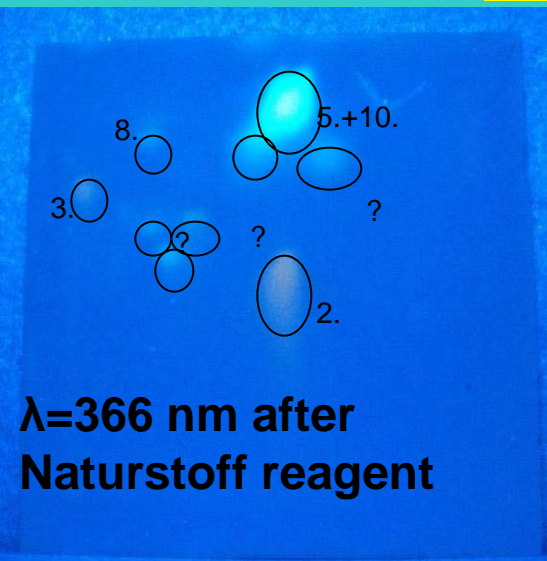
(CHEMICAL AND BIOLOGICAL FINGERPRINTING)

Fingerprint comparison for less polar fraction of
(*Salvia lavandulifolia* (a) i *Salvia atropatana* (b))



TLC FOR SCREENING NATURAL SAMPLES

Eupatorium cannabinum

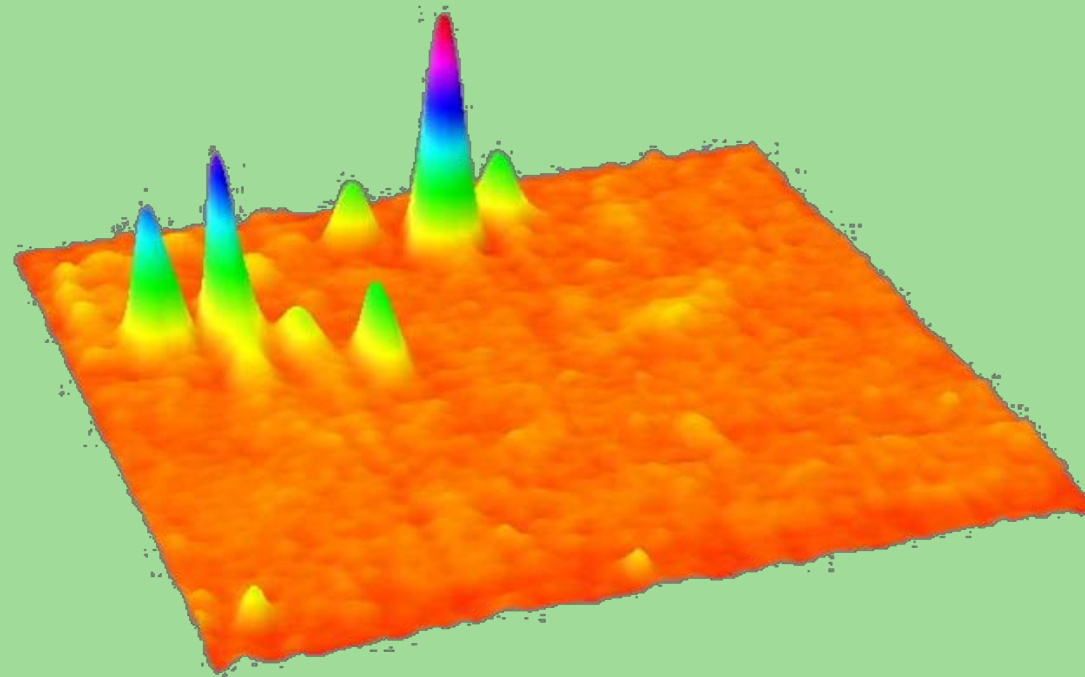


2D-TLC

DIOL plates

NP -70% AcOEt+n-Hp,

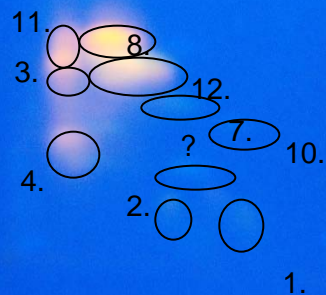
RP-30% MeOH+H2O



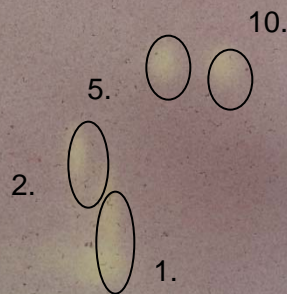
TLC FOR SCREENING NATURAL SAMPLES

Polygonum hydropiper

$\lambda=366$ nm after
Naturstoff reagent



after DPPH



2D-TLC

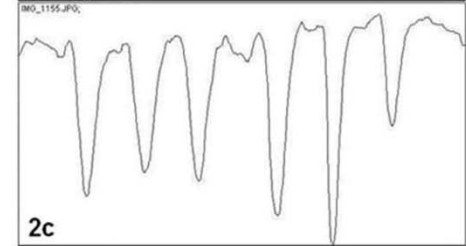
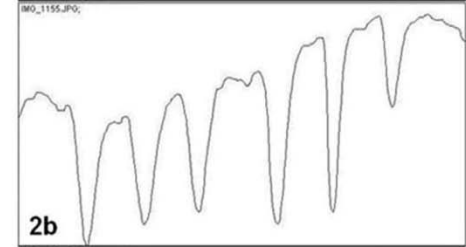
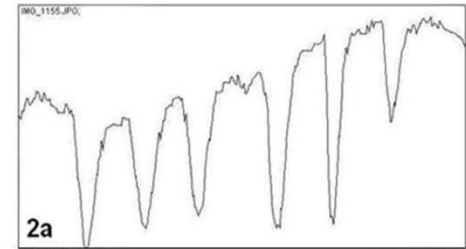
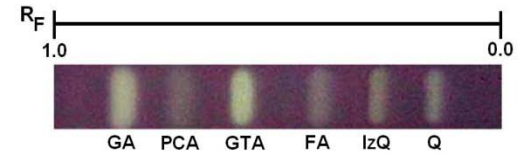
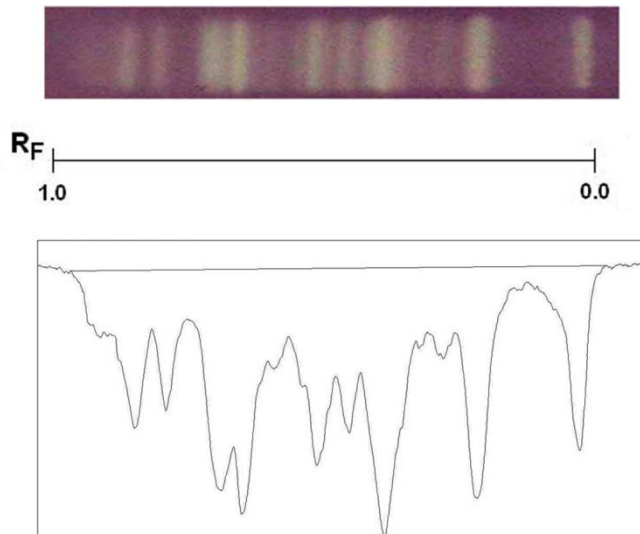
CN plates

NP - 30% iPrOH + n-Hp,

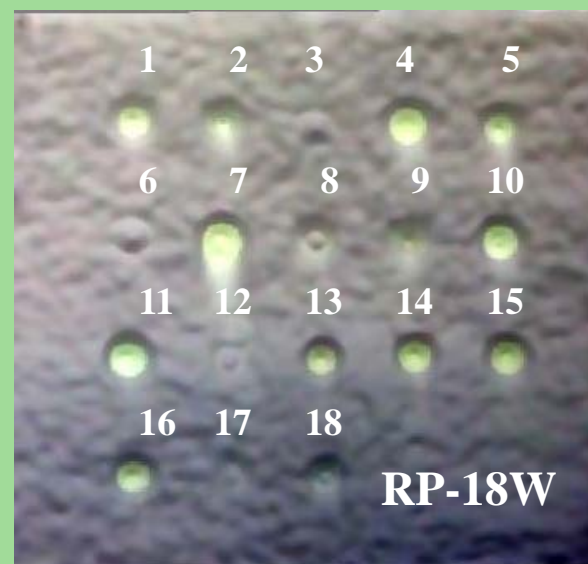
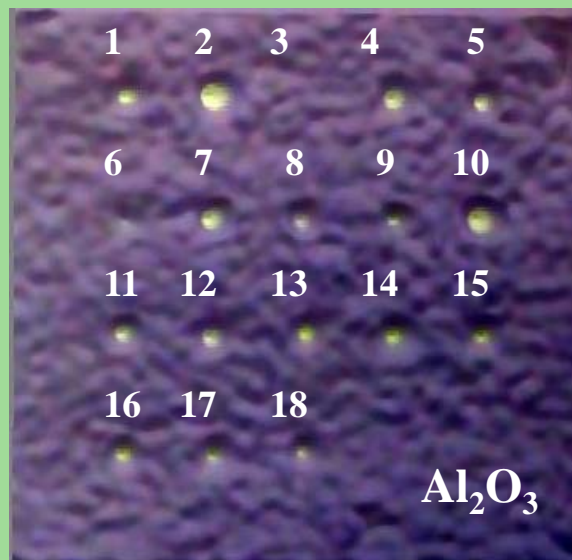
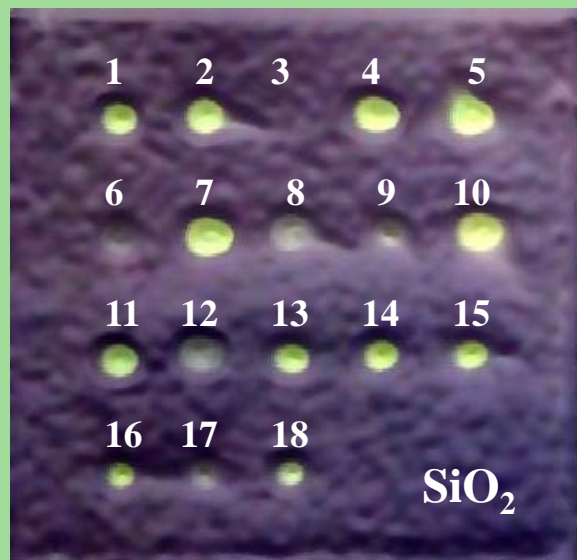
RP- 50% MeOH+H₂O



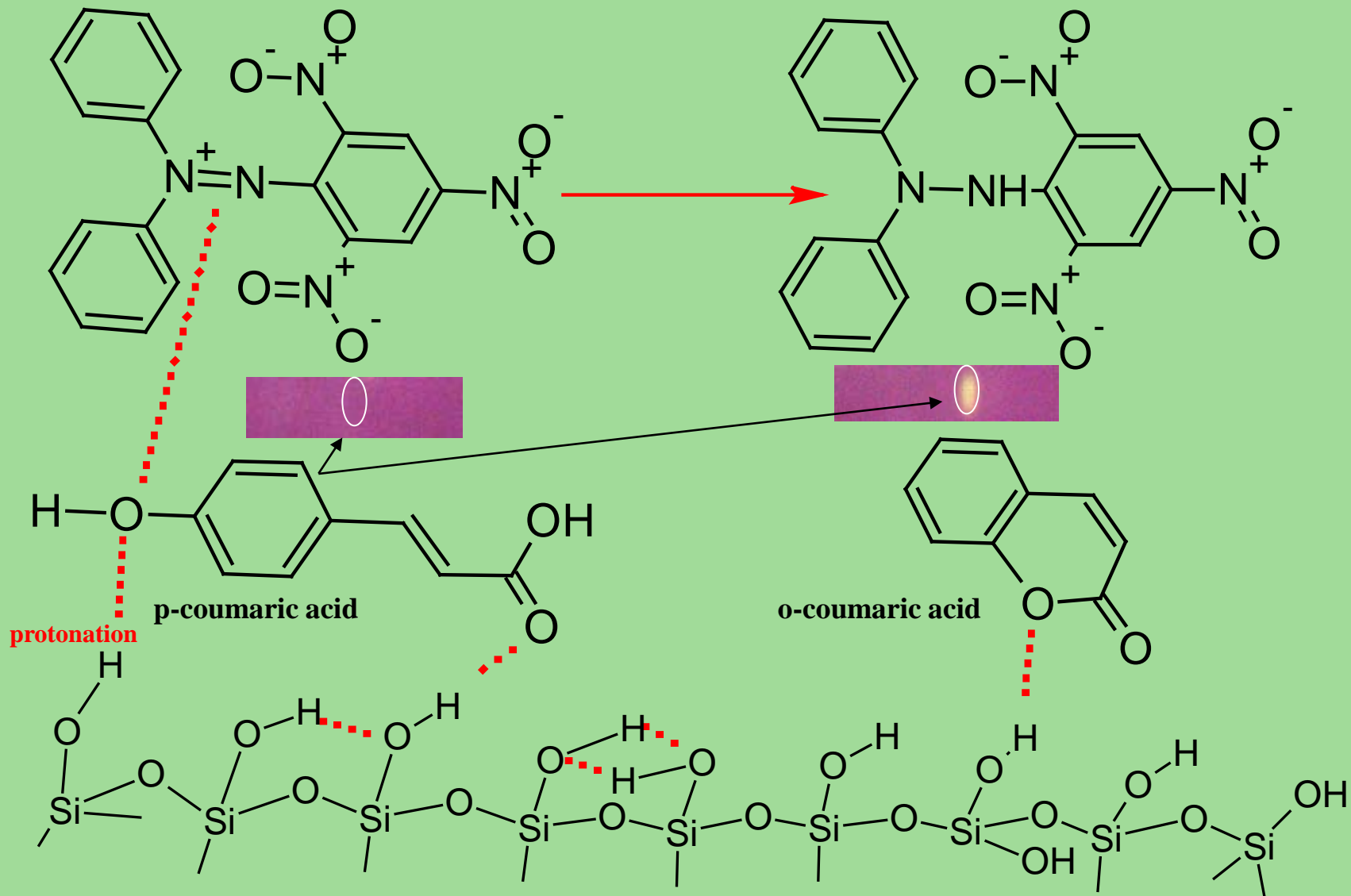
TLC FOR SCREENING NATURAL SAMPLES FOR THE PRESENCE OF ANTIOXIDANTS



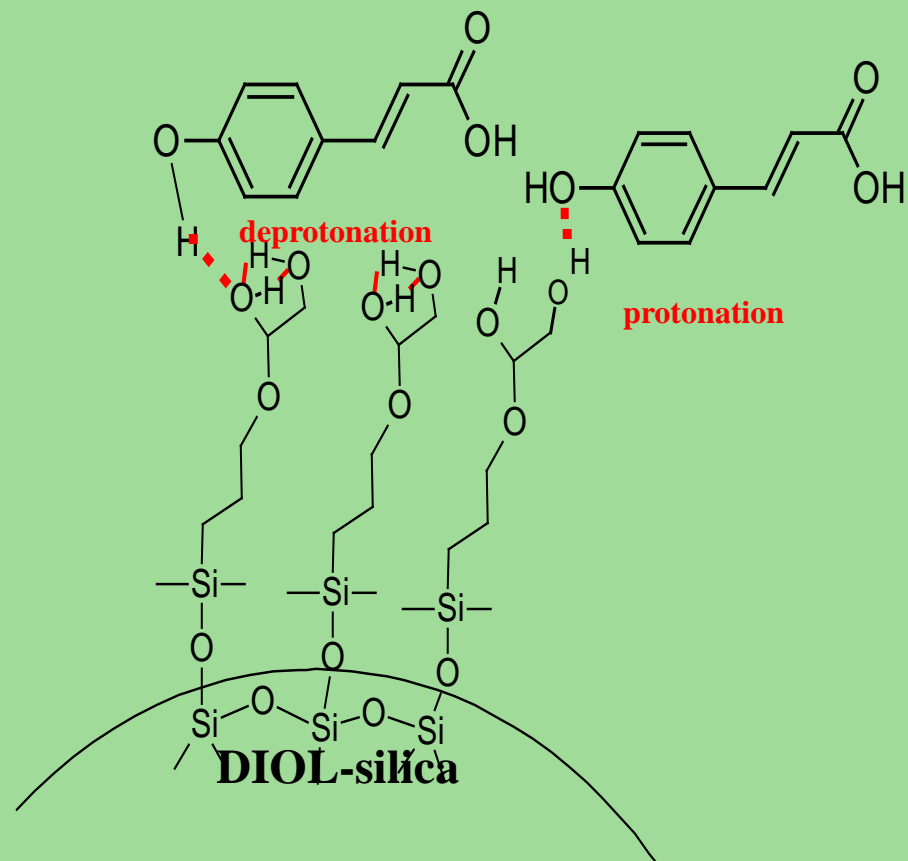
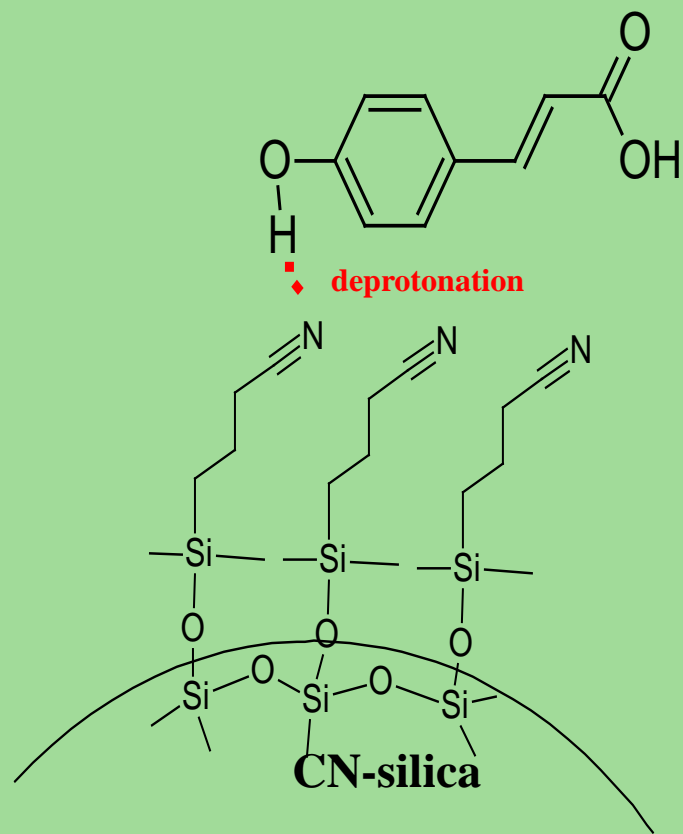
influence of analyte-adsorbent interactions on the results obtained in TLC-DPPH assay



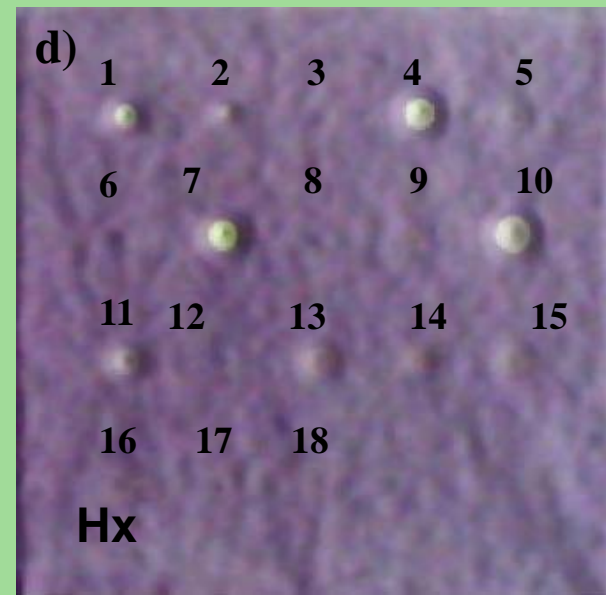
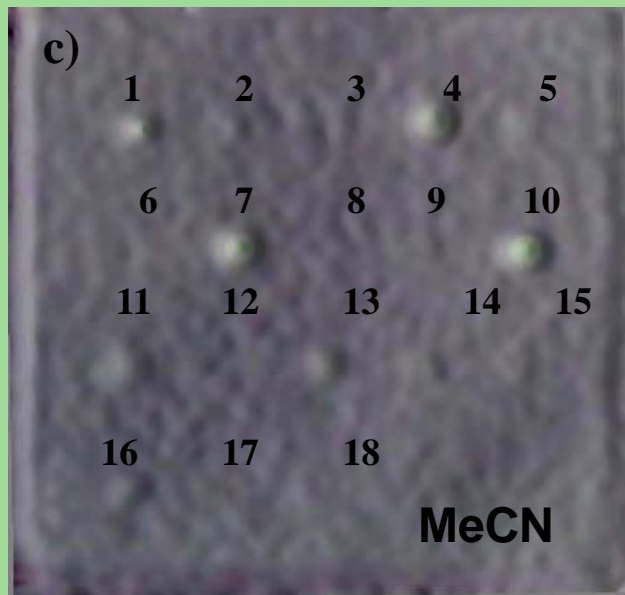
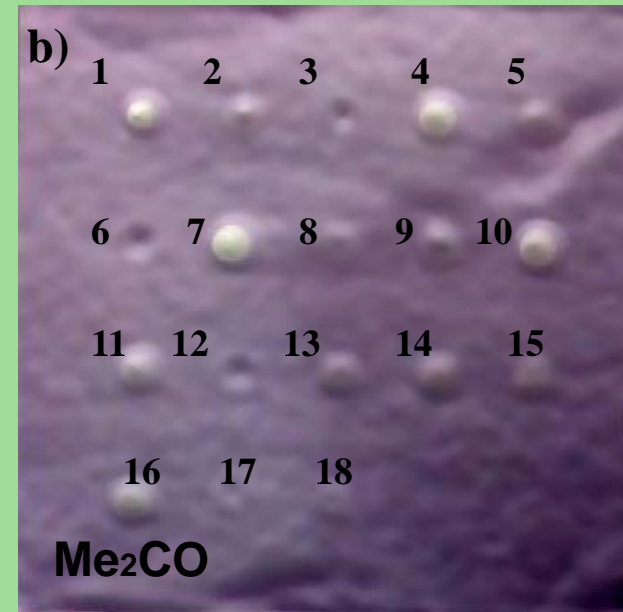
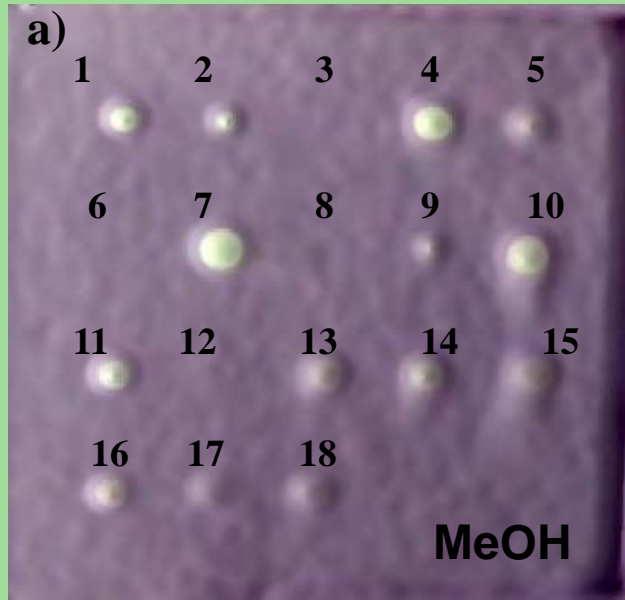
influence of analyte-adsorbent interactions on the results obtained in TLC-DPPH[•] assay



influence of analyte-adsorbent interactions on the results obtained in TLC-DPPH assay



The influence of analyte-solvent interactions on the results obtained in TLC-DPPH assay



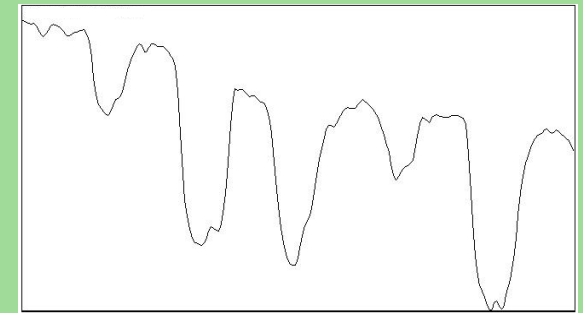
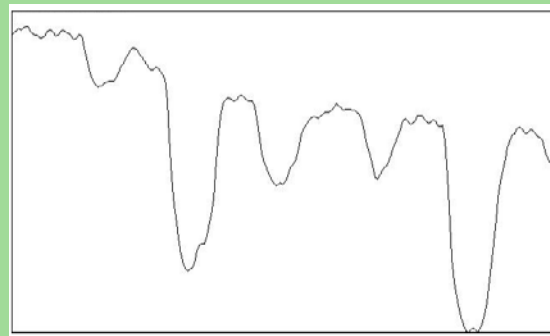
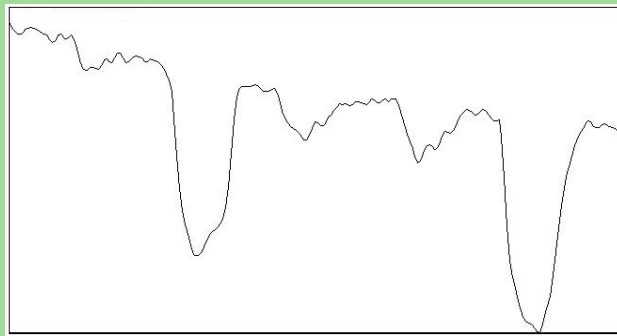
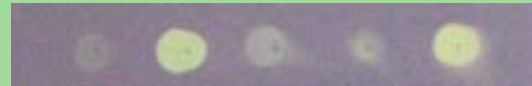
TLC-DPPH „dot-blot” test for selected phenolic compounds

Data comparison by means of ImageJ program

10 min.

15 min.

25 min.



o-Coumaric acid

Gallic acid

p-Coumaric acid

Luteolin

Synapinic acid

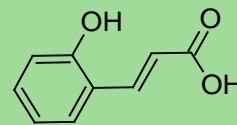
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2

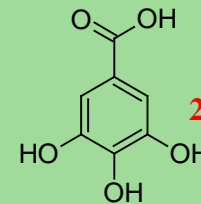
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4

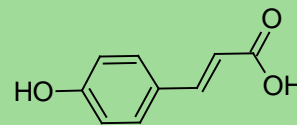
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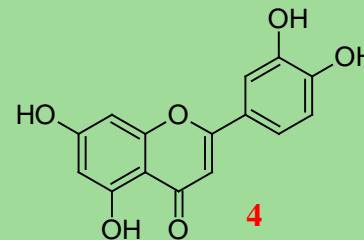
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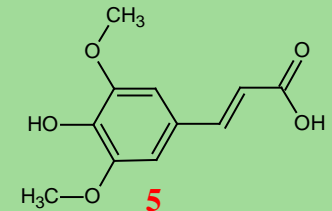
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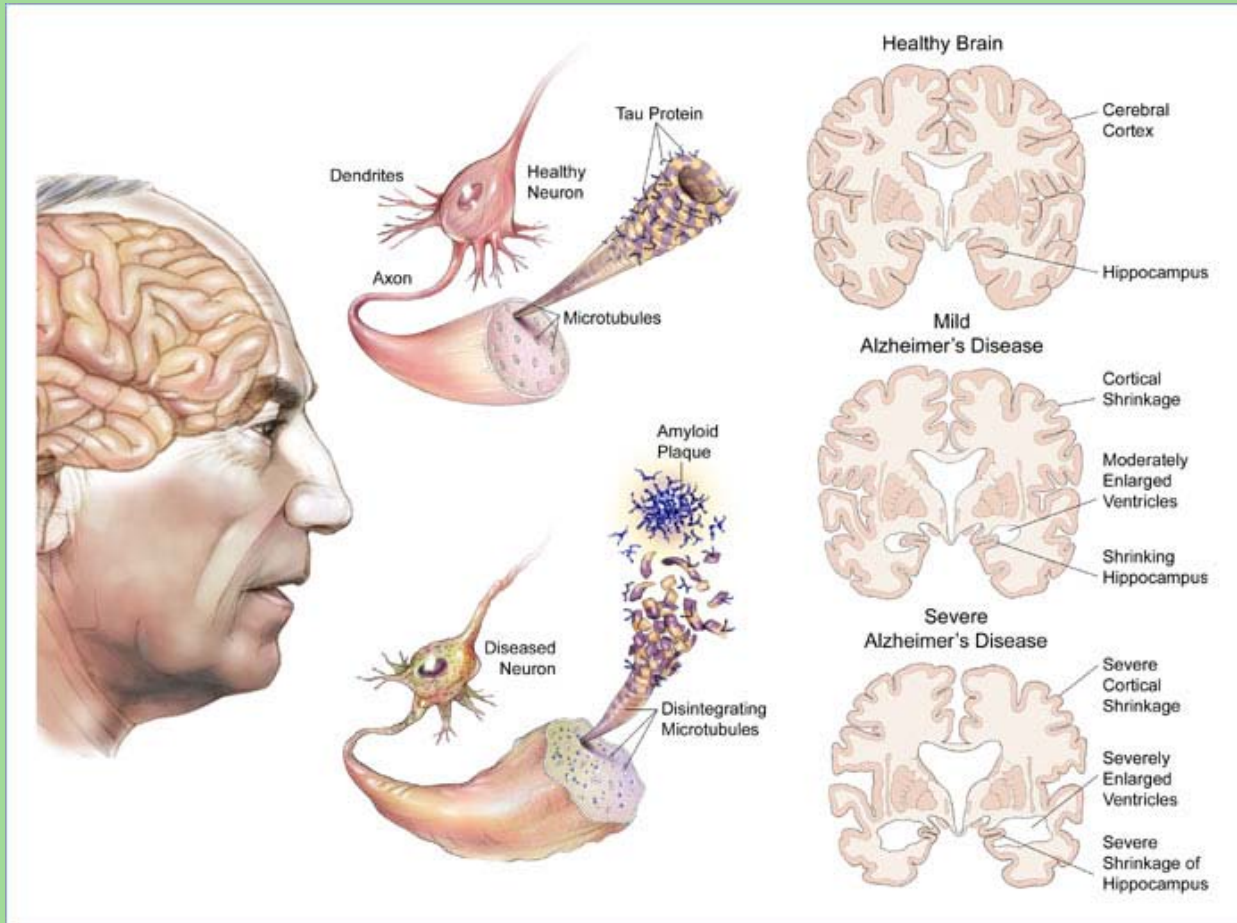


5

Cholinergic theory of AD dementia

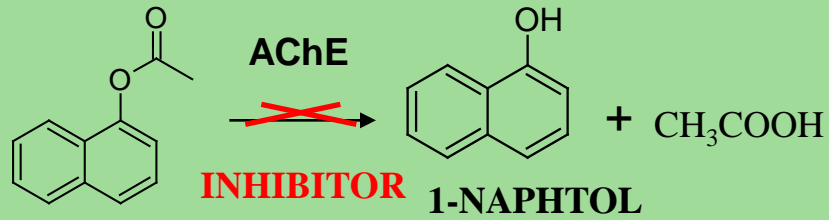
The majority of currently approved drugs are the AChE inhibitors.

GALANTHAMINE, HUPERZINE A are the examples of most recently investigated inhibitors of plant origin



TLC FOR SCREENING NATURAL SAMPLES FOR THE PRESENCE OF ACETYLCHOLINESTERASE INHIBITORS

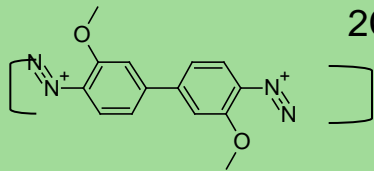
DIAZOTIZATION METHOD



NAPHTYL ACETATE

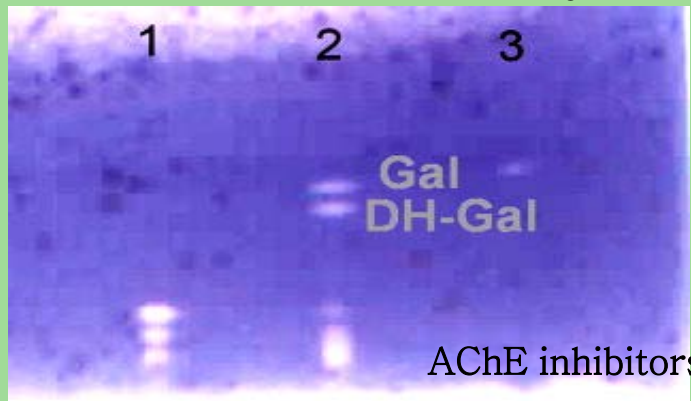
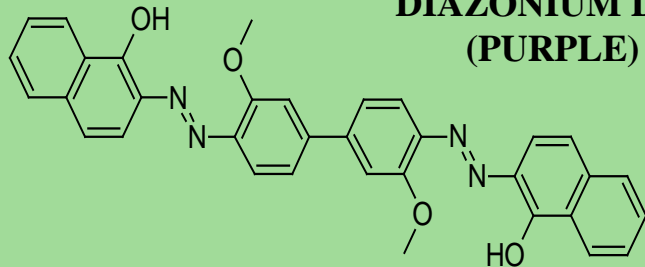
1-NAPHTOL

FAST BLUE B SALT

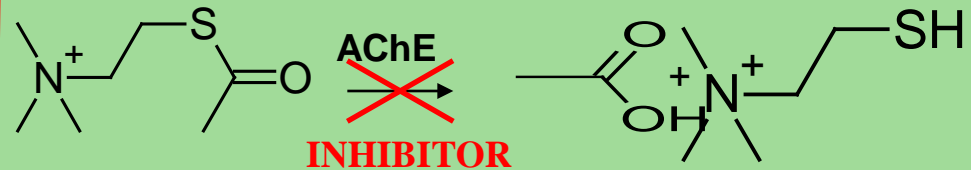


2Cl⁻

DIAZONIUM DYE
(PURPLE)

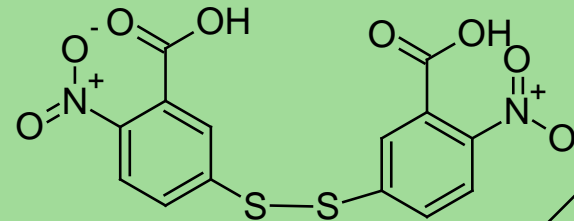


ELLMAN'S METHOD

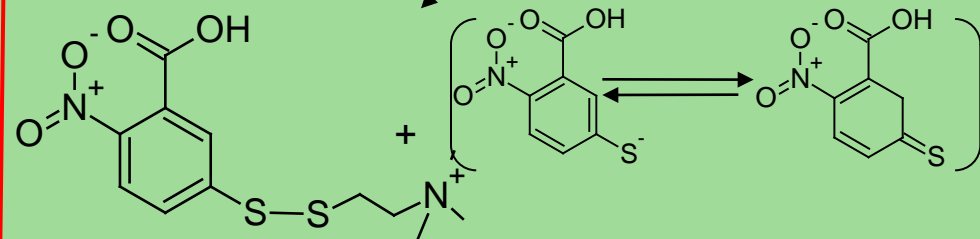


ACETYLTHIOCHOLINE

THIOCHOLINE



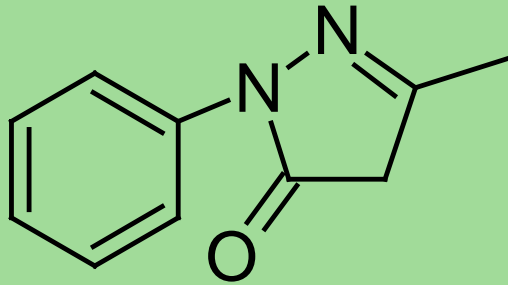
ELLMAN'S REAGENT



YELLOW ION

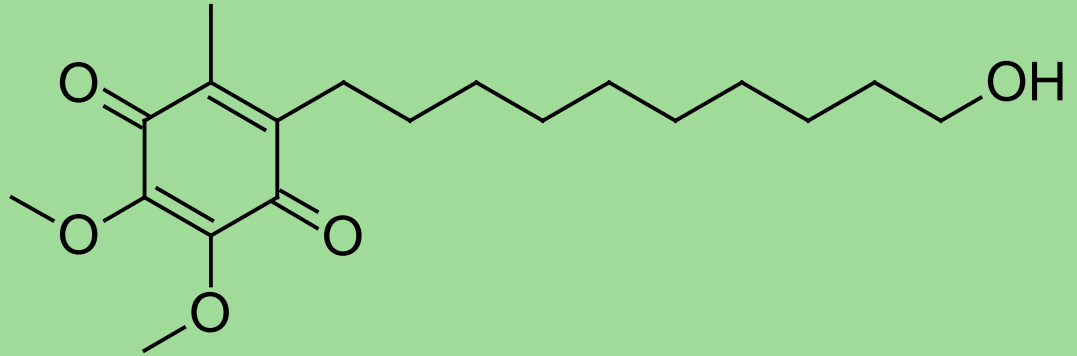
Nonphenolic antioxidant drugs

Edaravone



- * neuroprotective agent
- * aiding neurological recovery following acute brain ischemia and subsequent cerebral infraction
- * a potent antioxidant and strong free radical scavenger

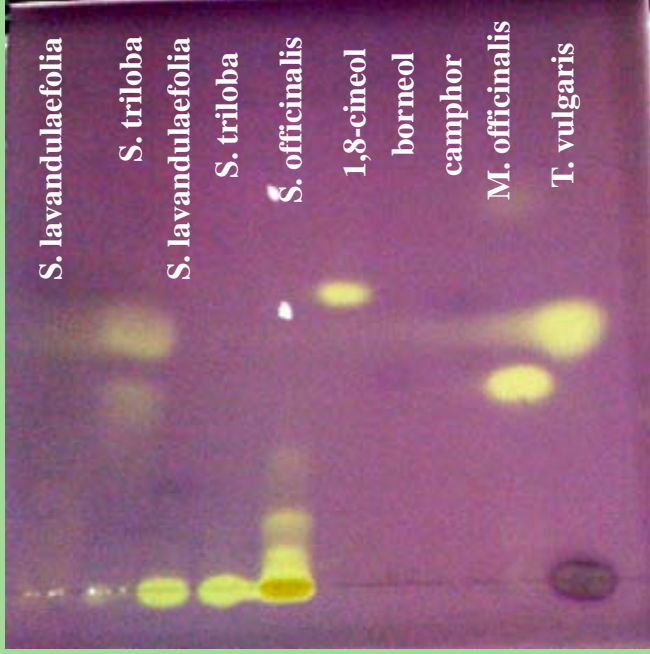
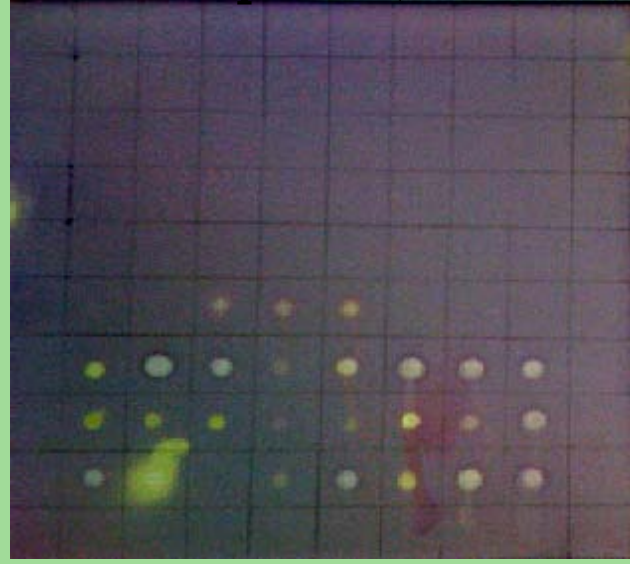
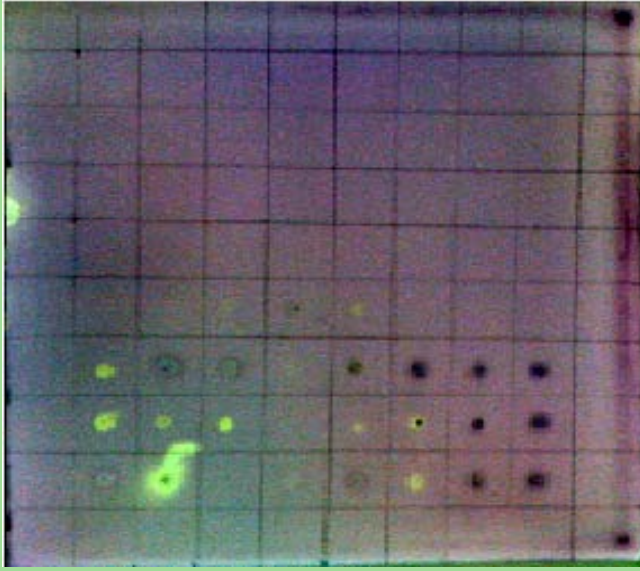
Idebenone



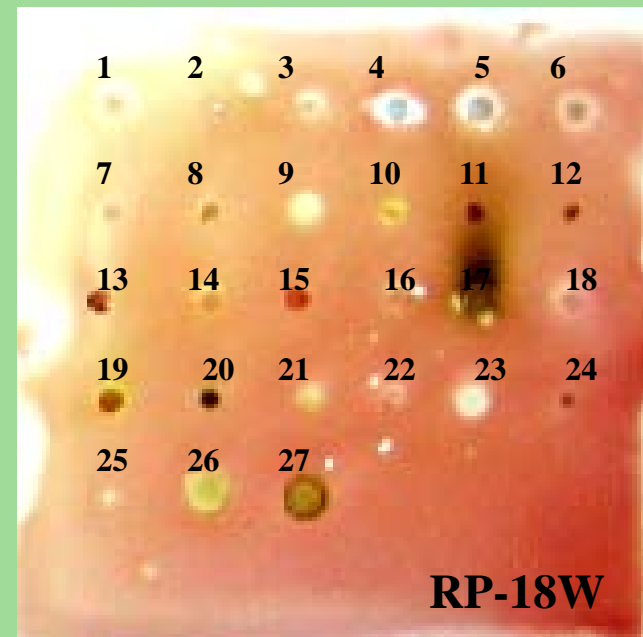
- * treatment of Alzheimer's disease and other cognitive defects
- * treatment of neuromuscular diseases (under investigation)
- * used in topical applications to treat wrinkles

TLC-DPPH test for volatile samples (low-temperature 7

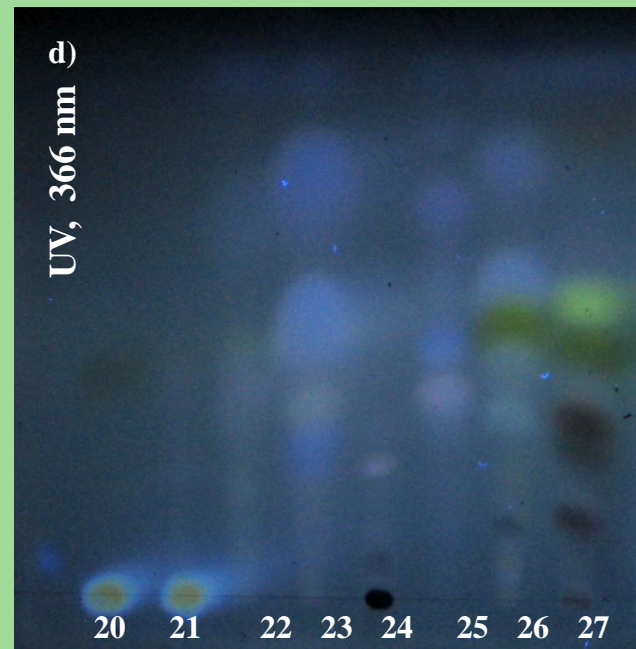
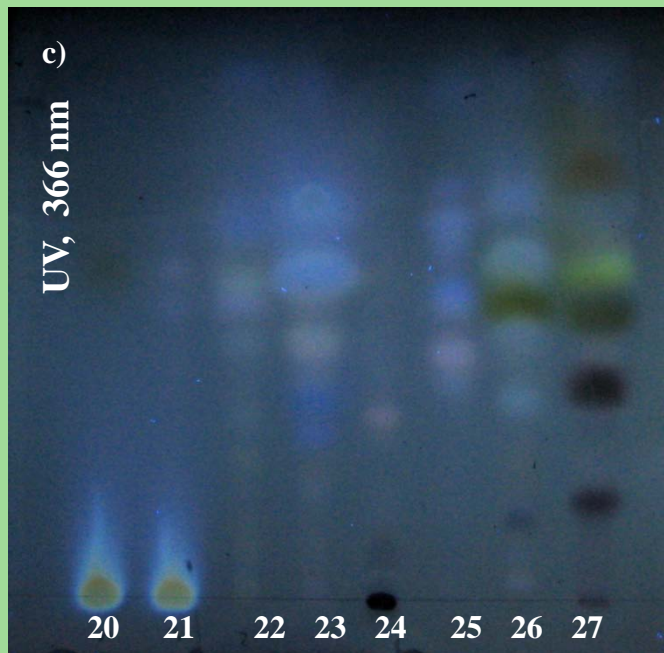
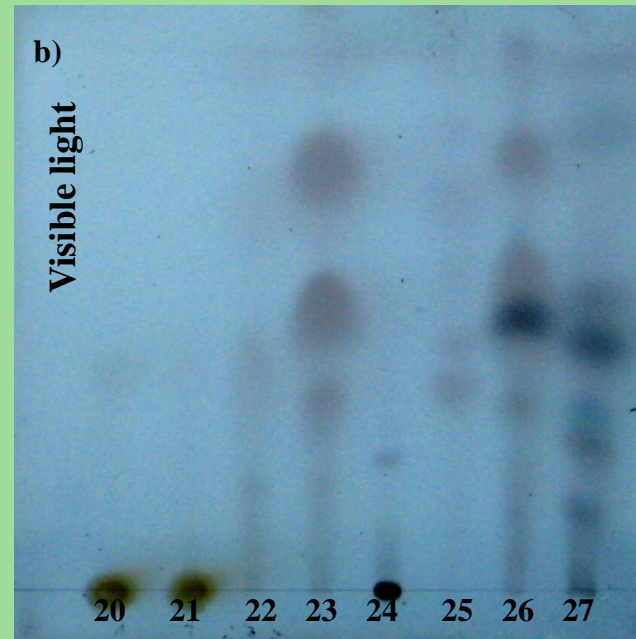
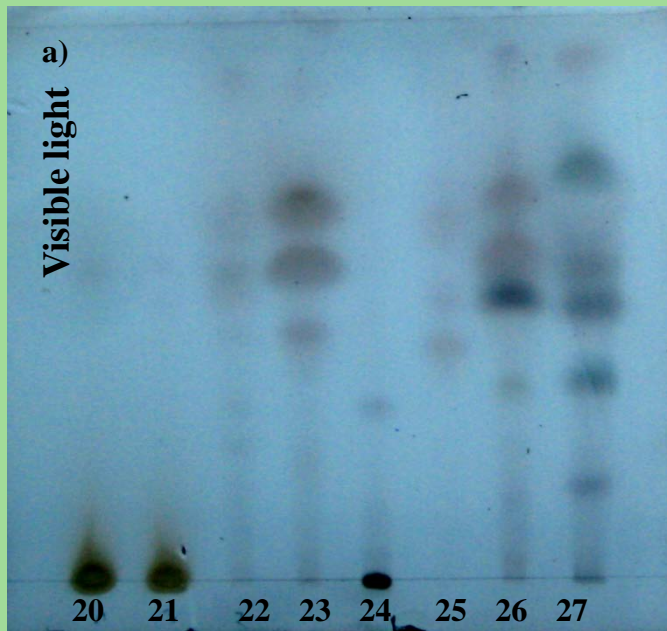
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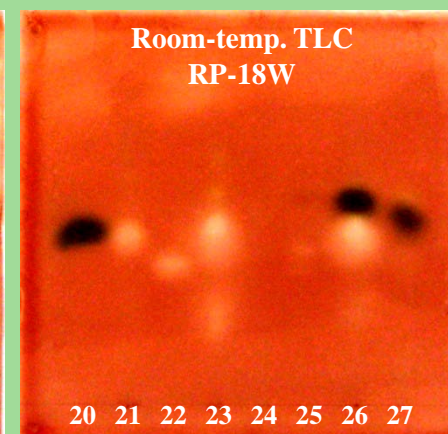
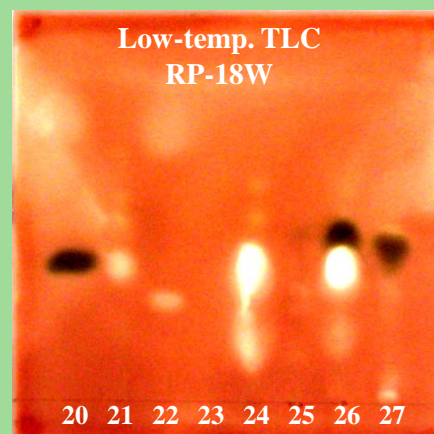
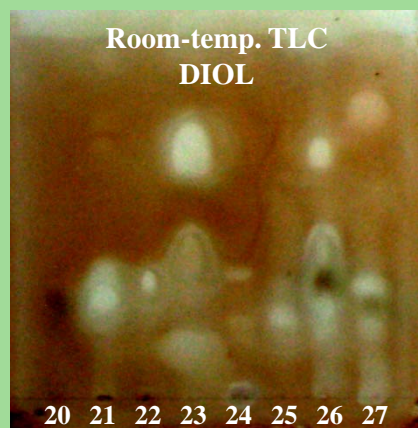
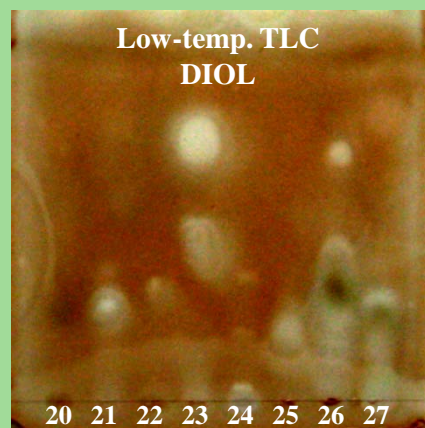
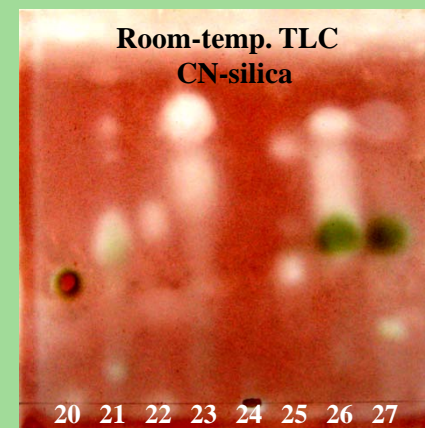
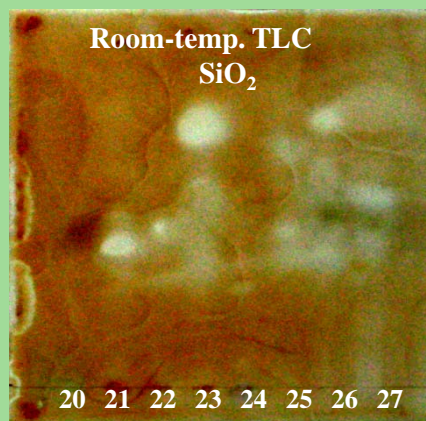
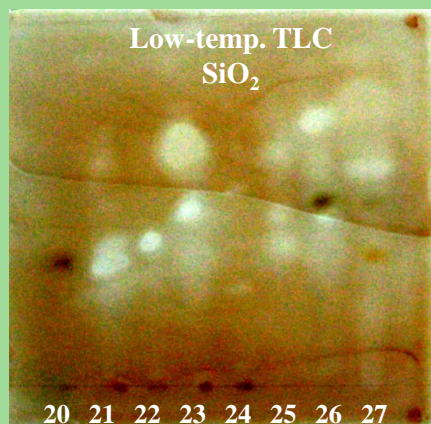
Dot-blot test to detect AChE inhibitors for volatile samples



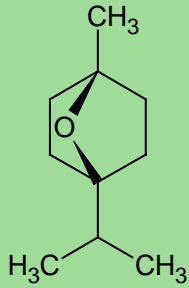
Low temperature TLC for volatile samples



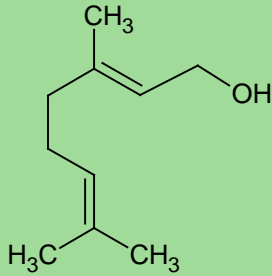
TLC test for identification of AChE inhibitors in volatile sam



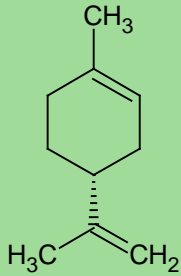
TERPENES



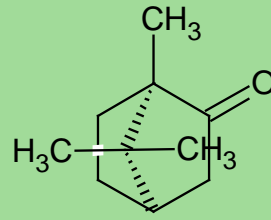
EUCALYPTOL



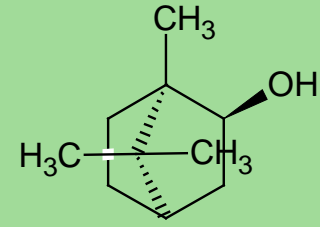
GERANIOL



LIMONENE

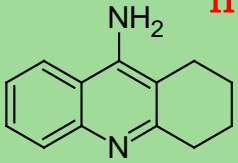


CAMPHOR

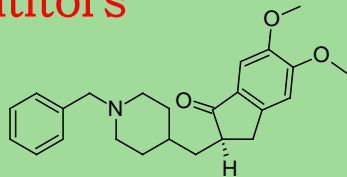


BORNEOL

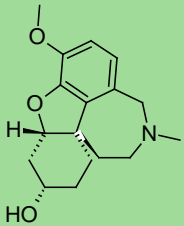
AChE inhibitors



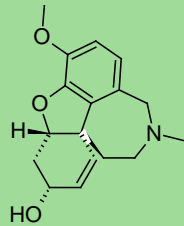
TACRIN



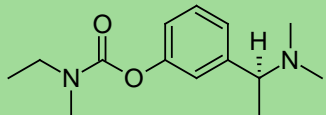
DONEPEZIL



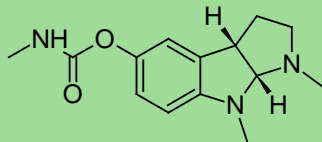
DIHYDROGALANTAMINE



GALANTAMINE

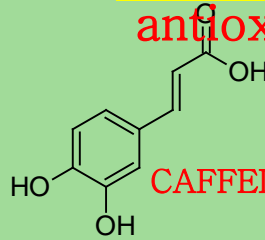


RIVASTIGMINE

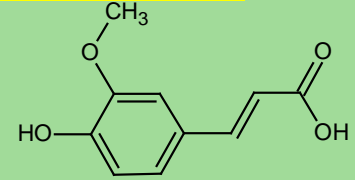


PHYSOSTIGMINE

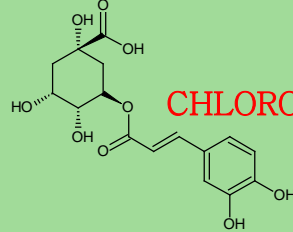
Polyphenolic antioxidants



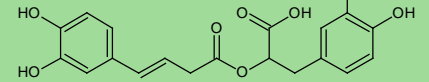
CAFFEIC ACID



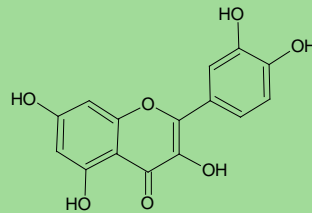
FERULIC ACID



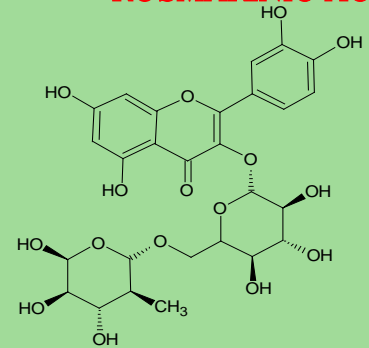
CHLOROGENIC ACID



ROSMARINIC ACID



QUERCETIN



RUTIN

Terpenes as potential drugs to treat Alzheimer's disease

- * this group of natural compounds is characterized with very interesting properties – both antioxidant and anticholinesterase, that are relevant to the treatment of Alzheimer's disease,
- * low molecular lipophilic compounds that can easily pass through the biological membranes,
- * they are absorbed through the lungs and may exert their activity in the brain,
- * it is assumed that a feature that is also associated with AChE inhibition is a hydrophobic ligand,
- * the major interaction of the enzyme–inhibitor complexes are due to hydrophobic and cation– π interactions; monoterpenes due to the presence of hydrocarbon skeleton may possess anticholinesterase activity,

**TKANK YOU
FOR YOUR
KIND
ATTENTION**