

Bioassays as a detection tool for thin layer chromatography

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Bioassays (biological activity tests) are screening or semi-quantitative methods, which are based on a measurement of an effect emerging in a given biological system as a result of biological action of the substances under investigation. Bio-detection enables searching for biologically active substances in complicated mixtures, what can be described as effect-directed analysis (EDA).

Biological activity tests are often hyphenated with separation methods, mostly with thin layer chromatography. The method is ideal for bioassays due to an open layer, which enables evaporation of solvents used as mobile phase components. The additional advantage of TLC is possibility of analyzing many samples in the same run.

Spectroscopic methods connected directly with TLC-bioassays can be used as confirmation methods giving full information about analytes.

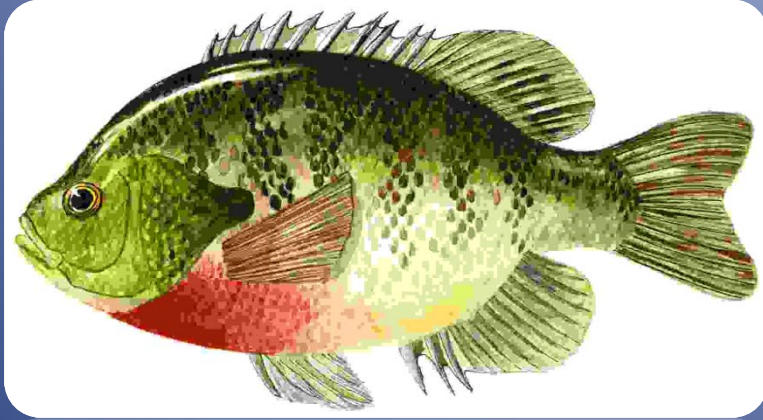




algae



daphnia



fish



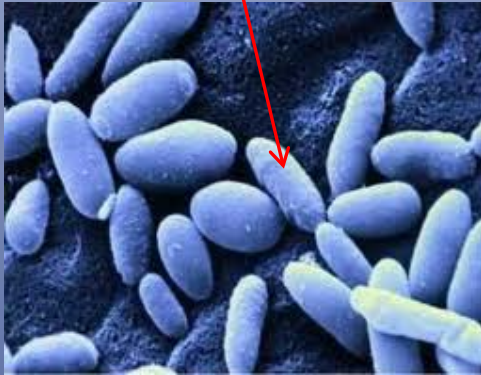
plants



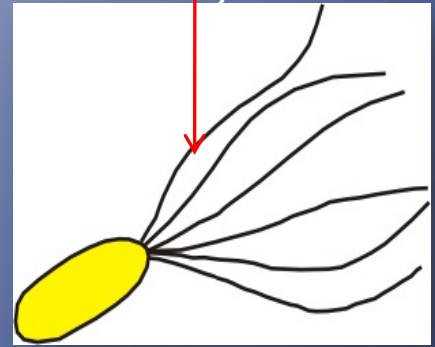
Cladosporium cucumerinum



Candida albicans



Vibrio fischeri



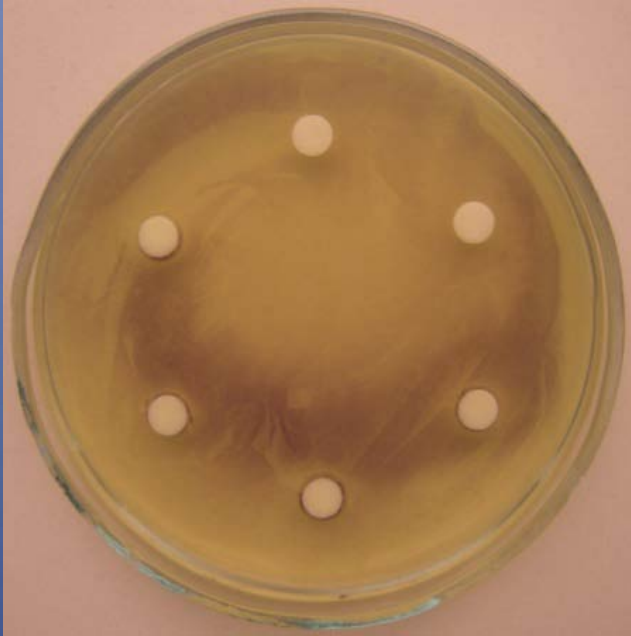
Escherichia coli



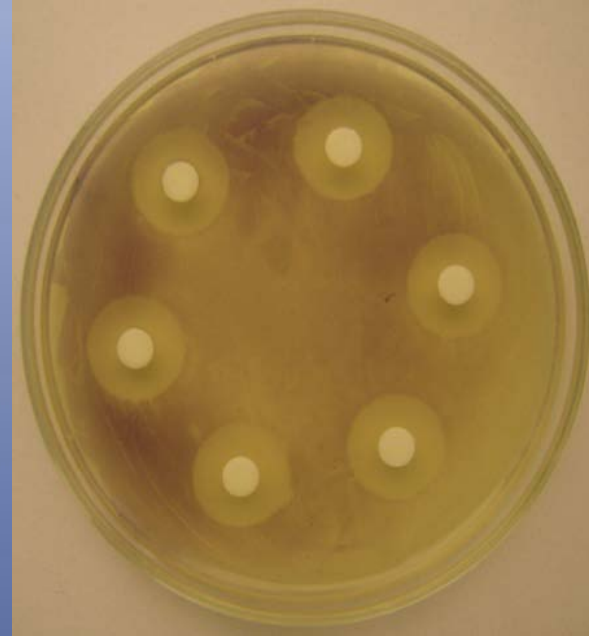
Frequently used tests

1. Mutagenicity and genotoxicity tests (Ames test, COMET, SOS - Chromotest, Umu-test, Mutatox).
2. Estrogenic activity (Yes test).
3. Enzymatic tests (acetylcholinesterase inhibition test).
4. Aryl Ah receptor binding (CALUX, EROD)
5. Antioxidant and radical scavenging activity tests (DPPH, ABTS)
6. **Microbial tests: dilution, diffusion and bioautographic.**

Disc test *E. coli* (ATCC 25922)



Flumequine - 0,05 µg per disc

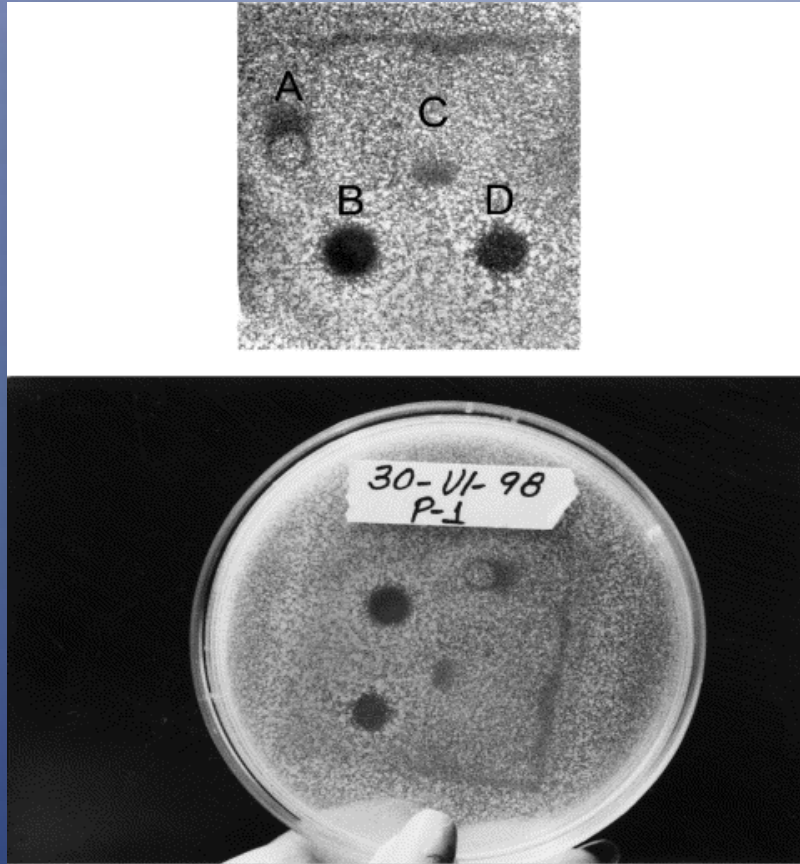


Flumequine - 5,0 µg per disc

Bioautography :

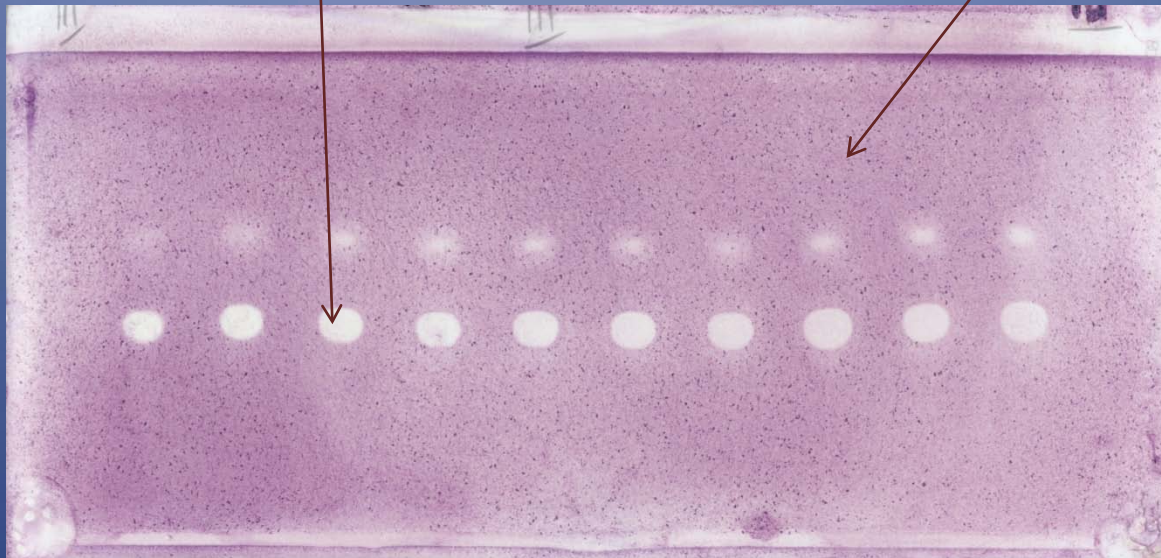
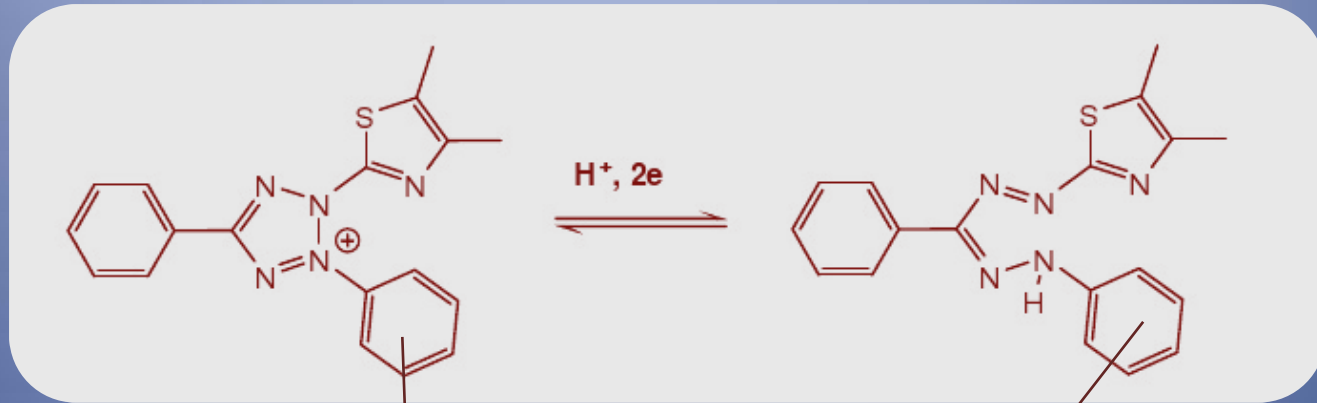
- Contact bioautography (agar diffusion),
- Immersion bioautography (agar-overlay),
- Direct bioautography – TLC-DB.

Contact bioautography

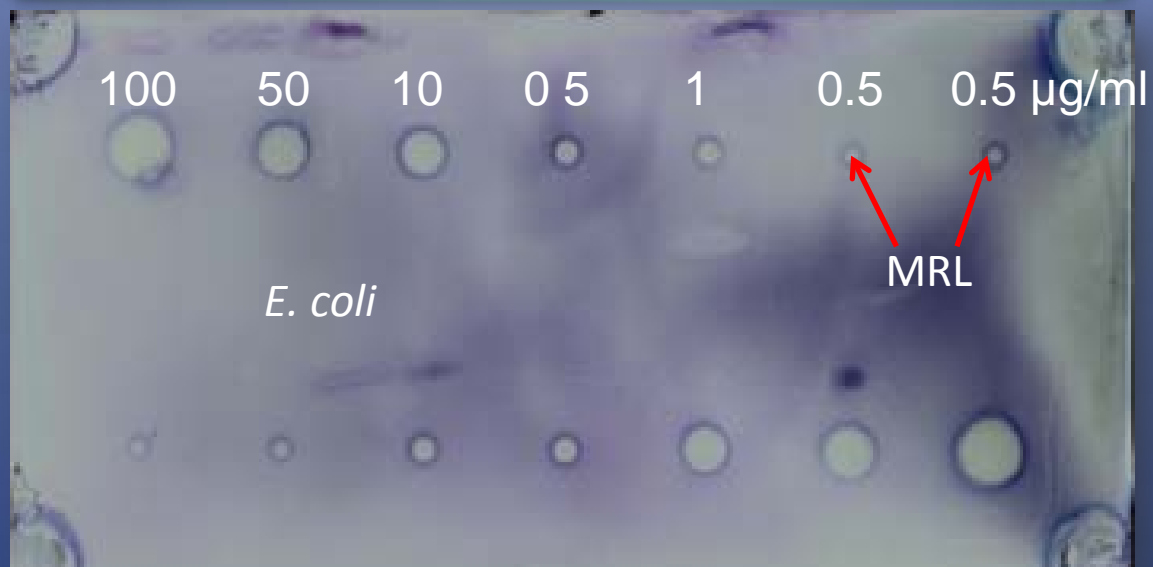


Direct bioautography

MTT



E.coli, cipro- and enrofloxacin

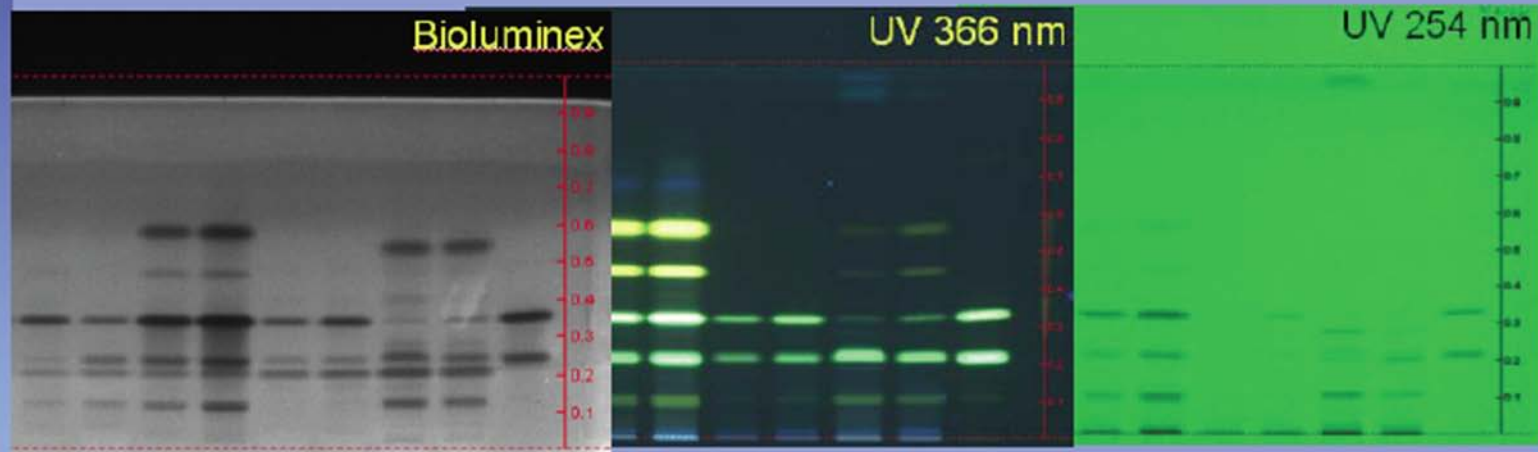


TLC-DB: standards of flumequine applied at 10 μl

Hyphenations:

- UV-VIS
- MS
- IR
- NMR

Natural Product Extracts



Track Assignment:

1-2: Mahonia stem

3-4: Golden Thread

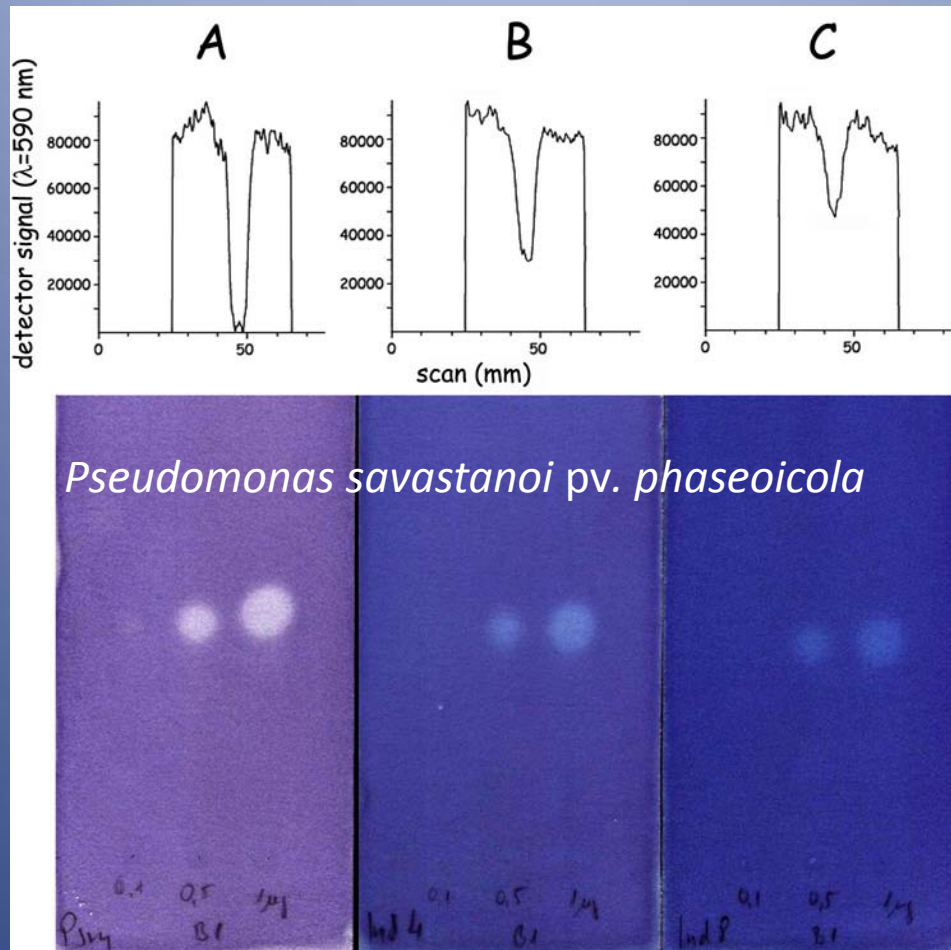
5-6: Chinese Cork Tree

7-8: Tinospora root

9: Palmatine, Berberine

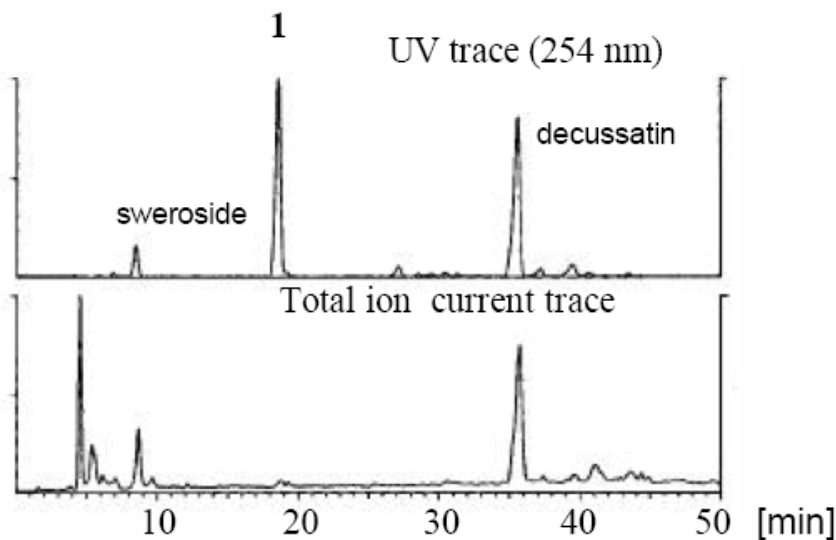
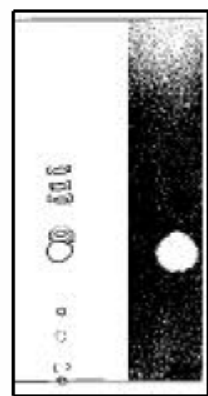
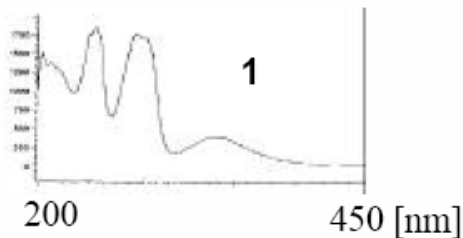
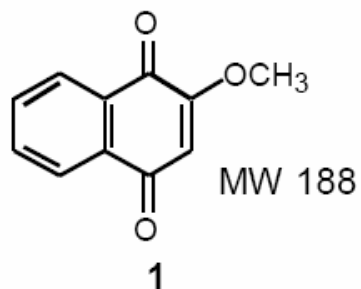
Additional zone (Rf 0.2) detected with Bioluminescence assay

TLC-DB BioArena, UV-VIS



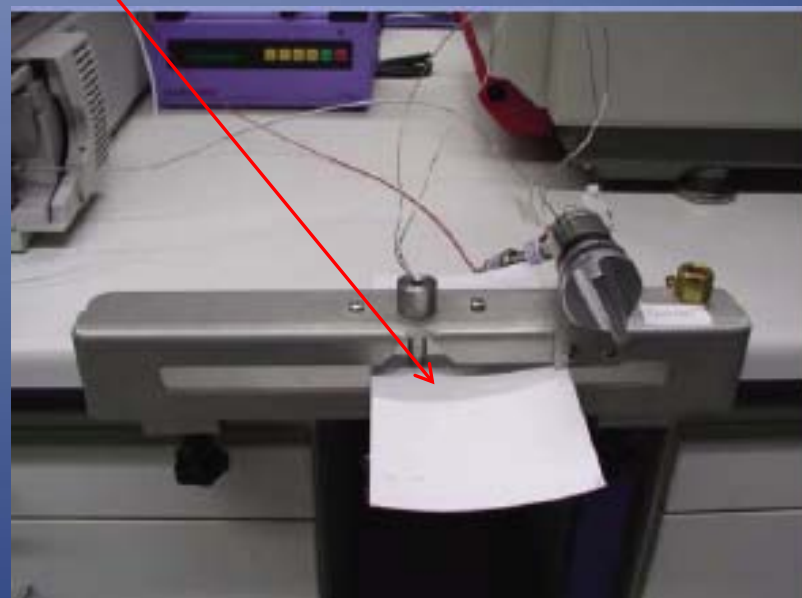
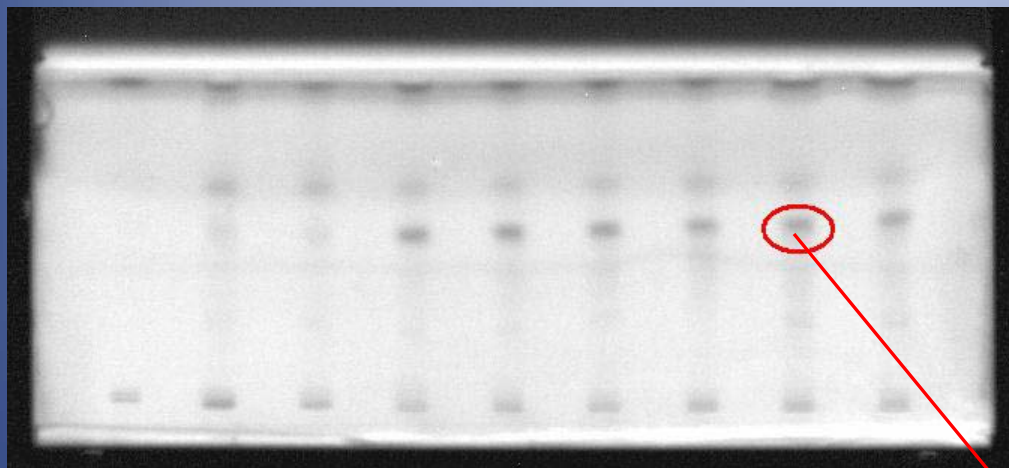
The influence of indigo carmine on antibacterial activity of aflatoxine B₁. A – control plate, B, C – 0.2, 0.4 mg/ml of indigo in a broth.

TLC-DB, HPLC-UV, HPLC-MS

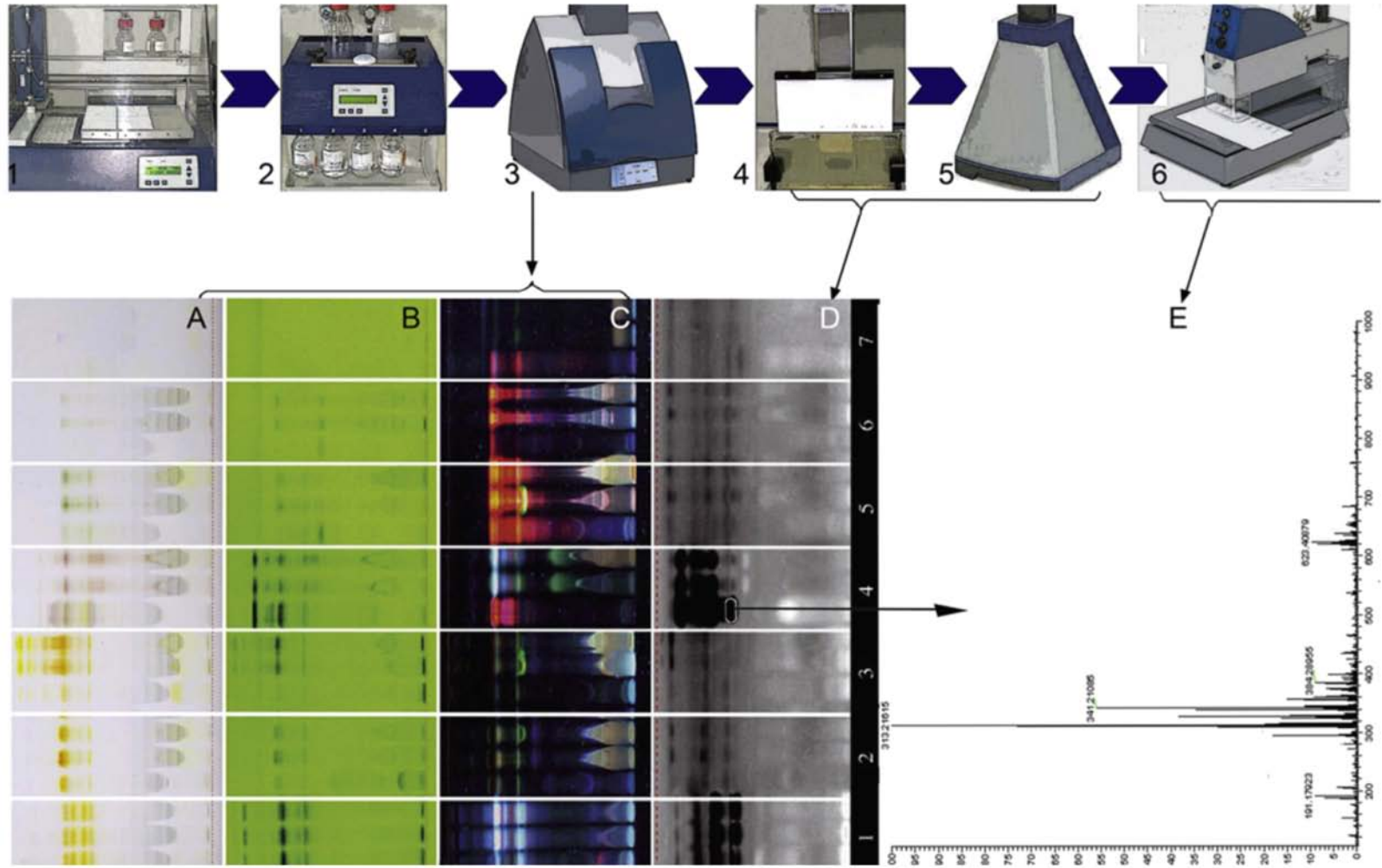


Unknown substance active against *Cladosporium cucumerinum* in *Swertia calycina* plant

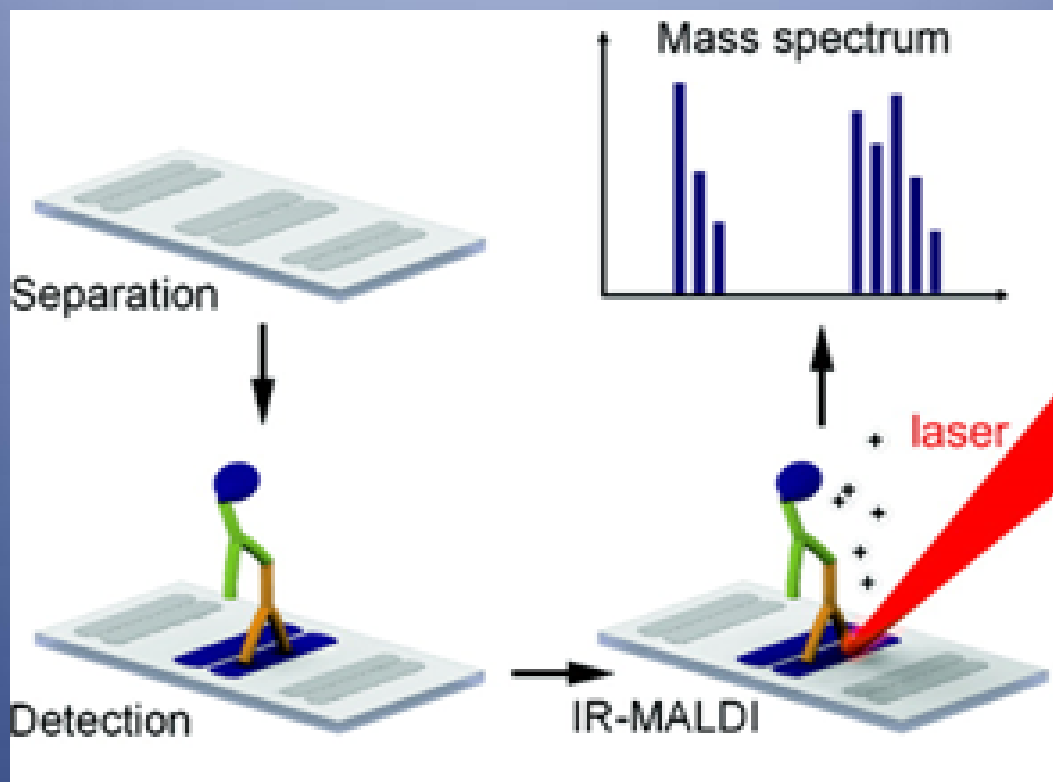
TLC – bioluminescence / MS



HPTLC-UV/Vis/FLD-bioassay-HPLC-HRMS

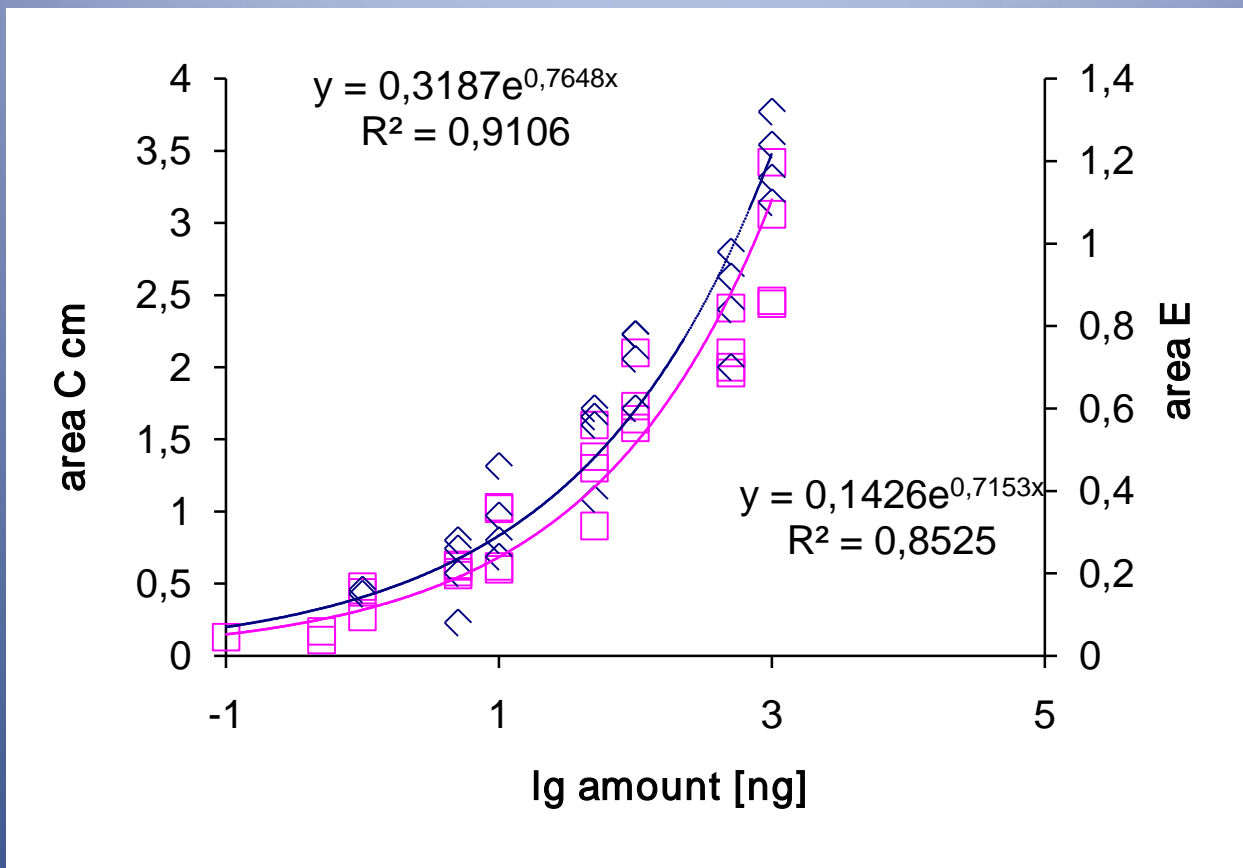


TLC Overlay Binding Assay / IR-MALDI-o-TOF MS

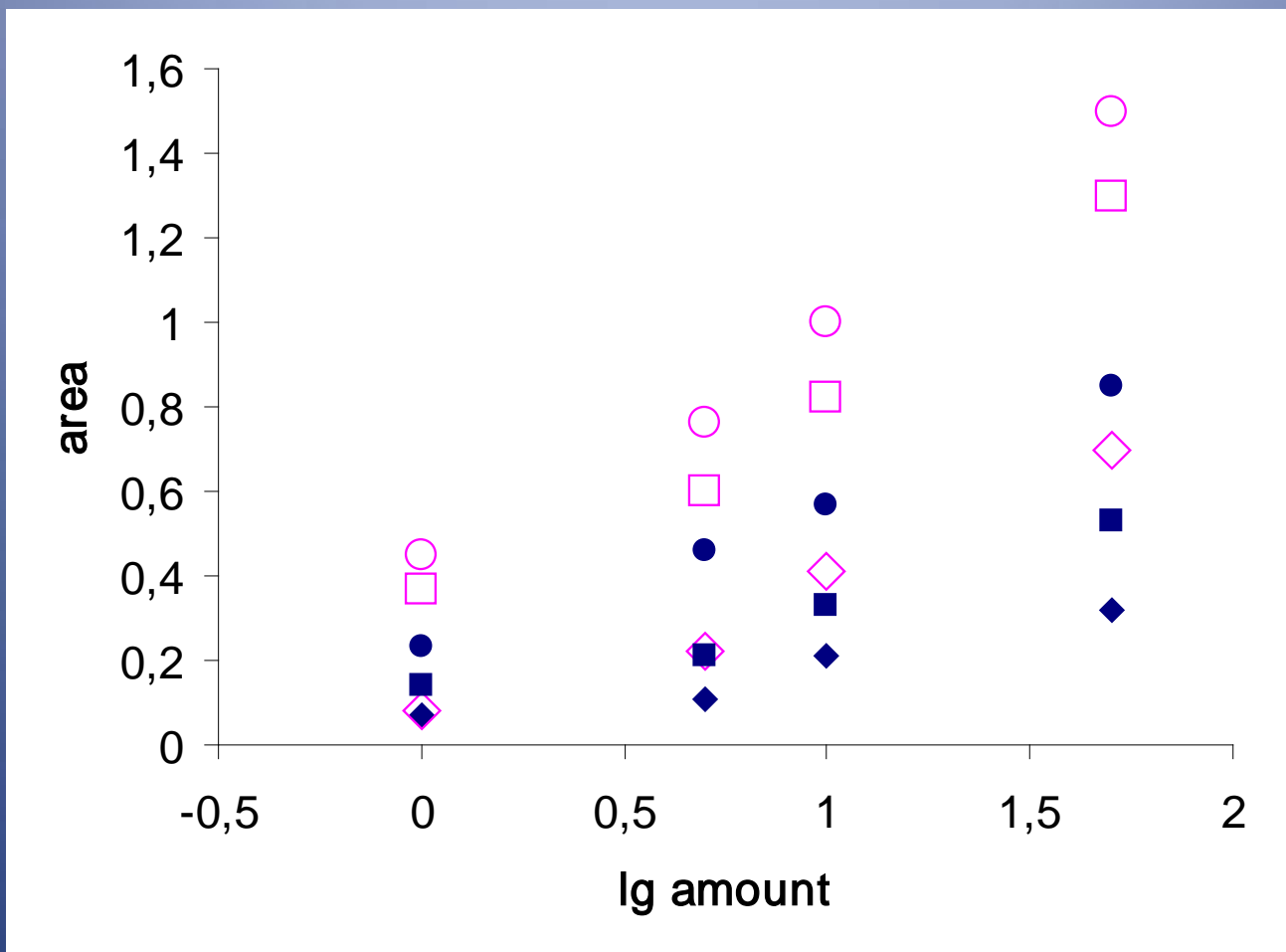


Results



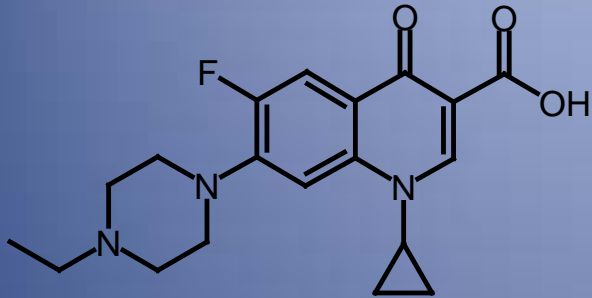


Exponential calibration curves: rhombus – enrofloxacin, square – ciprofloxacin. The data from four bioautograms.



Rhombus – 1, square – 10 , circle – 50 µl of enro- and ciprofloxacin.

Antibiotics residues in milk

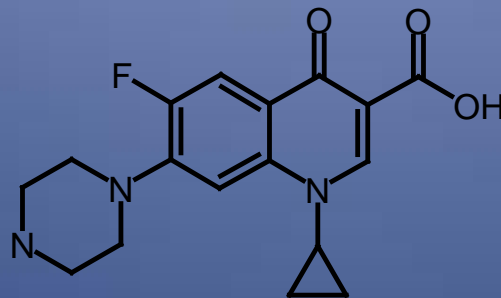


enrofloxacin

+

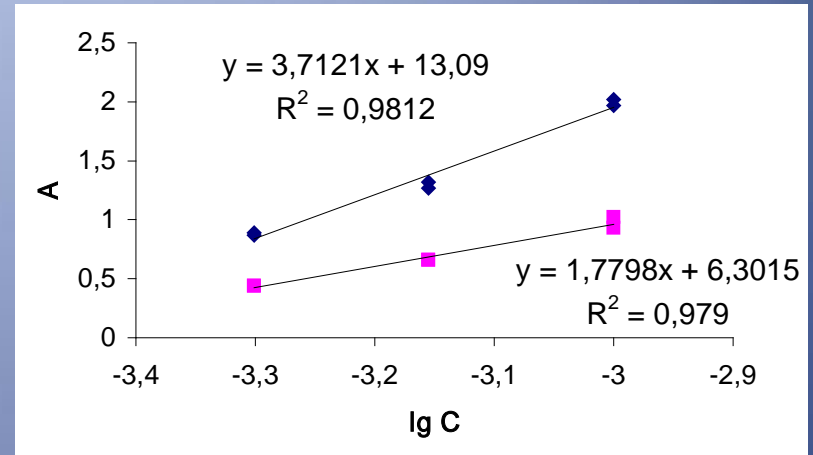
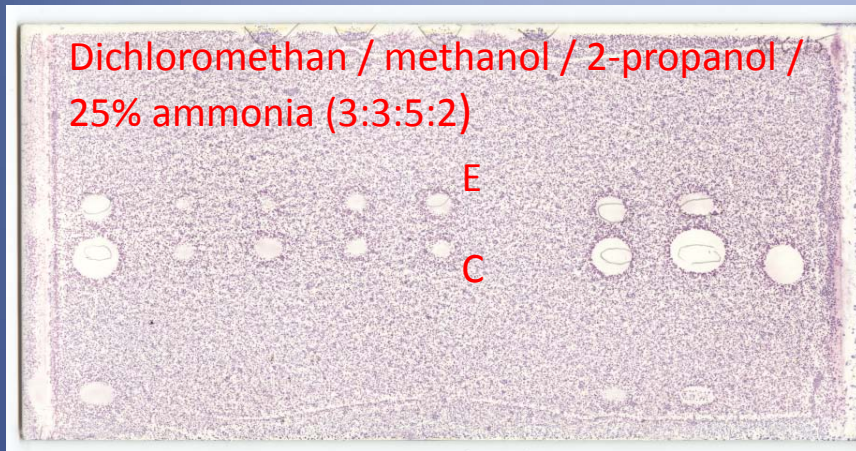


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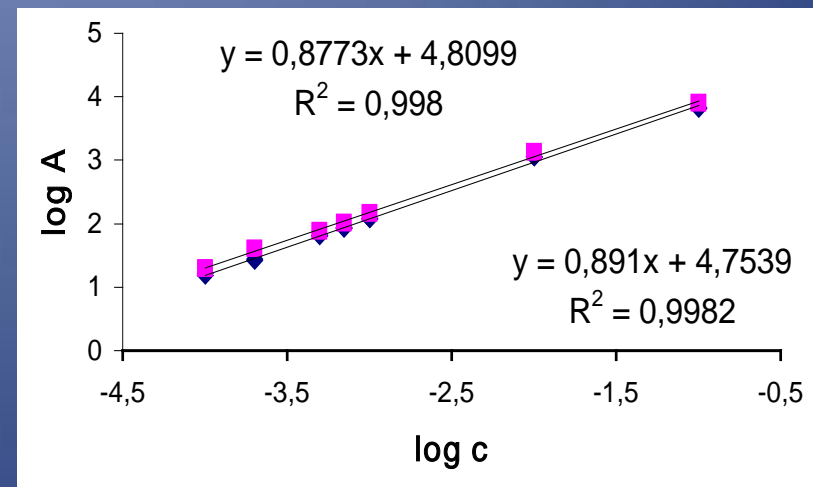
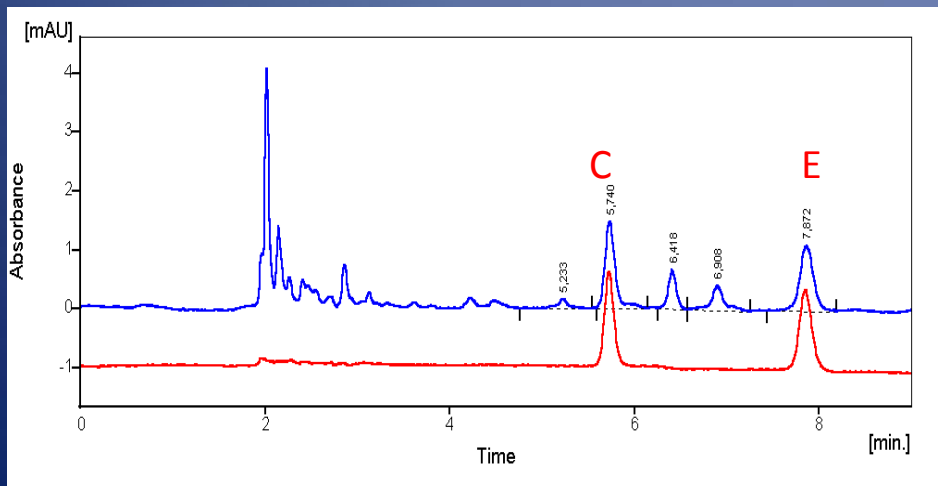


ciprofloxacin

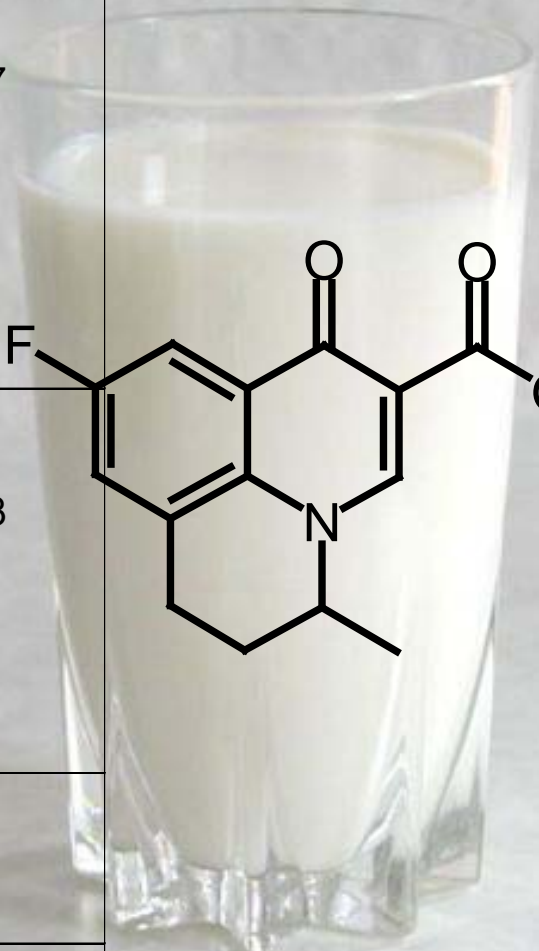
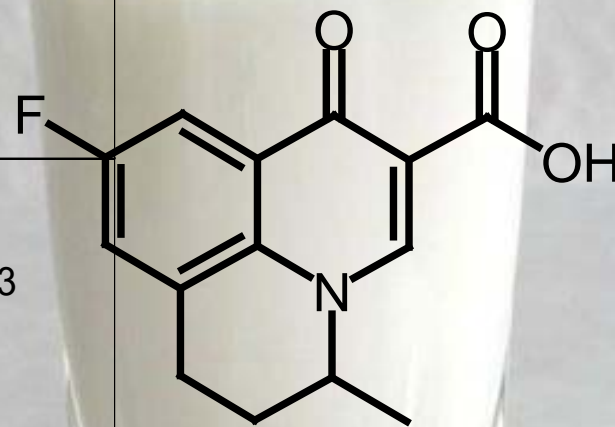
NP TLC DB



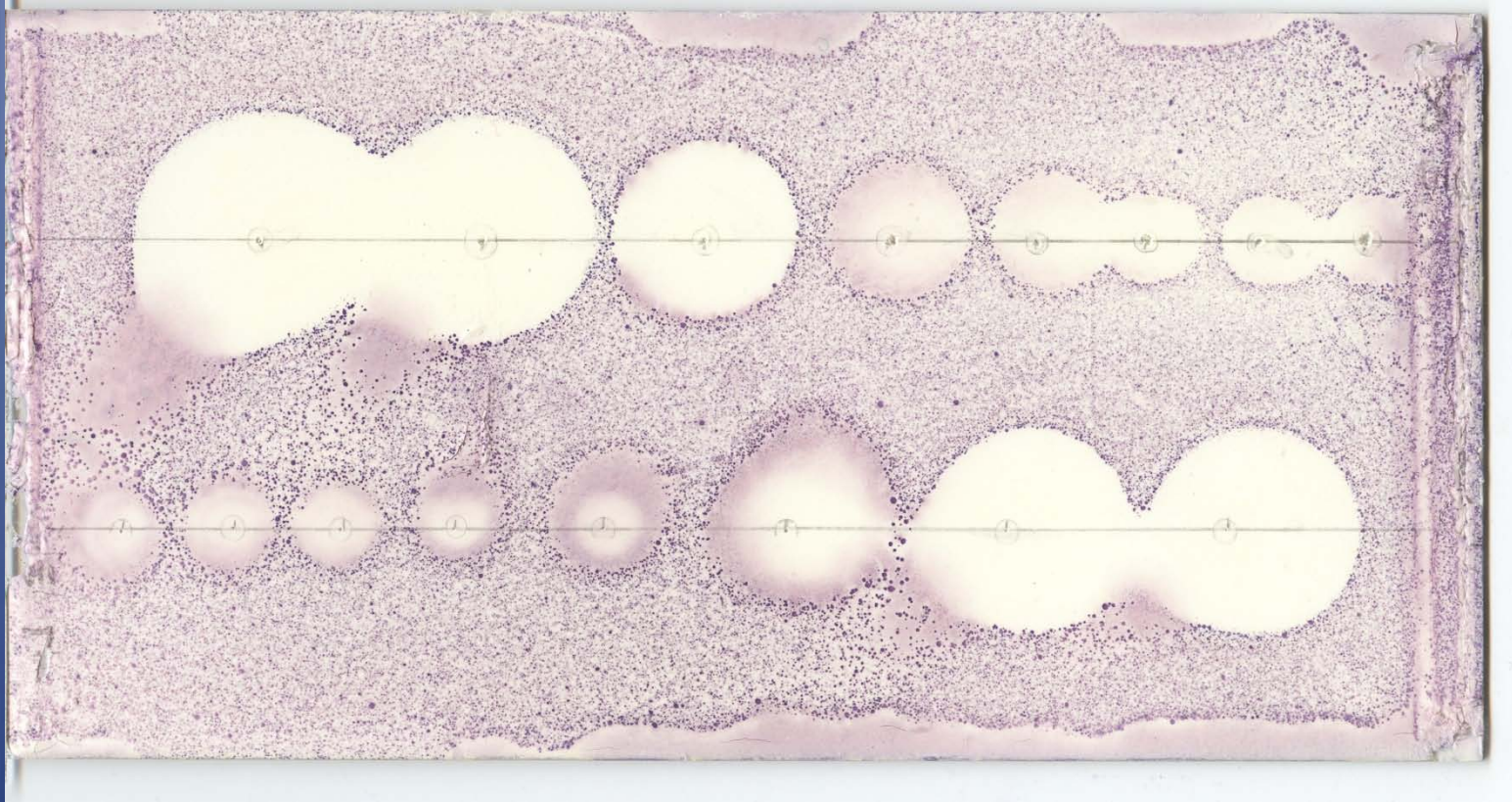
RP HPLC



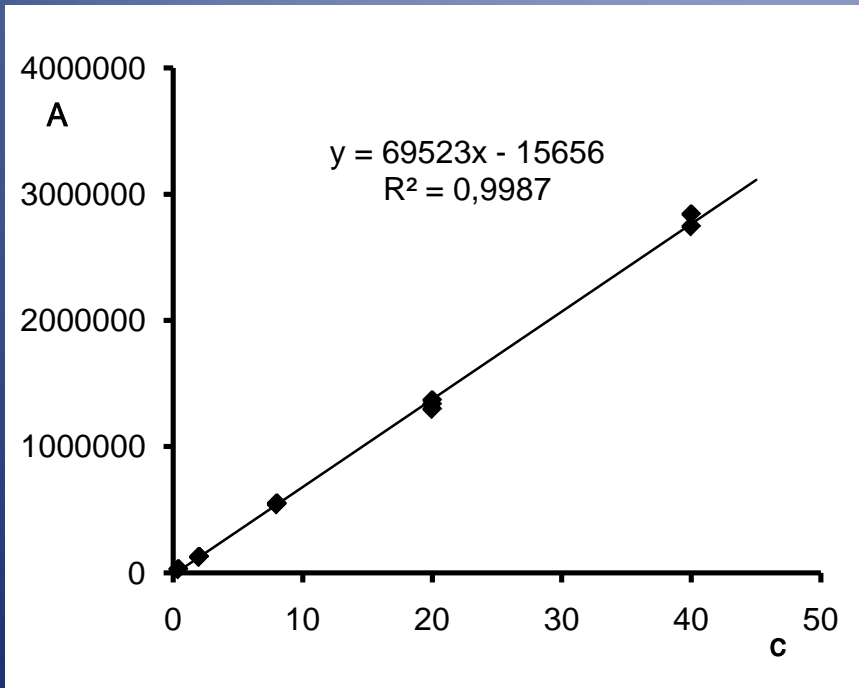
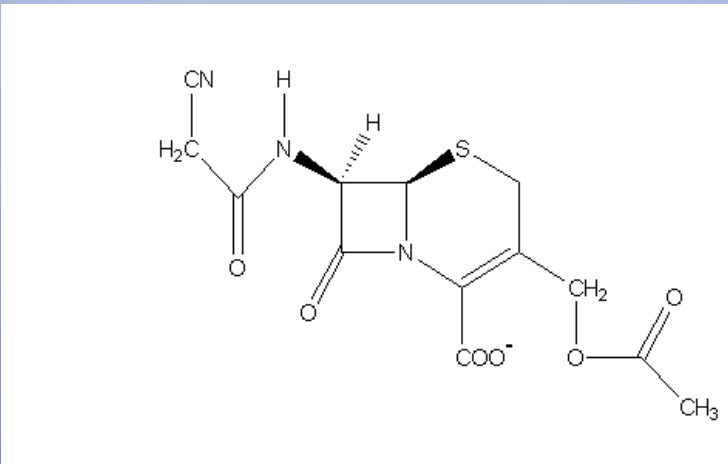
Concentration of flumequine in milk	Mean recovery % MSPD-TLC-DB	Mean of the means% (RSD)
10 ppm	56.02 (1 µl) 82.91 (2 µl) 66.88 (3 µl) 77.19 (4 µl) 64.75 (5 µl) 74.07 (1 µl) 79.44 (2 µl) 91.38 (3 µl) 83.74 (5 µl)	75.15±10.97 (14.6)
1 ppm	43.68 (5 µl) 83.77 (10 µl) 81.75 (15 µl) 49.29 (5 µl) 84.34 (10 µl) 89.0 (15 µl) 91.94 (20 µl)	74.82±19.73 (26.36)
0.1 ppm	113.0 (50 µl) 39.77 (50 µl)	76.0
0.05 ppm	70.0 (50 µl) 88.0 (50 µl)	79.0



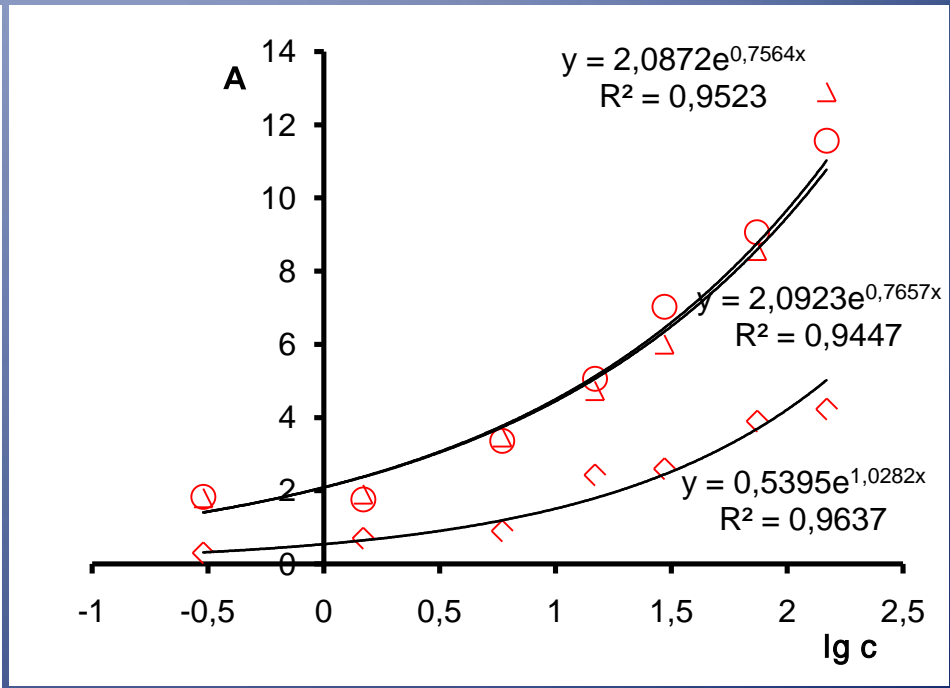
Cefacetril in milk



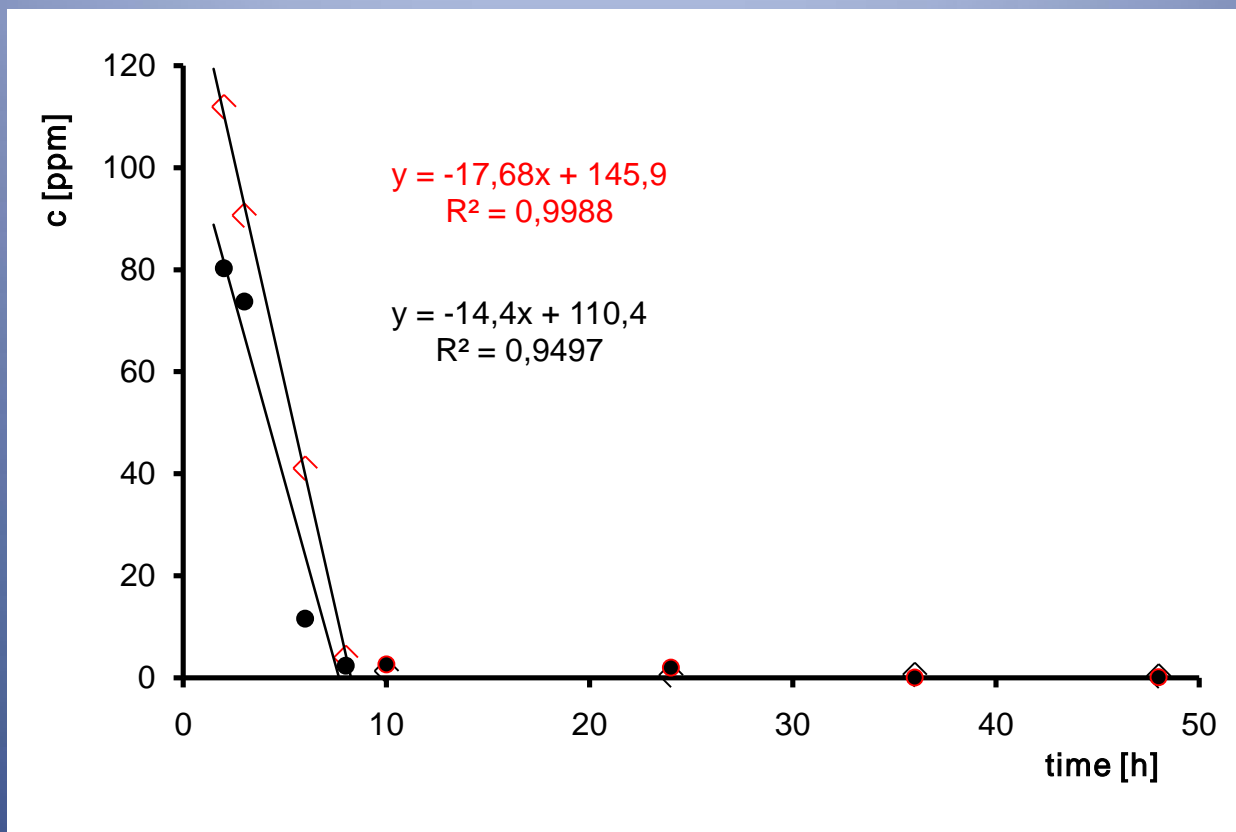
Excretion of cefacetril after intramammary application at 2, 3, 6, 8, 10, 24, 36 and 48 hours. Lower trial - 5 μ l, upper trial 10 μ l.



HPLC

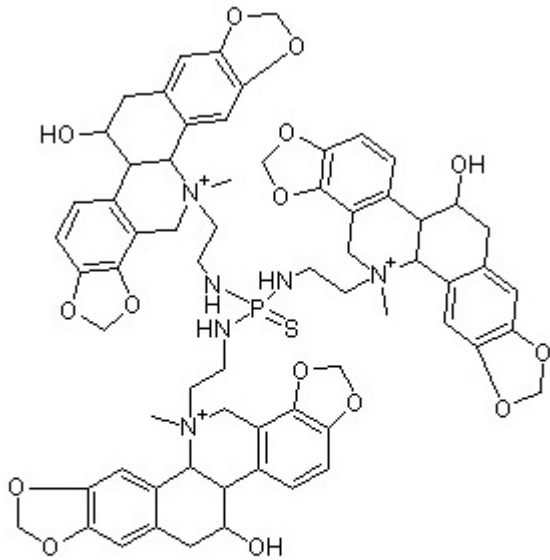


TLC - DB

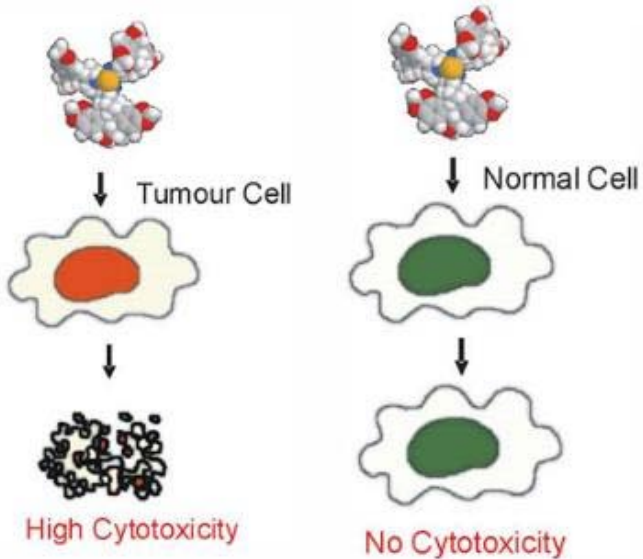
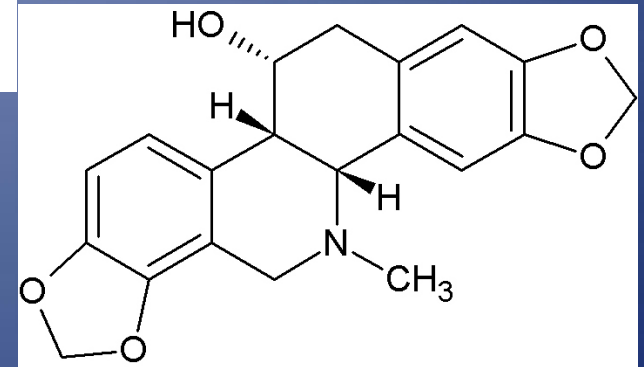
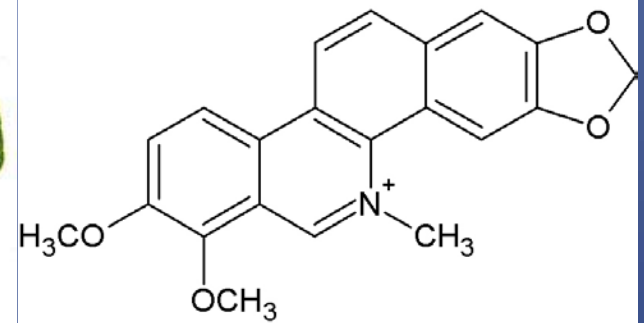
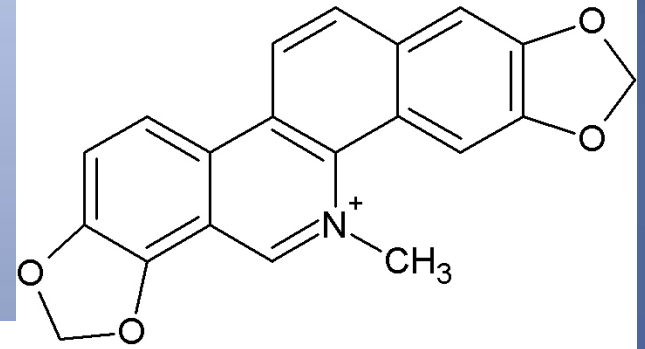


Concentration of cefacetril (ppm) in milk as a function of time after administration of the drug to a cow. Circle – HPLC data, rhombus – TLC - DB data.

Ukrain



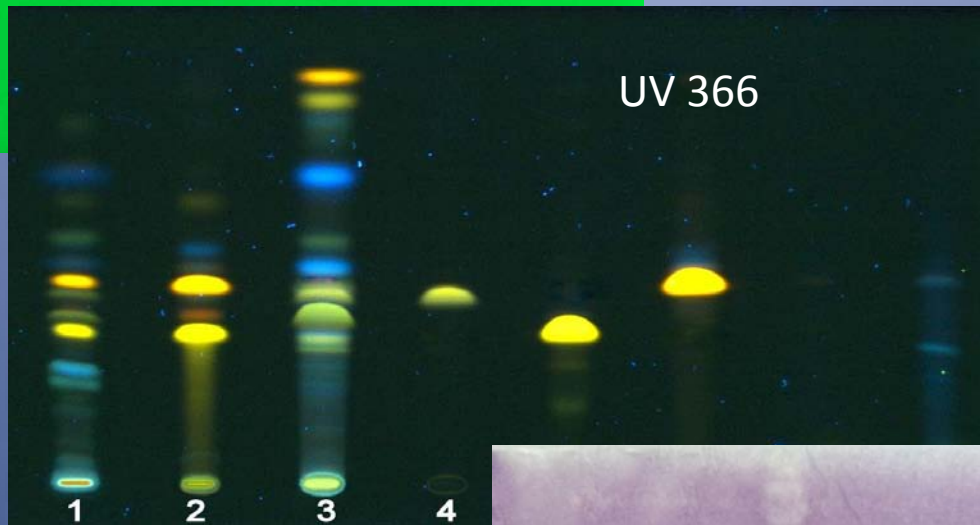
Molecular Formula: $C_{68}H_{72}N_6O_{15}PS$
1251.45 Da



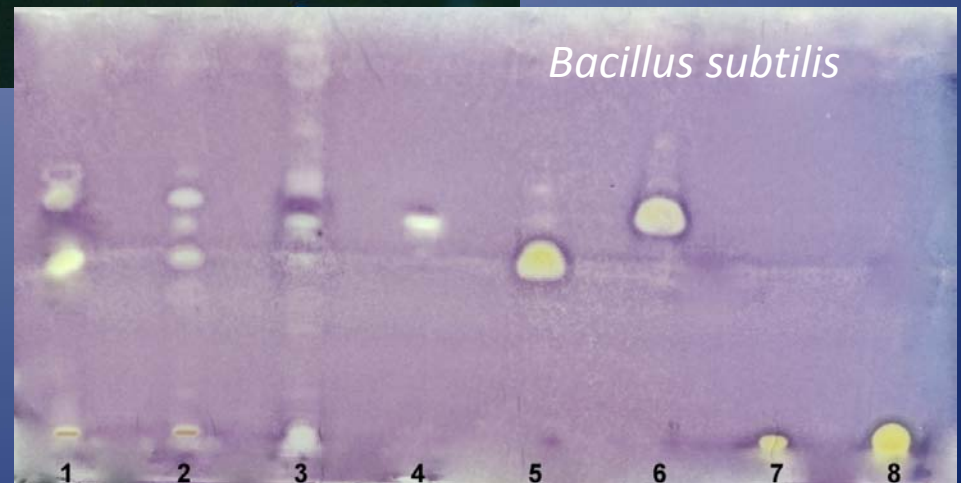
Chelidonium majus



1. Ukrain
2. Quaternary alkaloids
3. Tertiary alkaloids

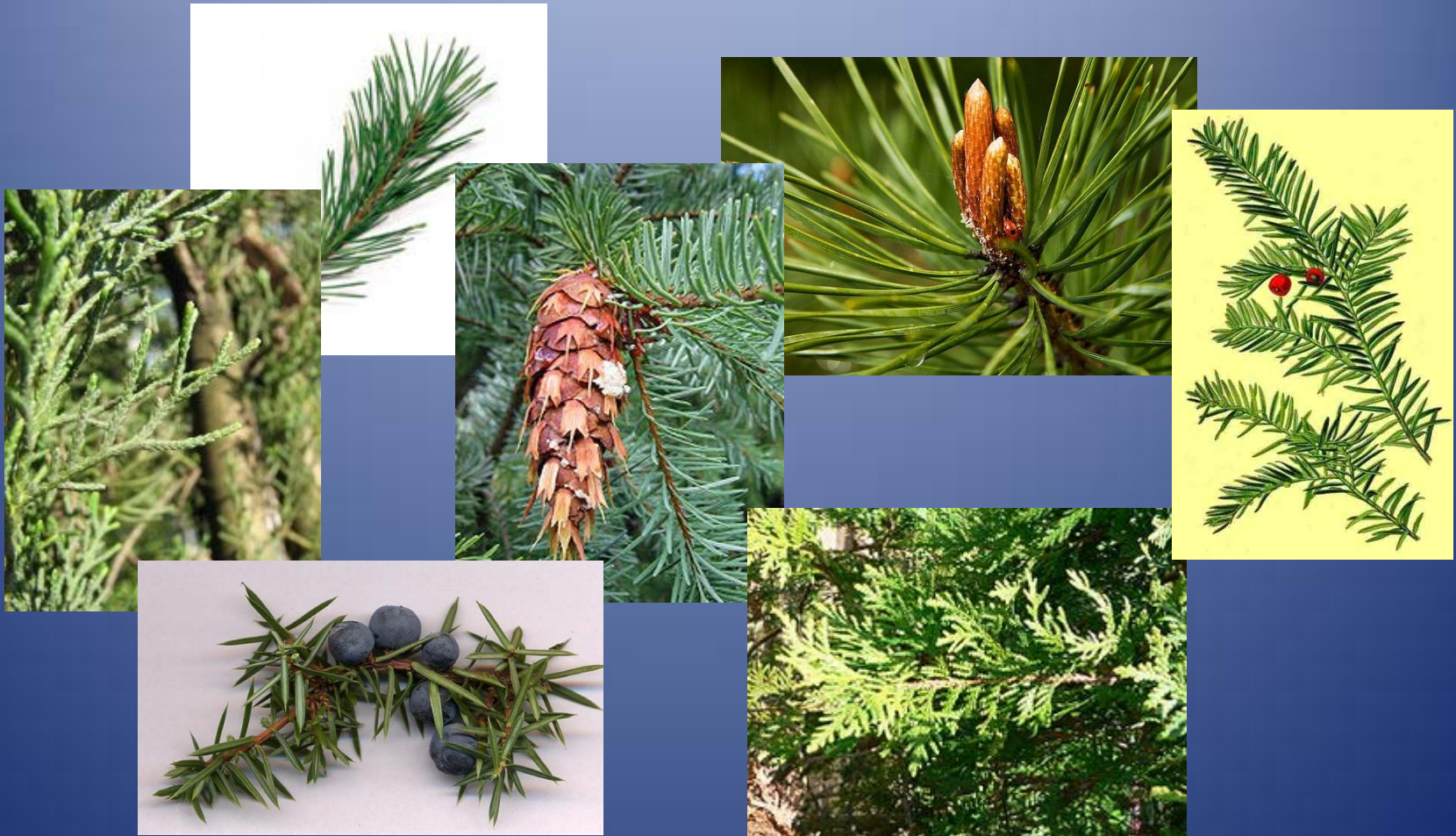


4. Chelidonine
5. Chelerythrine
6. Sanguinarine
7. Coptisine
8. Berberine



Mobile phase: chloroform : ethyl acetate : methanol (80:15:5)

Essential oils of conifers



TLC-DB: *E. coli*, *B. subtilis*

GC-MS

pine

cypress

green Douglas-fir

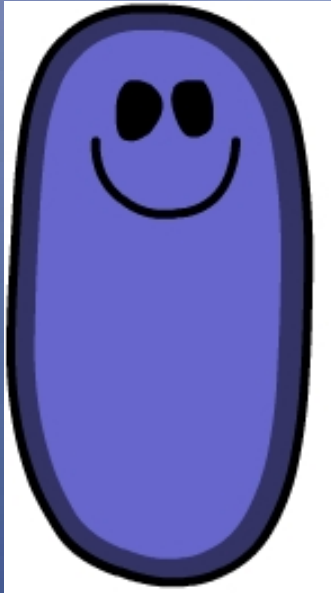
juniper

thuja



Compound	RI	Douglas (%)	Juniper (%)	Thuja (%)	Pine (%)	Cypress (%)
α - thujene	930	2,97	3,08	*	1,63	*
α - pinene	940	14,63	27,44	1,66	40,38	*
camphene	960	*	*	1,71	6,38	*
sabinene	979	1,75	40,92	*	*	*
β -pinene	987	20,07	1,30	*	2,2	*
β - myrcene	991	1,36	4,28	1,65	2,54	2,21
α - terpinene	1016	*	*	2,99	16,08	*
p- cymene	1024	0,65	1,20	*	*	*
D-limonene	1036	*	*	0,67	*	88,51
β - felandrene	1037	0,88	5,93	1,66	1,05	*
Z-ocimene	1049	5,10	0,69	*	*	*
γ - terpinene	1065	1,20	2,40	2,94	*	*
6- camphenon	1092	3,69	2,14	0,53	1,52	*
(+)-3-thujene	1100	*	*	38,16	*	*
α - thujene	1128	*	*	28,63	*	*
lavandulol	1165	*	*	9,48	*	*
Z-dihydrocarvon	1192	*	2,59	1,08	*	*
verbenone	1205	*	*	2,68	*	*
bornyl acetate	1294	45,34	*	3,05	*	*
butyl butanoate	1344	*	*	*	3,52	*
terpinyl acetate	1354	*	*	*	*	1,86
isolongifolene	1401	*	0,35	*	6,76	*
aromadendrene	1441	*	1,61	*	4,1	0,07
phenethyl isovalerate	1490	*	*	*	2,07	*
α - muurolene	1500	*	*	*	2,94	*
homo- γ -bisabolene	1515	*	*	*	7,96	*
Σ (%)		100	100	100	100	100

Spores are cool !!!



Bacillus subtilis

and

Escherichia coli



Thank you for your attention

Vibrio fischeri

