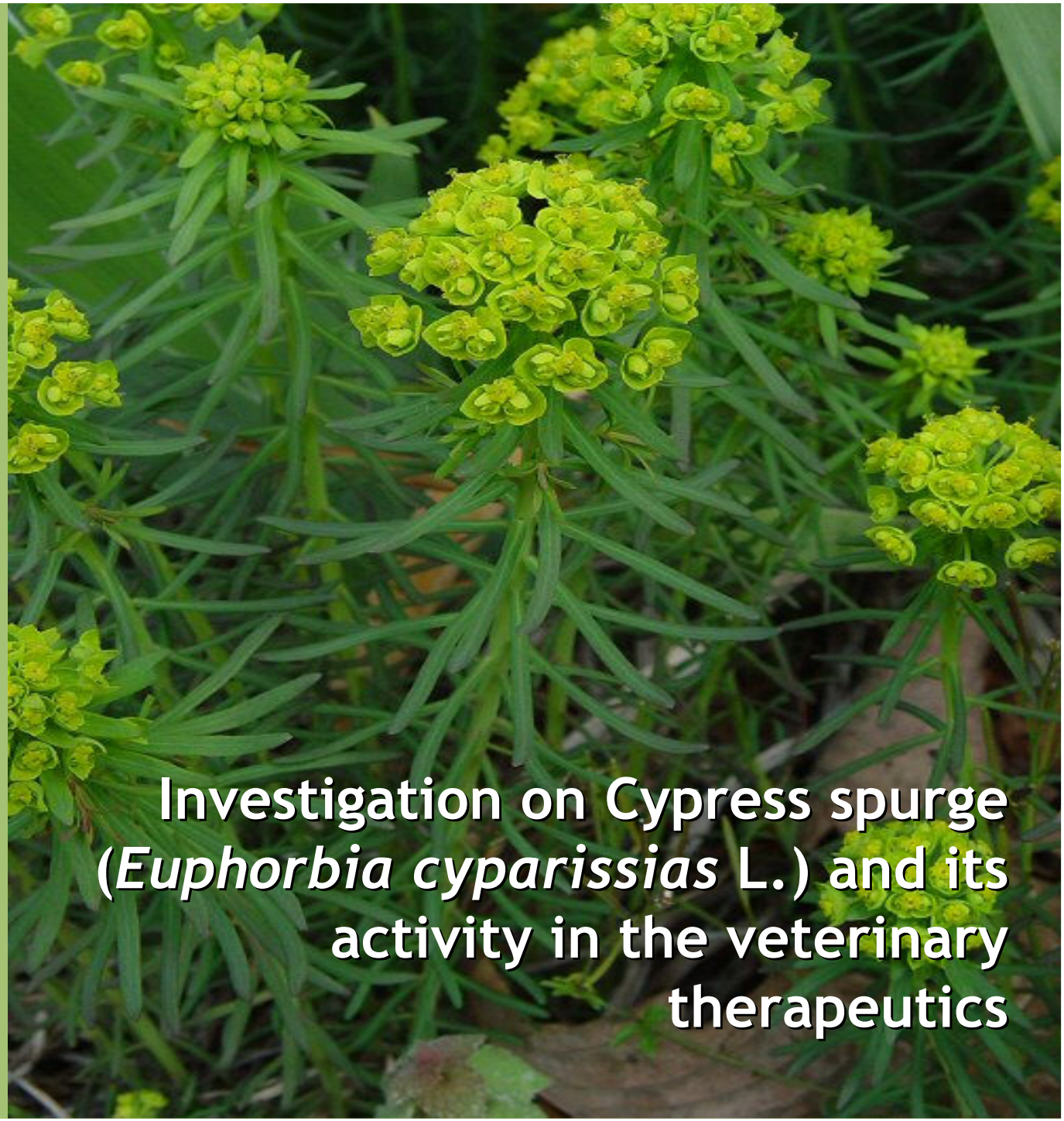


Investigation on Cypress spurge (*Euphorbia cyparissias* L.) and its activity in the veterinary therapeutics

Romeo-Teodor
Cristina

Numbering over 5000 species *Euphorbiacee* is a great plant family spread from sub arctic regions to Mediterranean areas on pastures, fields and ruderal areas.

Image of the entire plant

Source:http://botanika.wendys.cz/nahled/0189_2.jpg&imgrefurl

Between these *Euphorbia cyparissias* (common name - *Cypress spurge*) is easy to find and recognize in semi-woody Romania's spontaneous flora. Is an herbaceous to perennial plant about 15-30 cm. tall.

The flowers are nude, unnsexuate and monoic, being grouped in pleiocasium and cyantia. *E. cyparissias* is a helliophylic specia, having two flourishing periods: first in May – June and second in August – September accordingly with Geographic Region.

Why Euphorbia ?

Image of the stalk

Source: http://www.pharm1.pharmazie.uni-greifswald.de/gallery.jpg

Sources about the activity of different compounds extracted from *Euphorbia* revealed that this group is one of the contrasts, numerous elements being identified with the oriental principals of yin / yang.

Although the plant was presented as being a medical one, numerous botanists considering that the plant is a toxic one for humans and animals.

Pharmacochemical investigation of *E. cyparissias* is fully justified through multiple therapeutic features, yet unclear known of this plant.

Scientific papers emphasised that the plant is active especially

- against in: cancer, homeopathy,
- or as: molluscocide, insecticide, repellent, antiviral and galactagogue.

The Euphorbia's compounds activity in oncogenic process is proved in some studies started in this field.

Those come to reinforce the suppositions that this plant is a summum of activities with a great potential in cancer therapy and not only the deep knowledge of accurate compounding of this plant, especially, of the equilibrium between benefic and malefic determined by these two, being the key for the explanation why Euphorbia is in the same time toxic and medical.

Image of the inflorescence

Source: http://www.pharm1.pharmazie.uni-greifswald.de/gallery.jpg

Piceatanole from *E. lagascae* has proven antileukemic activity in rats. The mechanism is based on active inhibiting of the F0F1-ATP-aze, chimiopreventive activity mechanism being yet applied also for other species.

Antiquol C from the sap of *E. antiquorum* induced by the tumoral activator TPA (12-O-tetradecanoylphorbol-13-acetate, has a particular inhibitory activity in the activation of Epstein - Barr Virus Early Antigen (EBV-EA).

II-nd grade, coetaneous tumours emerged when euphole (853 µg/mouse) and other 12 compounds identified from the root of the *E. kansui*, determined high anti-inflammatory activity, suppressing the coetaneous tumoral proliferation.

E. Cyparissias after Uromyces pisi attack

Source: http://www.pharm1.pharmazie.uni-greifswald.de/gallery.jpg

Resiniferatoxine, a capsaicine analogue, present in the *E. resinifera* latex were investigated. Research revealed that the substance mediates the algic perception and neurogenic inflammation to the level of the specific receptor (denominated vanilloid).

Antinociceptive activity in rats was proven by the dosing *E. heterophylla* roots extracts in doses of 150-300 mg / kg.bw i.p.

E. Cyparissias' **gynaecium**

Source:
http://www.pharm1.pharmazie.uniqreifswald.de/gallery.jpg

E. splendens' latex was studied. The research confirmed the *cidal* activity on *Bulinus* (*B. glabrata*, *B. tenagophila* and *B. straminea*) snails, known as intermediate hosts of schistosomas (*S. mansoni* and *S. haematobium*).

Other activities

The control of ectoparasitic population is enough difficult, including numerous chemical substances and therapeutic techniques.

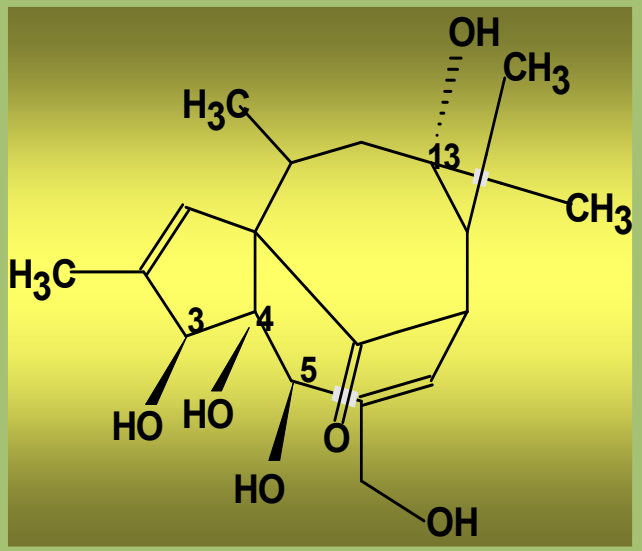
The used therapeutic arsenal's efficiency falling put serious problems to the practicians and makes the opportunity of new active substances discovery.

The investigations connected to the antiparasitary biocontrol have diversified at the moment being studied numerous control sources (e.g. fungus, vegetal extracts, volatile oils etc.).

In this respect, the plant extracts can be an important alternative source for the control of the acarians, being a rich source of efficient bioactive compounds.

Pharmaco-chemical studies of the E. *cyparissias extracts*

- active biological structures from *Euphorbiacee* certainly identified until now, are gliceroglicolipidic and glicosfingolipidic structures.
- the most important component of the plant is ingenole under its hydroxilated form.
- *phorbolester* (sin. *ingenole*, *euphorbone*, *triterpensaponine*, *diterpene*) exercises a high activity in cancer, as we have seen, the researches being in progress.
- The main interest now is the knowledge of the intimate active mechnism.



Hydroxyingenole, main component from *E. cyparissias*

What we know for sure

Glicosfingolipidics are present in the external membrane of the biological bi-lipidic membrane and are involved in the antigen - antibody reaction and in the biologic information transmission.

The final sfingolipides' products, *sfingozine* and *lisosfingolipides* are inhibiting proteinkinase C, an essential enzyme in the cells controlling and in transduction.

Sfingolipides and lisosfingolipides significantly affect the cell answer inducing recognized antitumoral activity on numerous cells of the mammalians.

These molecules can operate as cell function endogen modulators and, possible as secondary messengers.

Sfinx euphorbica moth visiting the plant

Source: http://bill.torque.net/aaf/archive/2004/vic_day/4694.jpg

Aim

to explore the composition in active compounds of Euphorbia cyparissias, using CG-MS methods.

Materials and Methods

Dried vegetal parts (after known methodology) of Euphorbia: inflorescence, herbaceous stalk and root, were extracted 10 days in hexane (Merck Co). (10g/sample in 100 ml solvent).

Analytic method required a GC Hewlett Packard HP 6890 analyzer, coupled with a mass spectrometer (Hewlett Packard 5973 Mass Selective Detector).

Obtained spectres were compared with NIST/EPA/NIH Mass Spectral Library 2.0 data base.

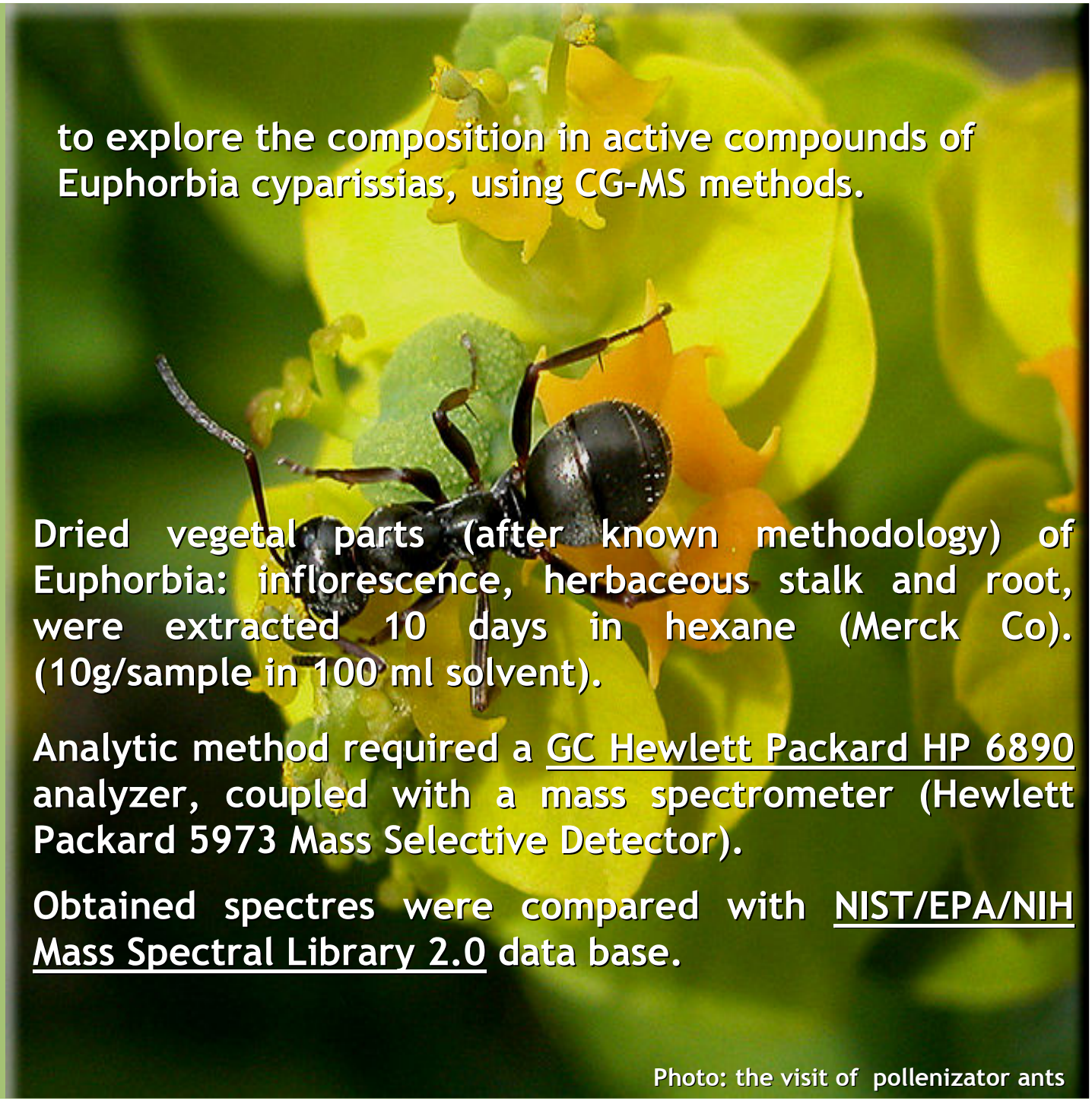


Photo: the visit of pollenizator ants

Analyse conditions:

- collumn (HP-5MS), lenght 30m, interiour diameter 0.25mm,
- thickness film: 0,25µm;
- temperature program: 50°C la 250°C with speed of 4°C/min;
- injector temperature: 280°C;
- detector temperature: 280°C; (with 70eV energy to 150°C)
- injection volume: 2 µl;
- carrier gas: He.
- scanning domanin: 50 - 300 amu,
- scanning speed: $1s^{-1}$ for MS.

The presentation of the plant by Linaeus (1707-1778)

Source: ww.agr.gouv.qc.ca/herbierv/ephcv/Page.htm

Results and disscutions

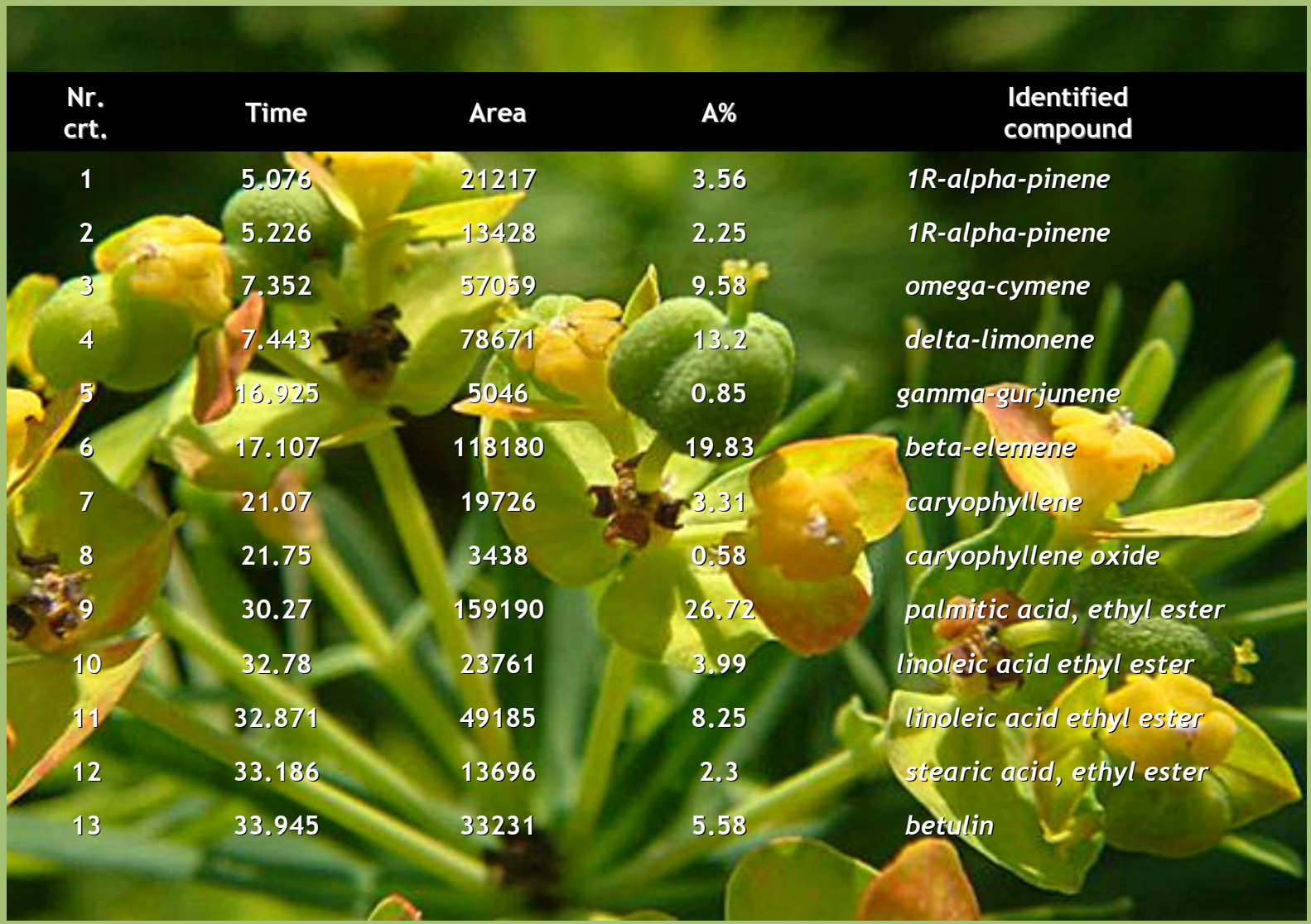
The identified compounds from *Euphorbia cyparissias* inflorescence extract were 13, majority of them being *sesquiterpenoids*:

- elemene (19.83%).
- beta-cariophyllene (3,31%) and epoxyde,
- cariophyllene-oxide (0,58%).

Also there were identiffied some *mono-terpenoids* and aromatic compounds in small amounts:

- *limonene* (13,3%),
- omega-*cimene* (9,58%),
- *alpha-pinene* (5,81%).

Composition of *Euphorbia cyparissias* inflorescence extract



Nr. crt.	Time	Area	A%	Identified compound
1	5.076	21217	3.56	*1R-alpha-pinene*
2	5.226	13428	2.25	*1R-alpha-pinene*
3	7.352	57059	9.58	*omega-cymene*
4	7.443	78671	13.2	*delta-limonene*
5	16.925	5046	0.85	*gamma-gurjunene*
6	17.107	118180	19.83	*beta-elemene*
7	21.07	19726	3.31	*caryophyllene*
8	21.75	3438	0.58	*caryophyllene oxide*
9	30.27	159190	26.72	*palmitic acid, ethyl ester*
10	32.78	23761	3.99	*linoleic acid ethyl ester*
11	32.871	49185	8.25	*linoleic acid ethyl ester*
12	33.186	13696	2.3	*stearic acid, ethyl ester*
13	33.945	33231	5.58	*betulin*

Similar compounds were also found in the stalk's extract from plant with the greater of concentrations of *sesquiterpenoids*:

- *elemene* 40,73%,
- *cariohyllene* 4,55% and its epoxide (0,97%),
- *selinene* and guainene.

Crt. Nb.	Time	Area	A%	Identiffied compound
1	17.104	97056	40.73	*beta-elemene*
2	19.679	8922	3.74	*delta-selinene*
3	19.914	5966	2.50	*delta-guaiene*
4	21.08	10843	4.55	*caryophyllene*
5	21.69	2310	0.97	*caryophyllene oxide*
6	30.28	98123	41.18	*palmitic acid, ethyl ester*
7	32.785	8563	3.59	*linolenic acid, ethyl ester*
8	32.892	6481	2.72	*linolenic acid, ethyl ester*

Composition of *Euphorbia cyparissias* stalk's extract

In the *Euphorbia's* root extract were identified 7 compounds the greater concentration being registered also for *sesquiterpenoids*.

- *elemene* 64,49%,
- *cariophyllene* 7,2% and its epoxide (1,53%),
- *selinene* 5,93% şi
- *guaiene* 3,96%.

To smaller concentrations were identiffied also monoterpenes:

- *cimene* 6,21% and
- *fenchene* 10,67%

Composition of *Euphorbia cyparissias* root's extract

Nr. crt.	Time	Area	A%	Identiffied compound
1	7.35	9345	6.21	*omega-cymene*
2	7.446	16059	10.67	*alpha-fenchene*
3	17.104	97056	64.49	*beta-elemene*
4	19.679	8922	5.93	*delta-selinene*
5	19.914	5966	3.96	*delta-guaiene*
6	21.08	10843	7.20	*caryophyllene*
7	21.69	2310	1.53	*caryophyllene oxide*

- The structure of Euphorbia was investigated by numerous authors who emphasised the correlation between the plants' composition (which can be different from a plant to the other) and the geographical region from where the plants were collected.
- Thus in a research EVANICS F and al. identified in *Euphorbiacea* 16 diterpenic polyesters, jatropanic similar structures and even named a new diterpenoide structure, *euphpekinezine.*
- In another study on *E. characias*, in Sardinia, APPENDINO and al. have identified 13 oxygenated terpenoids, among these four: *atisane*, *abietane*, *pimarane* and *kaurane* were identified as specific to that insular area.
- Until now researches from milky sap revealed numerous basic diterpenic components: *ingenole*, *euphorbone*, *piceatanole*, *aesculetine*, *jolkinol*, *hyperoside*, *kaempferol*, *acylphorbol*, *acylingenol* etc.

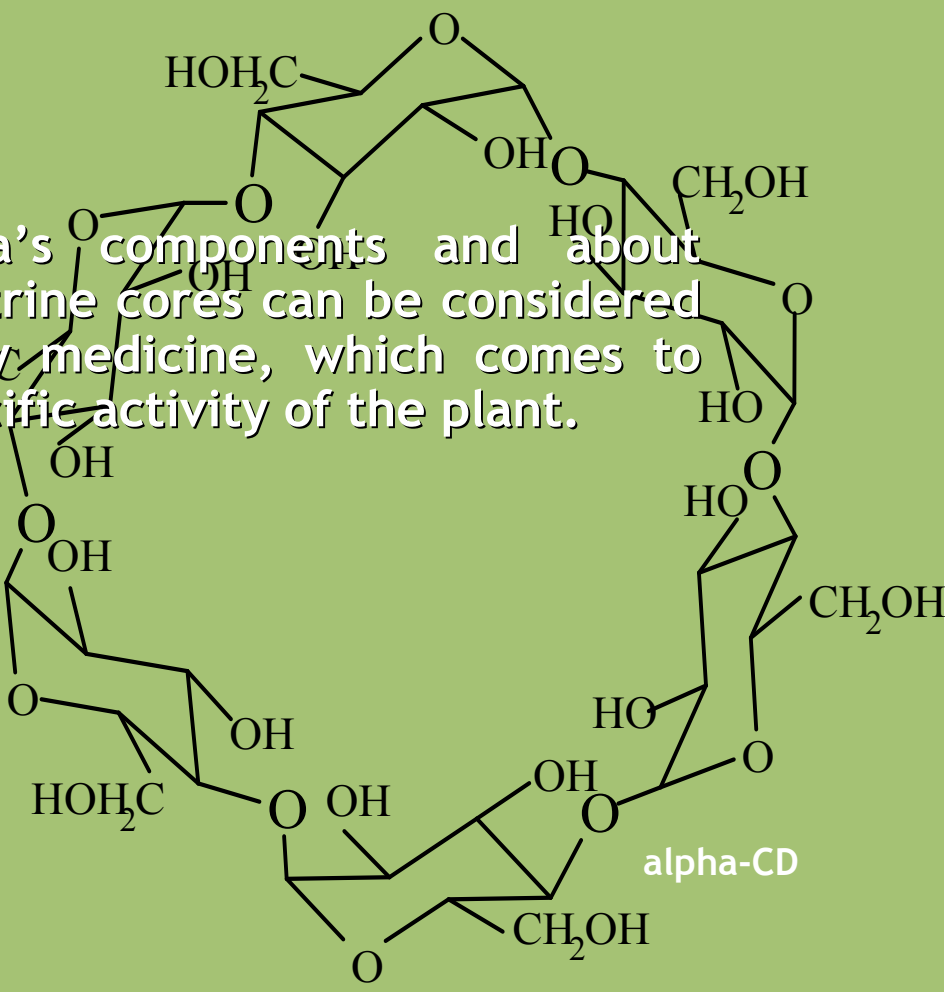
Conclusions

- ✓ In the E. cyparissias hexane extracts the bioactive compounds were found in all parts of the plant, the main components being *sesquiterpenoids*, with the preponderance of elemene and cariophylene;
- ✓ A great part of cariophylene was found in his epoxydic state (as cariophylene oxide);
- ✓ The sesquiterpenoids concentration diminished in the order: root - stalk - inflorescence (elemene concentration was reduced to one third in the inflorescence extract comparatively with the root's one), in the same time the monotherpene and aromatic concentration have grown in the inflorescence samples.

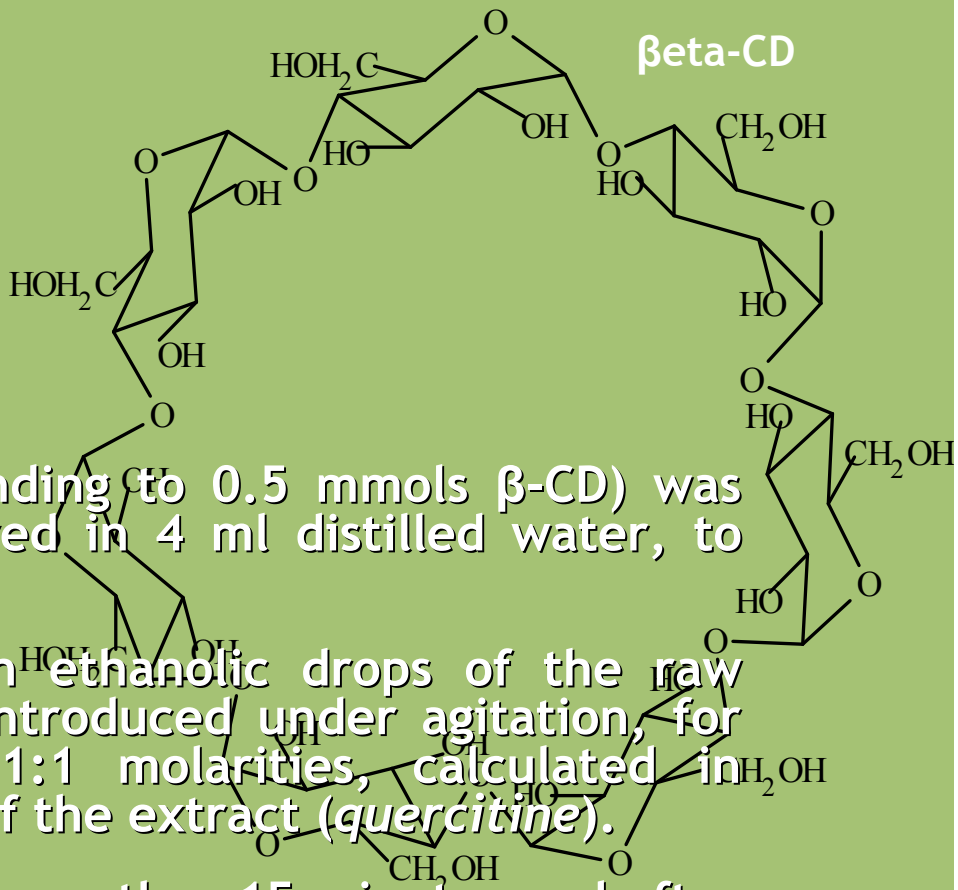
2. Study of the *Euphorbia* extract / β-*ciclodextrine* complex

Aim

a research about the Euphorbia's components and about inclusion of those on beta-ciclodextrine cores can be considered of a practical value for veterinary medicine, which comes to fulfil the gained data about the specific activity of the plant.



Materials and methods

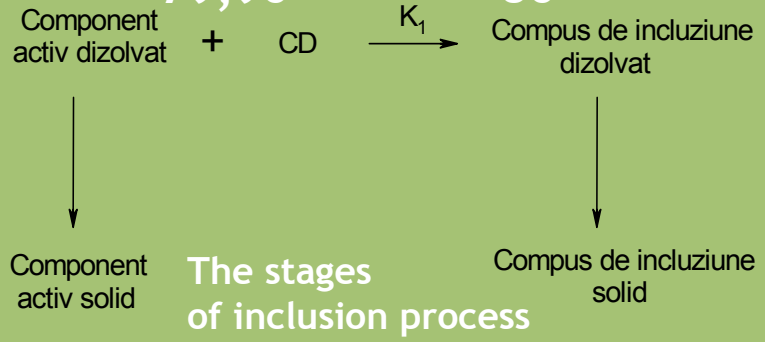


Complexion method

- *β*-ciclodextrine (β-CD) (corresponding to 0.5 mmols β-CD) was analytically weighted and dissolved in 4 ml distilled water, to 50±1°C.
- After dissolving, on this solution ethanolic drops of the raw *Euphorbia's'* concentrate were introduced under agitation, for 30 minutes, corresponding to 1:1 molarities, calculated in function of the main component of the extract (*quercitine*).
- Obtained solution was agitated for another 15 minutes and after that was chilled in four hours in a water bath and preserved for an additional 12 hours for the crystallisation of the complexes.
- The formed suspension was filtered and clarified with 1ml ethanol (96%) and dried out for the term gravimetric analyze.

Conditions and obtained results in the case of *Euphorbia* / β-CD extract complex

Code	T5	T10	T5c	10c
Description	Extract T5%	Extract T10%	Extract T5% conc. 1/5	Extract T10% conc. 1/5
m (βCD) (g)	0.671	0.671	0.671	0.671
V (water) (ml)	4	4	4	4
Temp.(°C)	50	50	50	50
V (EtOH)(ml)	4	4	4	4
Chill time (ore)	4	4	4	4
Time perf.(ore)	12	12	12	12
Efficacy.(%)	80	81	79,90	80



The stages of inclusion process

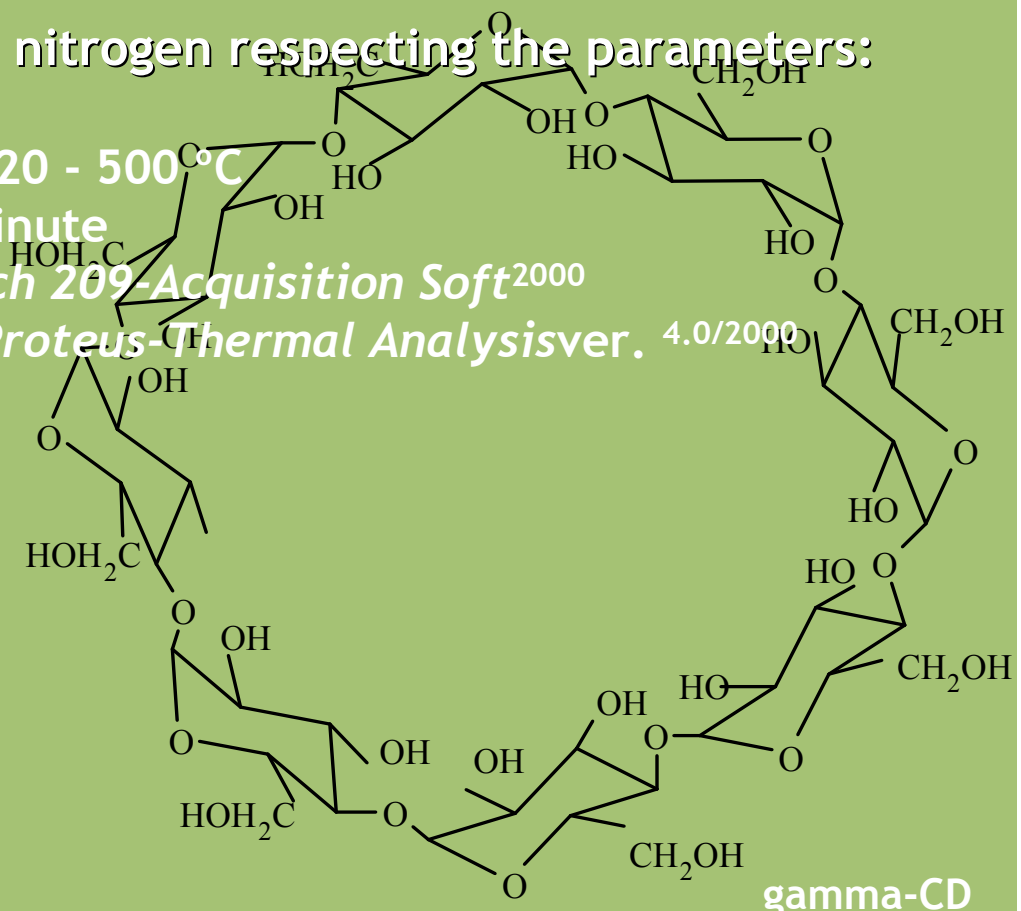
TG-DTG analyze of raw β-ciclodextrine

Method

- For the term gravimetric analyze (TG-DTG) of the obtained complexes term gravimeter TG 209 NETZSCH was used.

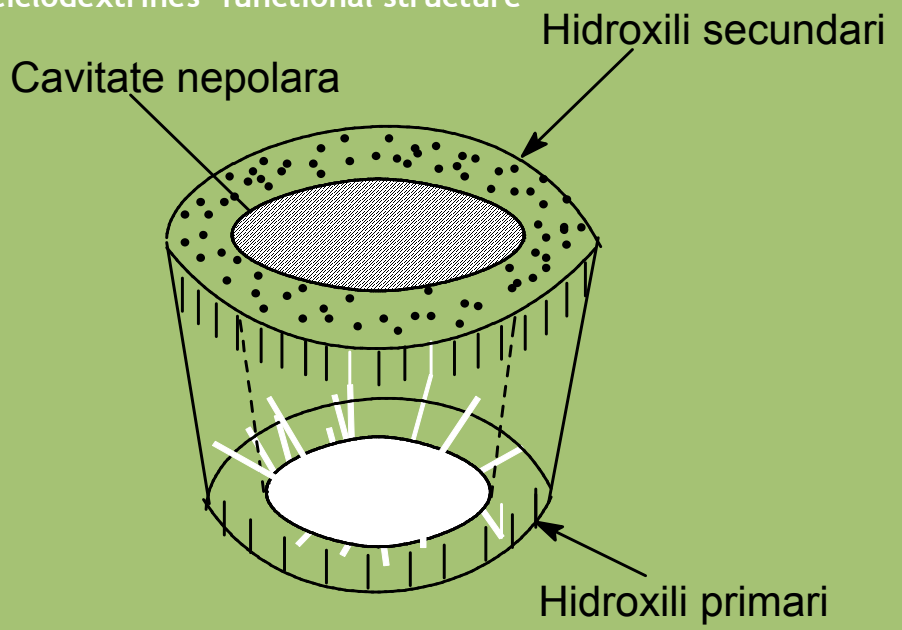
All determinations were realised in nitrogen respecting the parameters:

- temperature program: between 20 - 500°C
- heating speed: 10 °C / minute
- spectral data base: TG *Netzsch 209-Acquisition Soft*[2000]
- data analysing: *Netzsch Proteus-Thermal Analysis*ver. 4.0/2000



Results and discussions

ciclodextrines' functional structure



TG-DTG analyze of:

Pure β-*ciclodextrine*

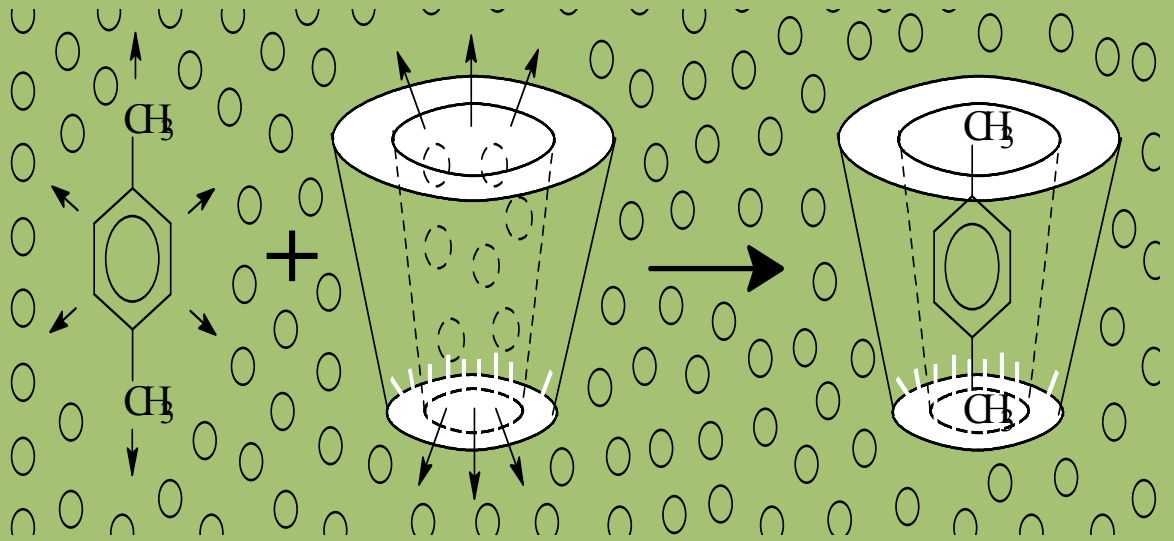
- The TG analyse of pure *β-ciclodextrine* denoted a 11.7% mass loss to 100°C, which is corresponding with loss of crystallizing water, loss rate augmenting to 76.3% in 100 - 500°C temperature interval, with β-ciclodextrine's decomposition.

Euphorbia T5/β-ciclodextrine complex raw extract.

- In the case of *Euphorbia* T5 / β-CD (5%) brute extract complex, the mass loss was lower than 8.9% at 100 °C, until 225°C, being registered 2.8% mass loss, probably corresponding to the encapsulated bio active's compounds de complexion phase.

Schematic process of ciclodextrines' incluzion:

(p-xylene is the host molecule, and the circles are representing the water molecules)



Euphorbia T10/β-ciclodextrine complex raw extract

• A similar behaviour was recorded for the *Euphorbia* T10 / β-CD (10%) brute complex, until 100°C, mass loss being of 8%, between 100 and 225°C of 2.9%, with a total mass loss of ~78%.

EuphorbiaT5 and T10/β-ciclodextrine co raw extract concentrate

• In the case of *Euphorbia* T5/T10/β-CD concentrated extracts complex, the mass loss until 100°C was up to 11.9%, between 100 and 225°C, being under 1%. This fact can be translated as a loss / degradation of initial complexable bioactive compounds from the initial extracts.

Conclusions

- ✓ TG of raw *β-ciclodextrine* indicated a 11.7% mass loss until 100ºC, loss rate augmenting to 76.3% in the 100 to 500ºC temperature interval, β-ciclodextrine decomposition.
- ✓ In the case of raw *Euphorbia* T5 / β-CD, mass loss until 100ºC was under 8.9%, until 225ºC being registered an additional 2.8% mass loss, due probably to the decomplexing phase of the extract encapsulated bioactive compounds.
- ✓ Similar behaviour to heating was registered also for *Euphorbia* T10 / βCD, extract raw complex, thus until 100ºC the mass loss being of 8% and between 100 and 225ºC of 2.9%, the decomposition determining a ~78% mass loss.
- ✓ In the case of *Euphorbia* T5/T10/β-CD, complexes, the mass loss until 100ºC was of 11.9%, and between 100 and 225ºC, this was under 1%. This fact can be translated as being a initial bioactive extracts compounds loss/degradation process.

Romeo, thanks for
Your's atention ♥

Cheers !