Participari la manifestari stiintifice

1. Conferinta Nationala de Fitoterapie, ed. a IV a, UMF Iasi, mai, 2008
Titlul lucrarii: Identificarea prin cromatografie pe strat subtire de inalta performanta(HPTLC) a unor compusi polifenolcarboxilici si a saponinelor cu actiune hipocolesterolemianta din *Medicago sativa si Trigonella foenum-graecum.*

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Abstract: Obiectivul acestui studiu este de a identifica prin cromatografie pe strat subtire de inalta performanta(HPTLC) principalele componente cu actiune hipocolesterolemianta din doua produse vegetale *Medicago sativa* si *Trigonella foenum graecum,* cunoscute pentru utilizarea lor in contracararea dezechilibrelor metabolice. Principalele clase de principii active studiate au fost compusii polifenolcarboxilici(izoflavone, flavone si acizi polifenolcarboxilici) si saponinele triterpenice din *Medicago sativa* si saponinele sterolice din *Trigonella foenul graecum.*
Pentru studiu s-au utilizat materiale vegetale autohtone, substante de referinta FLUKA, placi cromatografice MERCK si un sistem de cromatografie pe strat subtire CAMAG (aplicator LINOMAT IV si sistem REPROSTAR cu camera foto digitala CANON).
Cuvinte keie: hipocolesterolemiant, HPTLC, izoflavone, saponine, flavone, acizi polifenolcarboxilici.

INTRODUCERE

Colesterolul este unul din indicatorii biologici de apreciere a dezechilibrelor metabolice. Rezultatele „Studiului Național Transversal CARDIO-ZONE" publicate la 23 aprilie 2007 demonstreaza faptul că peste 30% din populația României are colesterolemia ridicată datorate in mare parte alimentatiei necorespunzatoare şi predispozitiei genetice.
Tendintele actuale in medicina pentru contracararea dezechilibrelor metabolice sunt de administrare in scop profilactic si curativ a micronutrientilor din extracte vegetale multicomponentiale(fitocomplecsi) obtinute din plante medicinale si aromatice.
Atat saponinele triterpenice (din lucerna) cat si saponinele sterolice (de tip furastanol) din schinduf (diosgenina) sunt cunoscute pentru efectele lor hipocolesterolemice.
Izoflavonele(genisteina, daidzeina, formononetina, biochanina A) din lucerna scad nivelul trigliceridelor si a colesterolului total, imbunatatind semnificativ profilul sanguin.
Acizii polifenolcarboxilici din lucerna sunt importanti pentru activitatea antioxidanta prin care se previne oxidarea acizilor grasi si a colesterolului.
Pornind de la compozitia celor doua materiale vegetale luate in studiu, respectiv *Medicago sativa*: saponozide (2-3 % in radacina, 0,5% in partea supraterestra),cumarine (esculetol, scopoletol), flavone (heterozide ale tricinolului, kempferolului, cvercetolului, miricetolului, naringenolului), izoflavone (genisteina, daidzeina, formononetina, biochanina A, sativan, 5- metoxisativan),cumestani (cumestrol si derivati, medicagol, sativol, lucernol, trifoliol), antociani (heterozide ale delfinidolului, malvidolului, petunidolului),taninuri (2-3 % catehice si galice),vitamine (beta-caroten, vitamina – B, C, D, E si K),poliholozide, minerale (potasiu, fier, calciu, fosfor) si *Trigonella foenum graecum*: apa -12%, 45-60% carbohidrati, in special fibre mucilaginoase (stahioza**,**

amidon, galctomanani, celuloza), 20-30% proteine (bogate in lizina si triptofan), uleiuri grase si lipide steroidice - 9% (lecitina, lipide mono si polinesturate, gliceride saturate, fitine) 0,6-1,7% saponine (diosgenina, tigogenina, iamogenina, neotigogenina), flavonoide (apigenina, luteolina, cvercetina, etc), 0,5% colina, aminoacizi liberi (0,09% 4-hidroxileucina, arginina, histidina, lizina), protide -25%, alcaloizi piridinici (0,2-0,36% trigonelina, gentianina), uleiuri volatile, tanin, substante amare, vitamine (B1 ,C, E, nicina, colina,acid nicotinic si beta-caroten), micro si macroelemente (calciu, cupru, fosfor, fier, siliciu, sodiu),tiamina, 0,6-1,7%,sitosterol, s-a urmarit identificarea prin metoda cromatografica pe strat subtire de inalta performanta a compusilor polifenolcarboxilici(izoflavone, flavone si acizi polifenolcarboxilici) si a saponinelor triterpenice din *Medicago sativa* si a saponinelor sterolice din *Trigonella foenum graecum.*

MATERIALE SI METODE

Au fost studiate doua materiale vegetale: *Medicago sativa, herba(Lucerna, parte aeriana)* si *Trigonella foenum graecum, semen(Schinduf, seminte),* s-au utilizat substante de referinta FLUKA, placi cromatografice HPTLC silicagel G60 si G60F254 MERCK si un sistem de cromatografie pe strat subtire CAMAG (aplicator LINOMAT IV si sistem REPROSTAR 3 cu camera foto digitala CANON.

Pentru identificarea ***saponinelor sterolice*** din semintele de schinduf s-a folosit un extract metanol-cloroform obtinut din seminte degresate si hidrolizate la cald cu solutie de acid sulfuric, solutie de diosgenina 0,1% in metanol ca substanta de referinta, developant diclormetan : acetone si reactiv de identificare acid sulfuric in alcool etilic . Examinarea s-a efectuat la lumina zilei dupa incalzire la 105^0 C timp de 15 minute.

Pentru identificarea ***saponinelor triterpenice*** din iarba de lucerna s-a folosit un extract hidroalcoolic, solutii de β sitosterol, stigmasterol, colesterol, acid oleanolic 0,1 % in cloroform si solutie de saponin 0,1% in alcool etilic 30% ca substante de referinta, developant benzen : metanol si reactiv de identificare anisaldehida - acid sulfuric . Examinarea s-a efectuat la lumina zilei dupa incalzire la 105^0 C timp de 15 minute.

Pentru identificarea ***izoflavonelor*** din iarba de lucerna s-a folosit un extract metanolic, solutii de genistin, daidzein si biochanin A 0,01% in metanol ca substante de referinta si developant cloroform : methanol: apa:acid acetic. Examinarea s-a efectuat in lumina ultravioleta la 254 nm.

Pentru identificarea ***acizilor polifenolcarboxilici si a flavonelor*** s-au folosit extracte hidroalcoolice si metanolice din iarba de lucerna declorofilata, solutii de acid cafeic, acid clorogenic, rutin, hiperozida, quercetin, apigenin si kaempferol 0,01% in metanol ca substante de referinta, developant acid formic anhidru : apa : metal-etil-cetona : acetat de etil si reactivi de identificare solutie de diphenylboric acid aminoethyl ester 1% in metanol si solutie de polyethilenglycol 400 5% in alcool etilic. Examinarea s-a efectuat in lumina ultravioleta la 254 nm inainte si la 366 nm dupa pulverizarea cu reactivii de identificare.

REZULTATE SI DISCUTII

Studiul calitativ efectuat pe cele doua materiale vegetale a scos in evidenta prezenta principalelor componente cu actiune hipocolesterolemianta.

In *Trigonella foenum graecum* s-a identificat prezenta semnificativa a ***diosgeninei*** printr-un spot la acelasi Rf si cu aceeasi intensitate de culoare ca substanta de referinta - figura 1.

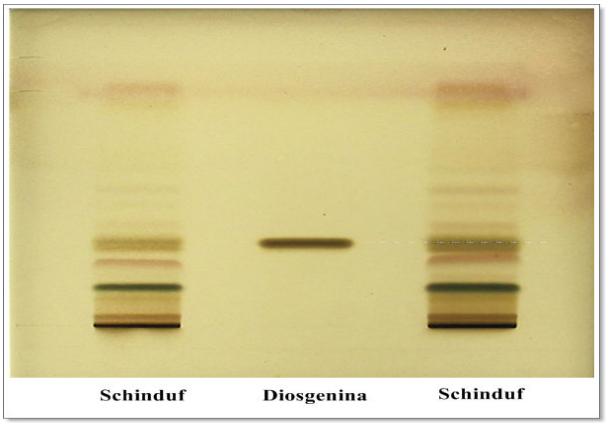


fig 1

In *Medicago sativa:*

- prezenta ***izoflavonelor*** *genistin, daidzein si biochanin* ***A*** este indicata prin spoturile din solutiile de proba care se afla la aceleasi Rf-uri cu cele din solutiile de referinta – figura 2;

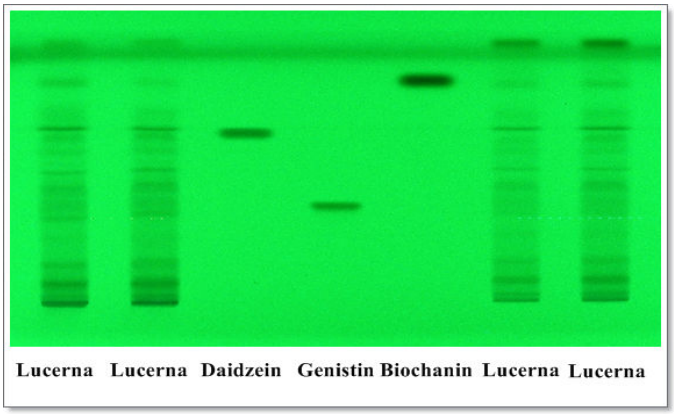


fig 2

- preznta ***saponinelor triterpenice*** β sitosterol, stigmasterol, colesterol si saponin este indicata prin spoturile din solutiile de proba care se afla la aceleasi Rf-uri cu cele din solutiile de referinta . In solutia de proba exista si alte spoturi de culoare specifica saponinelor triterpenice, la alte Rf-uri fata de solutiile de referinta aplicate – figura 3;

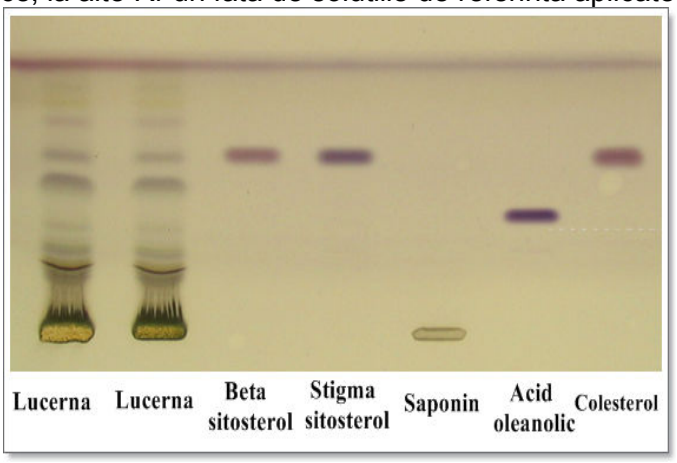
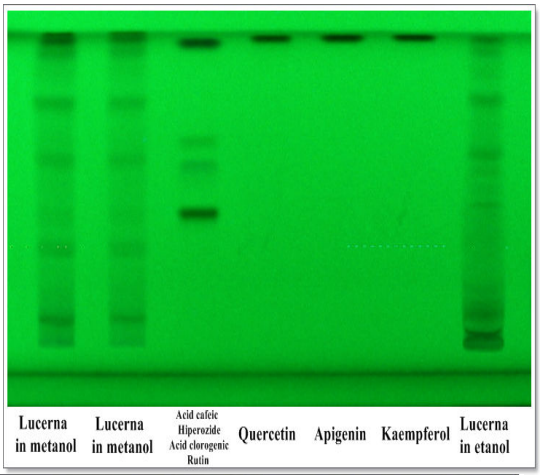


fig 3

- cromatogramele din figura 4 si 5 indica prezenta ***flavonelor si acizilor polifenolcarboxilici*** in toate solutiile de proba, comparativ cu spoturile specifice substantelor de referinta utilizate: apigenin, quercetin, kaempferol, rutin, hiperozioda, ac. cafeic si ac.clorogenic precum si a altor derivati ai acestora.



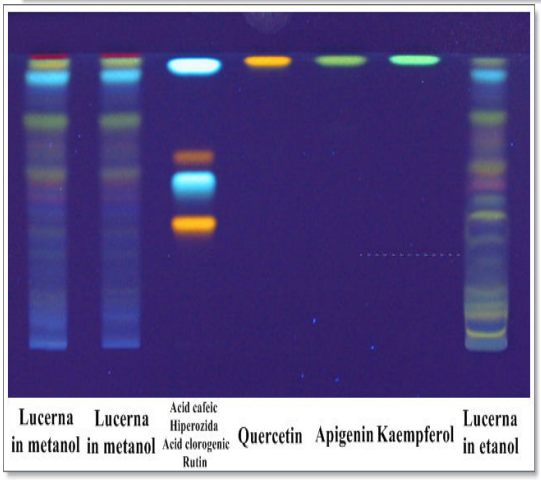


fig 4-examinare in UV la 254 nm

fig 5- examinare in UV la 366 *nm*

CONCLUZII

Studiul efectuat pe cele doua materiale vegetale a scos in evidenta prezenta componentelor cu actiune hipocolesterolemianta. Acest studiu sta la baza stabilirii unor formule de asociere pentru obtinerea de suplimente alimentare cu rol in contracarea dezechilibrelor metabolice.

BIBLIOGRAFIE

1. WAGNER H., BLADT S., ZGAINSKI E.M. , Plant Drug, Analysis, Springer-Verlag, Berlin Heidelberg New York Tokyo, 1984
2. European Pharmacopoeia, ed. 6.0, 01/2008
3. ISTUDOR V., Farmacognozie Fitochimie Fitoterapie, Ed. Medicala, Bucuresti 1998, vol. 1
4. FRIED B., SHERMA J., Practical thin-layer chromatography, CRC Press, Boca Raton, New York, London, Tokyo, 1996
5. CAMAG BIBLIOGRAPHY SERVICE PLANAR CHROMATOGRAPHY, Switzerland

2. La **Conferinta plantelor medicinale si aromatice a tarilor din sudestul Europei, editia a V a(5thCMAPSEEC)** care a avut loc la Brno, Cehia au fost prezentate doua lucrari elaborate in colaborare cu partenerii:

2.1. The qualitative and quantitative determination of diosgenin from trigonella foenum-graecum by high performance thin-layer chromatography and densitometric methods.

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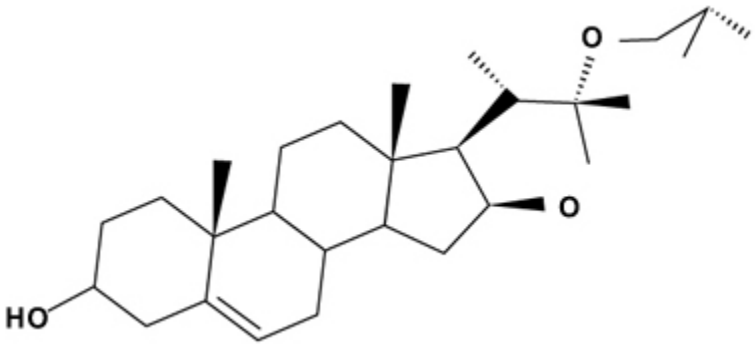
OBJECTIVES

Sterolic saponins of furastrol type (diosgenin, tigogenin, yamogenin, neotigogenin) from dry seeds of *Trigonella foenum-grecum* are known for their hypocholesterolemiant action. The most important compound from the total sterolic saponins is diosgenin.
The objective of this study was to elaborate an original method which permits the identification and quantification of diosgenin from total the sterolic saponins by High Performance Thin Layer Chromatography (HPTLC).



diosgenin chemical structure

SAMPLE PREPARATION

Figure 1

HPTLC method – equipment and materials

– Automatic TLC Sampler LINOMAT IV – application of varying amounts of standard solution and a single concentration of sample, band application;

- CAMAG TLC SCANNER 3 and WINCATS software, at λ=397 nm, evaluation of peak height and aria with linear regression;
- HPTLC plates 20 x 20, Silica gel 60 F254 Merck;
- sample;
- diosgenin (Fluka), 0,05% in methanol;
- mobile phase: petroleum ether: acetone (30:7,5);
- detection reagent: sulphuric acid 10% V/V in methanol.

Fig.2. HPTLC Chromatogram of standard Diosgenin and *trigonella foenum graecum* sample: track 1, 5, 6, 10 - *trigonella foenum graecum* sample - application volume 2 µl in 4 replicates ; track 2, 3, 4, 7, 8, 9 - standard Diosgenina application volume 4, 6, 8 µl in 2 replicates

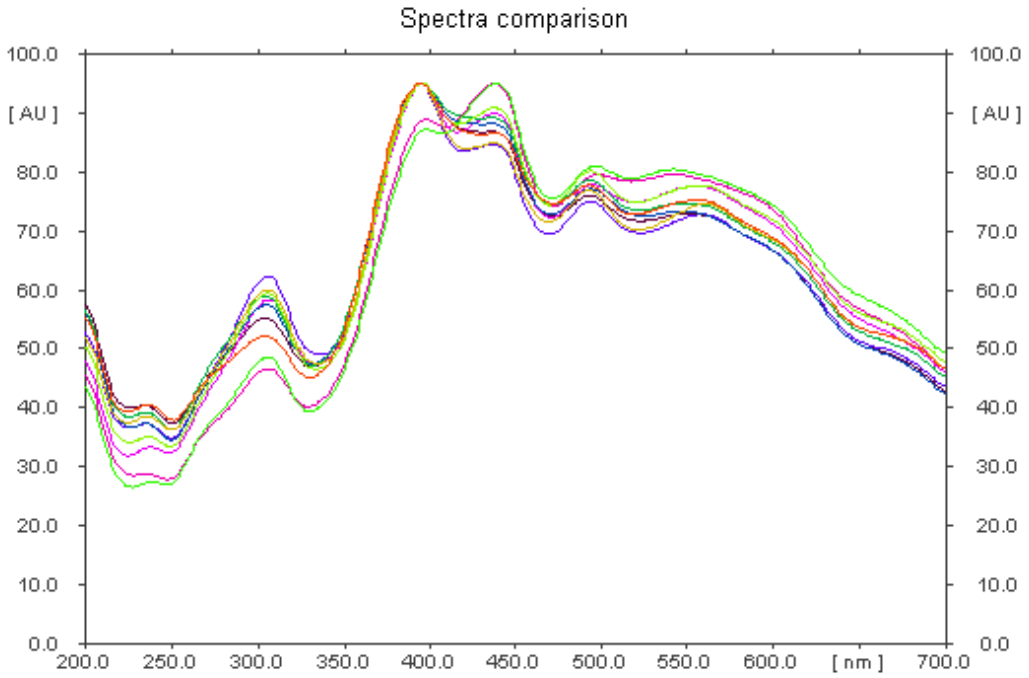


Fig.3. UV Spectrum of standard Diosgenin and sample after derivatization with sprayng reagent(sample - orange, green, blue, dark red and standard -beige, light green, fluorescent green, purple, pink, red)

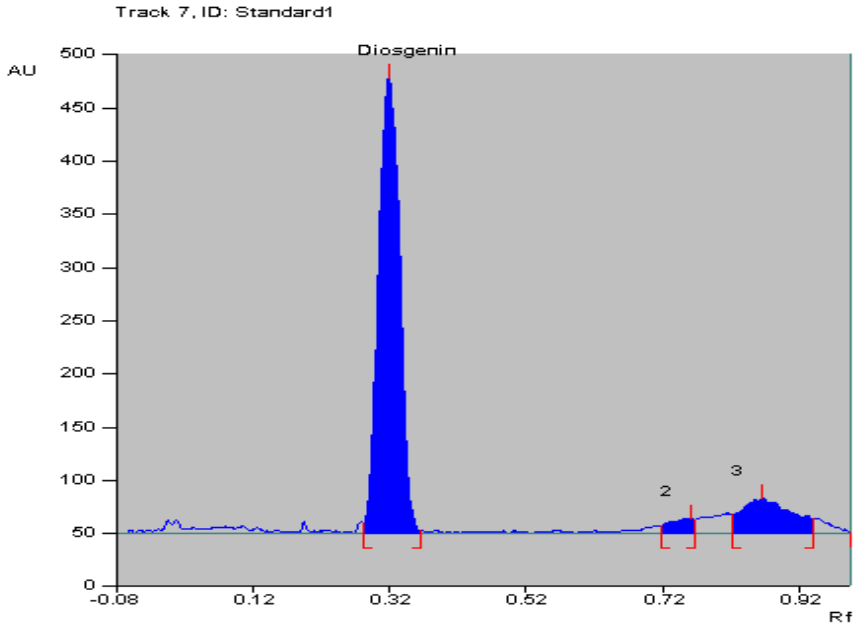


Fig.4 Densitogram of standard Diosgenin (2 µg)

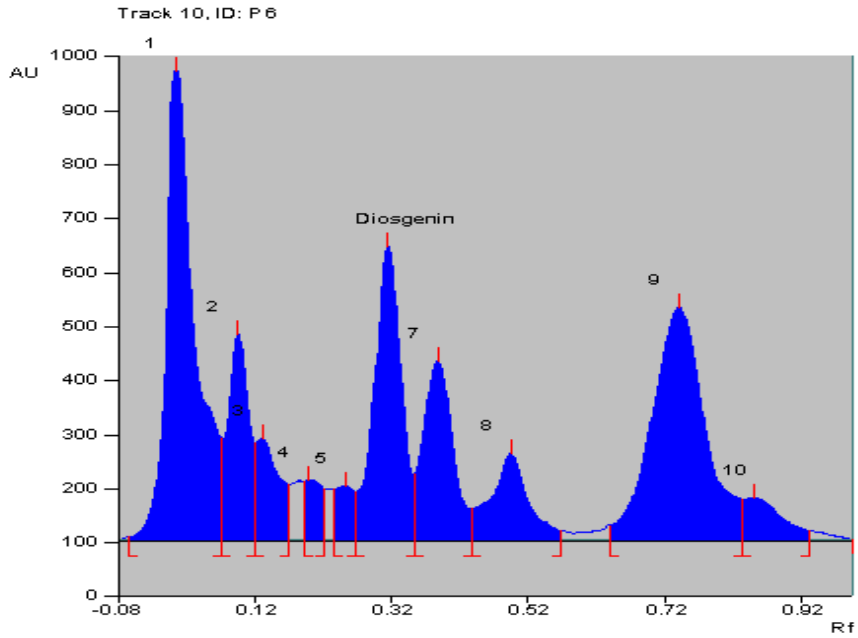


Fig.5 Densitogram of the *trigonella foenum graecum* sample containing Diosgenin

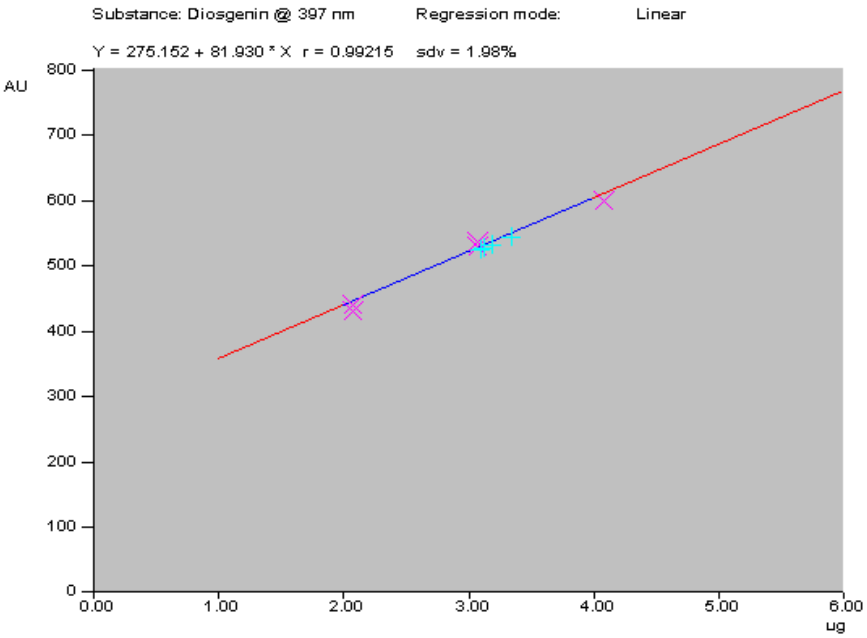


Fig. 6. Calibration curve of standard Diosgenin in the concentration 2,3,4µg, with 2 replicate analysis for each concentration (red) and with the sample volum 2 µl in 4 replicate analysis (blue)- height evaluation by linear regression . Correlation coefficient obtained is 0,99215.

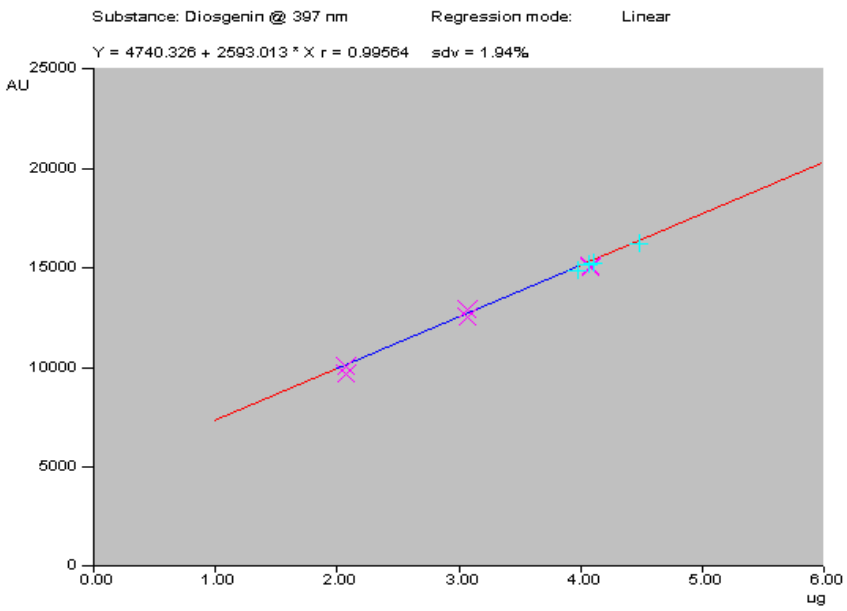


Fig. 7. Calibration curve of standard Diosgenin in the concentration 2,3,4µg, with 2 replicate analysis for each concentration (red) and with the sample volum 2 µl in 4 replicate analysis (blue)- area evaluation by linear regression . Correlation coefficient obtained is 0,99564.

UV-VIS SPECTROPHOTOMETRIC method – equipment and materials

- CINTRA 101 UV-VIS spectrophotometer
- glass cuvettes 1 cm
- diosgenin (Fluka), 0,01% in methanol;
- sample;
- perchloric acid 70 %;
- absorption measurement at 410 nm.

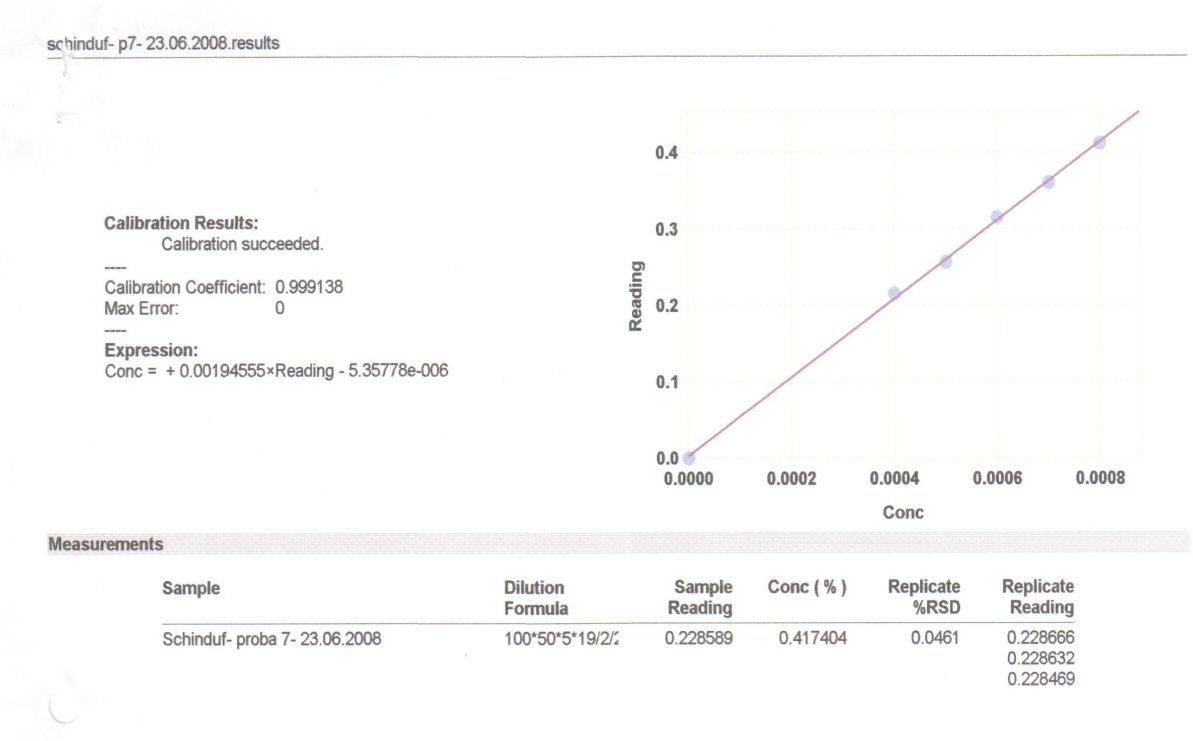


Fig. 8. Calibration curve and analysis report of Diosgenin in sample of *Trigonella foenum graecum* seeds

CONCLUSION

The proposed HPTLC method is rapid, simple and accurate for qualitative and quantitative monitoring of Diosgenin in *Trigonella foenum graecum* seeds.
The qualitative determination stresses that the other phytoconstituents present in the seeds did not interfere with the peak of Diosgenin.
Using HPTLC densitometric method, a content of 017% Diosgenin was separated from 0,417% total saponins content obtained by UV VIS spectrophotometric method.
Therefore the method is specific in separation of Diosgenin from other constituents of *Trigonella foenum graecum* seeds and help to determinate the content of Diosgenin.

2.2. Studies in obtaining of phytotherapeutical bioproducts with hypocholesterolemiant action.
Note 1. The qualitative and quantitative assays of the active principles with hypocholesterolemiant action from Trigonella foenum-grecum, Medicago sativa, Helianthus tuberosus and Vitis vinifera.

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OBJECTIVE

Identification and quantification of bioactive principles: sterolic saponins, aminoacids, polyphenolcarboxylic compounds, oligo and polysaccharides and minerals from Trigonella foenum-grecum, Medicago sativa, Helianthus tuberosus and Vitis vinifera wich recommended them for hypocholesterolemiant action.

Qualitative assay
HPTLC method – equipment and materials

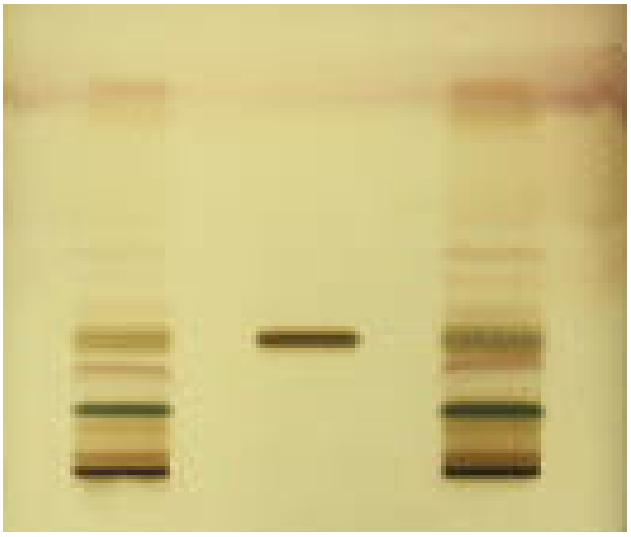
– Automatic TLC Sampler LINOMAT IV CAMAG – application of standard solutions and of samples, band application;
- DIGISTORE 2 Documentation system CAMAG
- HPTLC plates 20 x 20, Silica gel 60 F254 Merck;

Trigonella foenum-grecum, seeds

Composition: sterolic saponins, aminoacids and minerals

- sterolic saponins

Samples: methanolic-chloformic extract from defatted seeds(S1, S2)
Reference substance: diosgenin Fluka 0,1% in methanol
Mobile phase: methylene chloride : acetone
Detection reagent: sulphuric acid 10% in ethanol
Examination: after heated for 10 minutes at 120°C in the oven

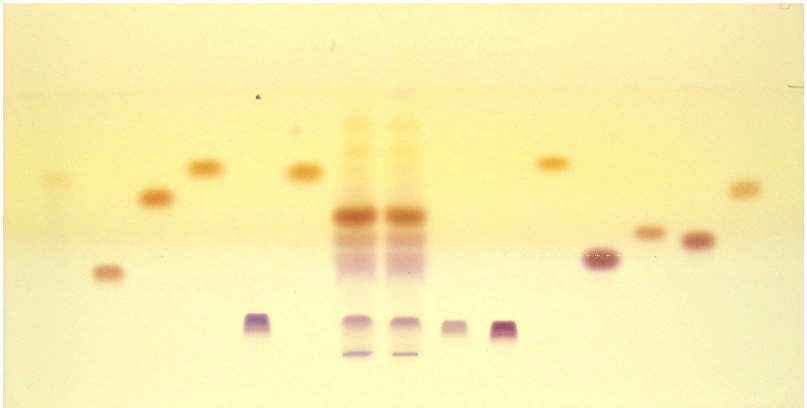


S 1 Diosgenin S 2

Fig.1. HPTLC Chromatogram of Diosgenin standard and trigonella foenum graecum samples

- aminoacids

Samples: isopropanolic-aquos extract from defatted seeds(S1, S2);
Reference substances Fluka : L-tyrosine (track 1), L-proline(track 2), L-valine(track 3), L- leucine (track 4),L-arginine (track 5), L-isoleucine(track 6), L- hystidine (track 9), L-lysine(track 10), L-tryptophan(track 11), glycine (track 12), L- glutamic acid(track 13) ; L-threonine(track 14); L- methionine (track 15) 0,1% in methanol 80%;
Mobile phase: n-buthanol: acetic acid: aqua;
Detection reagent: ninhydrin 0,25% in acetone;



1 2 3 4 5 6 S1 S2 9 10 11 12 13 14 15

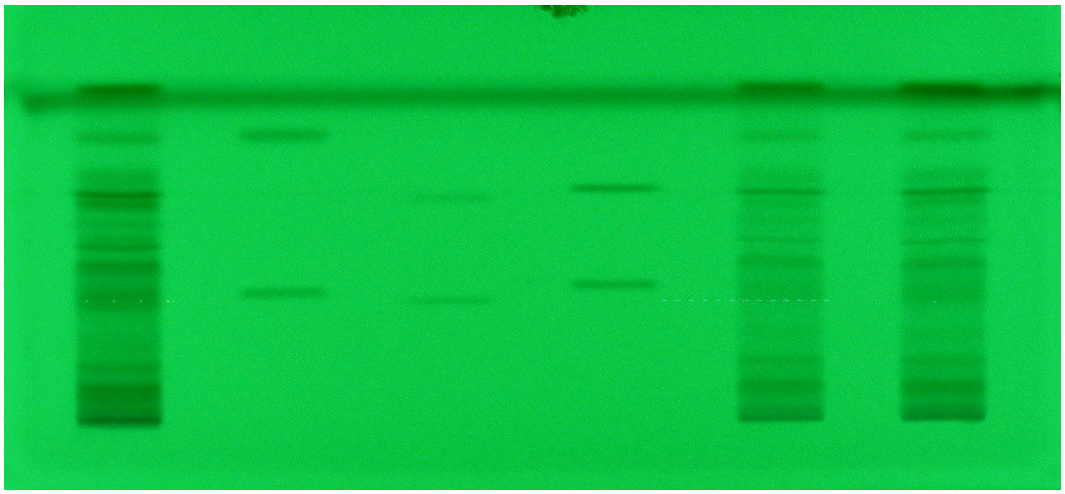
Fig.2. HPTLC Chromatogram of aminoacids standards and trigonella foenum graecum samples

Medicago sativa, aerian parts

Composition: polyphenolcarboxylic compounds(isoflavones), aminoacids and minerals

- isoflavone

Samples: methanolic extract from aerian parts (S1, S2, S3);
Reference substances Fluka : adenosine, biochanin (track 2), daidzin, daidzein (track 3), genistin, genistein (track 4) 0,002% in methanol;
Mobile phase: chloroform: methanol: aqua: acetic acid;
Examination in UV at 254 nm.

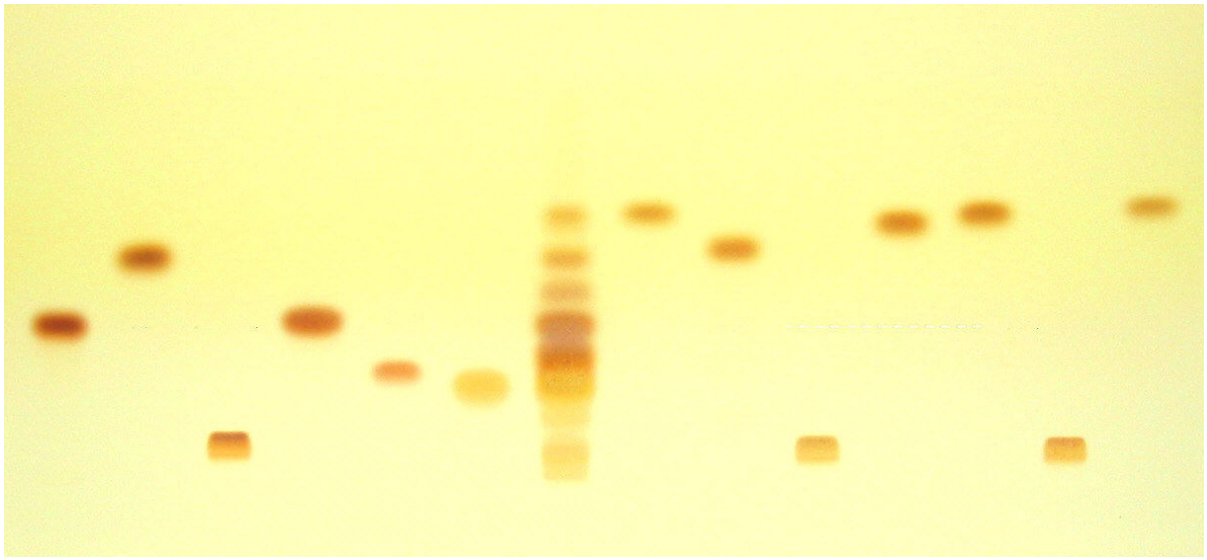


S1 T2 T3 T4 S2 S3

Fig.3. HPTLC Chromatogram of isoflavones standards and Medicago sativa samples

- aminoacids

Sample: methanolic-aquos(50%) extract from aerian parts (S);
Reference substances Fluka : L-treonina (track 1), L-valina (track 2), L-arginina (track 3), L- acid glutamic (track 4), L-prolina (track 5), asparagina (track 6), L- fenilalanina (track 8), L-metionina (track 9), L-histidina (track 10), L-izoleucina (track 11), L-izoleucina (track 12) ; L- lizina (track 13); L-triptofan (track 14) 0,1% in methanol 80%;
Mobile phase:n-buthanol: acetic acid: aqua;
Detection reagent: ninhydrin 0,25% in acetone;



T1 T2 T3 T4 T5 T6 S T8 T9 T10 T11 T12 T13 T14

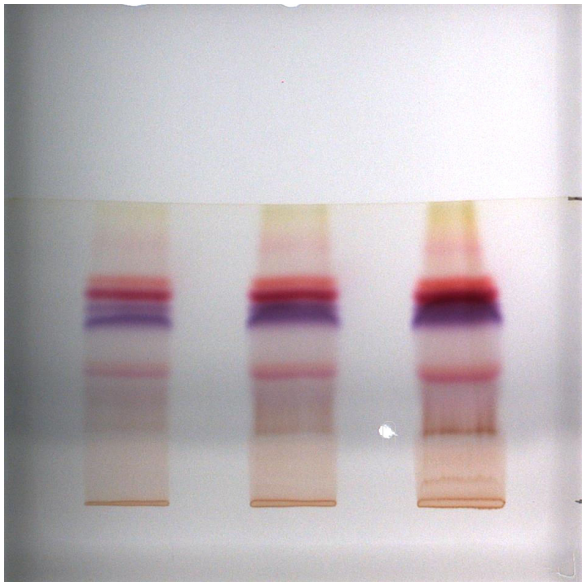
Fig.4. HPTLC Chromatogram of aminoacids standards and Medicago sativa samples

Vitis vinifera, grapes

Composition: polyphenolcarboxylic compounds (anthocyanins) and minerals

- anthocyanins

Sample: acidulated(HCL 1%) methanolic extract from pomace grapes(S1-8µl, S2-11µl, S3-14µl);
Mobile phase: formic acid : n-buthanol;
Examination: after heated for 10 minutes at 120°C in the oven.



S1 S2 S3

Fig.5. HPTLC Chromatogram of anthocyanins from Vitis vinifera-pomace grapes

Helianthus tuberosus, tubers

Composition: oligo and polysaccharides and minerals

- oligo and polysaccharides

Samples: ethanolic extract from tubers (S1, S2)
Reference substance: raffinose pentahydrate + D- froctose (track 1), β- D- lactose + D-maltose+ D- galactose (track 3), sucrose+ D-glucose (track 5) 0,05% in ethanol 50%;
Mobile phase: n-buthanol: acetic acid : aqua;
Detection reagent: anisaldehyde in sulphuric acid;
Examination: after heated for 10 minutes at 120°C in the oven

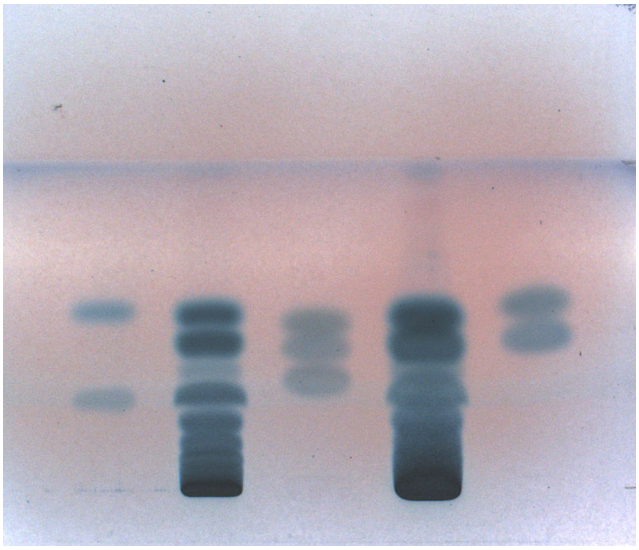


Fig.6 HPTLC Chromatogram of oligo and polysaccharides from Helianthus tuberosus, tubers

Quantitative assay

- UV-VIS SPECTROPHOTOMETRIC method

- equipment - CINTRA 101 UV-VIS spectrophotometer

Medicinal plants	Aminoacids (glutamic acid),%	Sterolic saponins (diosgenin),%	Anthocyanins,%	Polysaccharides,%
Trigonella foenum-grecum, seeds	1,65	0,417	-	-
Medicago sativa, aerian parts	2,7	-	-	-
Vitis vinifera, greeps	-	-	0,58	-
Helianthus tuberosus, tubers	-	-	-	66

Tab.1 Active principles of the analysed samples

- Atomic absorbtion spectrofotometry method

- equipment - SHIMADZU AA-6200 spectrophotometer

Medicinal plants	Mn, ppm	Cu, ppm	Zn, ppm	Fe, ppm	Ca, ppm	Mg, ppm	K, ppm
Trigonella foenum-grecum, seeds	5,6	7,13	11,14	159,58	317.26	399,07	1802,88

Medicago sativa, aerian parts	7,55	2,22	7,02	62,88	2331,83	518,02	7334.11
Vitis vinifera, greeps	2,53	5,46	1,98	41,66	611,54	133,28	2774,06
Helianthus tuberosus, tubers	1,13	3,53	7,84	48,26	139,00	183,72	5815.28

Tab.2 Mineral content of the analysed samples

Conclusions

The results of the study confirm the chemical composition from the four medicinal plants analysed. These results constitute the basis for establishing the optimum parameters for the decisive active principles extraction and for herbal drugs association formulas in order to obtain phytotherapeutical bioproducts with hypocholesterolemiant action.

3. Expoziția realizărilor cercetării româneşti SALONUL CERCETĂRII - 2008
Perioada : 7 – 11 octombrie 2008
Locul desfasurarii: Pavilionul 13, Complexul Expozițional ROMEXPO