Utilization of HPTLC for the Authentication and Identification of Botanical Specimens

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National Center for Natural Products Research

- Research Institute of Pharmaceutical Sciences – 1964
- NCNPR proposal developed in mid 1980s
- USDA-ARS initiated site visit in 1988
- Facility planning funds appropriated, 1989
- Construction 1990 - 2001
- Launch/partial occupancy - July 1995
- USDA-ARS Natural Product Utilization Research Unit (NPURU) established, CRA initiated 1996
- Facility Dedication 1998 – ‘Thad Cochran Research Center’
- FDA/CFSAN CRA 2001
- NCNPR 10th Anniversary - 2005

National Center for Natural Products Research - 2009

Primary Appointments

- ***11 Research Faculty***
- ***22 Research Scientists***
- ***48 Staff***
- ***27 Postdoctoral, Visiting Scientists***
- ***35 USDA/ARS personnel***
- ***30-40 student workers***

NCNPR Programmatic Emphases

Natural Product Discovery and Development

- Natural Product Sourcing/Repository
- Pharmaceutical
- Agrichemical

Medicinal Plant Research

- Phytochemistry
- Botanical Supplements
- Agronomics
- Cannabis Chemistry, Production, Analysis

Natural Resources and Environment

- Center for Water and Wetlands Resources
- Environmental Toxicology
- Ocean Biotechnology Center and Repository

Drug Discovery

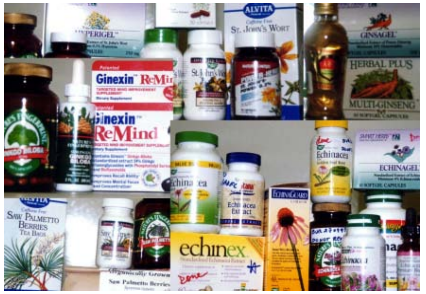
Dietary Supplements

Agrichemicals

Medicinal Plant Research

- BOTANY
- HORTICULTURE
- AGRONOMICS
- PLANT GENOMICS
- PLANT TISSUE CULTURE
- NATURAL PROD. CHEMISTRY
- CHEMOTAXONOMY

- ETHNOMEDICINE/BOTANY
- ANALYTICAL CHEMISTRY
- REFERENCE STANDARDS
- BIOLOGICAL ASSAY
- INFORMATICS
- TOXICOLOGY
- ADME PROFILING



NEW or IMPROVED CROPS
NEW COMPOUND SOURCES
SAFER, MORE EFFECTIVE DIETARY SUPPLEMENTS


50,000 – 70,000 medicinal and aromatic plants are estimated to be used world-wide.


Some 3,000 species of medicinal and aromatic plants are traded internationally.
HERBA CENTAUR
HERBA THYMI
WELEDA
Arnika

“Dietary Supplement’s” in the US

Regulated by the FDA under the Dietary Supplement Health and Education Act of 1994 (DSHEA).

- Classifies what the rest of the world considers “Traditional Medicine” as Dietary Supplements.
- The FDA’s main concern for Dietary Supplements is safety.

Recent, cGMP regulations (21 CFR § 111) require that the manufacturers of dietary supplements provide 100% identity for botanical ingredients within a product.


PERIGEL™
Extract of St. John's Wort
um 0.3% Hypericin
APSULES
250 mg
30 SOFTGELS
ALVITA
Caffeine Free
ST. JOHN'S WORT
GINSAGEL
Standardized Extract of Panax Ginseng
Minimum 14% Ginsenosides
FTGEL CAPSULES
HERBAL PLUS
MULTI-GINSENG
30 SOFTGEL CAPSULES
Patented
Ginexin™ ReMin
TARGETED MIND IMPROVEMENT SUPPLEMENT†
Dietary Supplement
Ginexin™
ReMind
RGETED MIND IMPROVEMENT SUPPLEMENT
etary Supplement
Contains Ginexin™ Ginkgo Biloba
standardized extract 24% Ginkgo
Flavonglycosides with Phosphatidyl Serine
and Bioflavonoids
mproves Recall Ability†
mproves Mental Focus
and Concentration†
NATURE'S FINGERPRINT
GINKGO BILOBA
SUBLINGUAL
2 FL. OZ. (60 ml)
Echinacea
Golden Seal Root
ECHINAGEL
Standardized Extract of Echina
Minimum 4% Echinacoside
30 SOFTGEL CAPSULES
ECHINAGUARD
LIQUID
Echinacea Extract
echinex
Standardized Echinacea Extract
Dietary supplement to
support natural resistance
against infection
ALVITA
Caffeine Free
SAW PALMETTO
BERRIES
TEA BAGS
SAW PALMETTO
Organically Grown
Saw Palmetto Berries
Serenoa repens
ECHINACEA

FDA-UM Center of Excellence

CFSAN has responsibility for safety of dietary supplements, including botanicals

The NCNPR on its third 5-yr cooperative research agreement cycle to study botanical dietary supplemnts.

Recognized as 1 of 4 ‘Centers of Excellence’ for the FDA

- The only one established for botanical dietary supplement work

In June 2009 the NCNPR was awarded FDA Commissioner’s Special Citation

FDA-UM Center of Excellence

- Specific Aims
  - Identify botanical dietary supplements (BDS) of priority concern to FDA from a public safety perspective and determine research needs.
  - Acquire and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA for evaluation of safety.
  - Exchange technical and scientific information, methods and reference material with FDA scientists. Collaborate with FDA scientists in research areas of mutual interest.
  - Hosts an Annual International Conference on the Science of Botanicals (ICSB) in Oxford, MS. 11th ICSB, April 16th-19th 2012. www.OxfordICSB.org.
  - Training of US FDA CFSAN inspectors - cGMP (21 CFR § 111).

Factors that Affect Botanical Product Quality

- **Adulteration** - Intentional
- **Substitution** - Accidental/inadvertent
  - Other plant species
  - Other plant parts
- **Contaminiation**
  - Other foreign matter
  - Heavy metals and pesticides
  - Microbial/microtoxins

FDA's Botanical Dietary Supplement Center of Excellence

Plant Sourcing

ADMET and Safety Profiling

Classical Identification

Phytochemical Profiling

Genetic Profiling

Utility of HPTLC in Botanical Dietary Supplements

Ideal analytical tool for the rapid analysis of complex herbal mixtures

Offers the ability to present the results as an image

Can be used for qualitative as well as quantitative analyses

- **Qualitative analysis**
  - **Fingerprinting**
  - **Screening**
  - **Identification**

- **Quantitative analysis – Precise marker determination**

Adaptable – single sample or multiple (parallel) samples

Advantages of HPTLC:

Simplicity and cost efficiency

Parallel analysis of multiple samples is possible

Sample capacity is high and results are rapid

Multiple detection options

It is an ideal tool for screening, identification, quantification and determination of adulteration of botanical products

Can provide a precise method for robust routine analysis

Disadvantages:

The separation power of HPTLC is lower than that of HPLC and UPLC. Particularly for complex samples like botanicals it is often difficult to achieve sufficient resolution for all components

TLC is an open system. The plate is exposed to environmental and climatic factors (temperature, light, fumes) and chemical/mechanical stress.

Volatile and sensitive samples require special care. In order to achieve reproducible results.

HPTLC for Identification of Chemical Constituents from Botanicals and Dietary Supplements

- **HOODIA**
- **BLUE COHOSH**
- **CHAMOMILE**
- **ACAI**

Chemical fingerprint of *Hoodia* species, dietary supplements, and related genera

Scientific Name: ***Hoodia gordonii***

Family: **Asclepiadaceae**

Reported Active Principle: **P57 (appetite suppressant)**

Origin: **South Africa, Namibia and Botswana**

Major Class of Compounds: **Pregnane Glycosides**

Purpose: **Authentication and Standardization of** ***Hoodia gordonii***

C. S. Rumalla et al /J. Sep. Sci.31(2008) 3959-3964.

Sample Preparation: Dry plant samples (0.3 g) or an adequate amount of powdered products extracted with 10 mL methanol. The supernatant was used for analyses.

Standards: pregnane glycosides related to P57: hoodigoside M (1), hoodigoside L (2), hoodigoside P (3), hoodigoside U (4), hoodigoside O (5), hoodigoside E (6), hoodigoside F (7), hoodigoside J (8), hoodigoside N (9), P57 (10), and hoodigoside C (11)

Stationary phase : HPTLC Glass plates with silica gel 60F254 (Merck, Darmstadt, Germany)

Mobile phase: Dichloromethane/methanol/water (75:17:2.2)

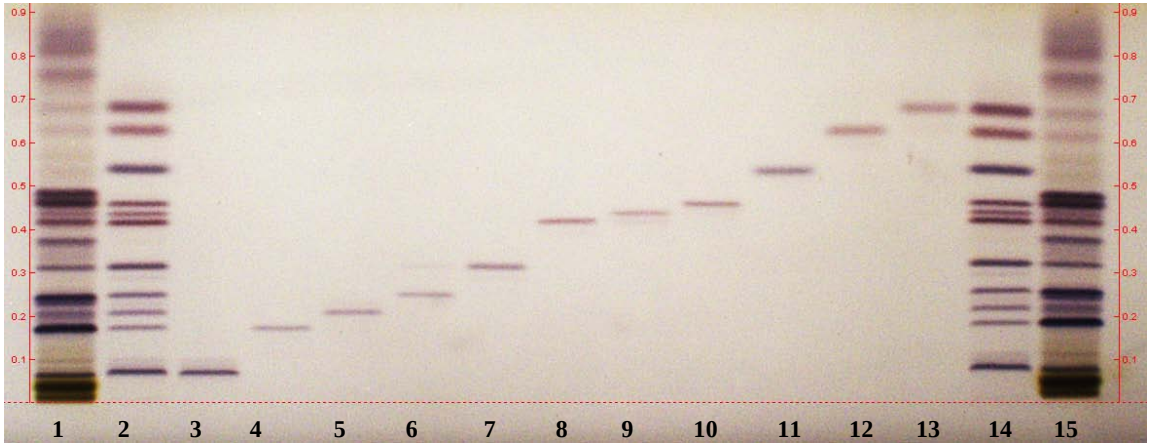
Sample application: All samples were applied according to the following settings: 8 mm from the bottom of the plate, band width 8 mm; distance between bands 10 mm; application volume 1–4 µL

Derivatization reagent : Anisaldehyde reagent

C. S. Rumalla et al /J. Sep. Sci.31(2008) 3959-3964.

Results

Method developed for standard compounds and Image after derivatization with anisaldehyde reagent white light.

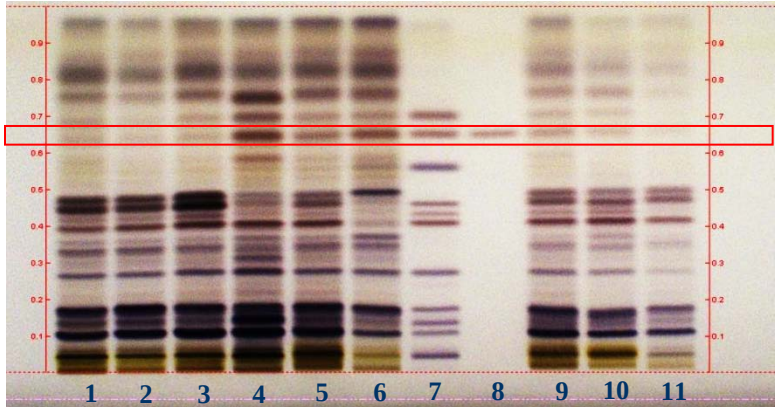


Tracks : 1, 15, *H. gordonii*; 2,14, Std Mix 1-11; 3, Hoodigoside M; 4, Hoodigoside L; 5, Hoodigoside P; 6, Hoodigoside U; 7, Hoodigoside O; 8, Hoodigoside E; 9, Hoodigoside F; 10, Hoodigoside J; 11, Hoodigoside N; 12, P57; 13, Hoodigoside C.

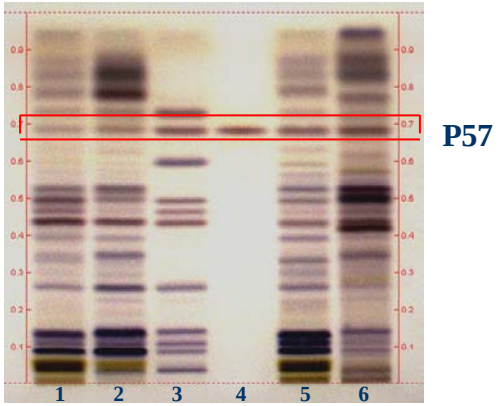
C. S. Rumalla et al /J. Sep. Sci.31(2008) 3959-3964.

Method applied for different populations of *Hoodia gordonii* and various species of *Hoodia*

Image after derivatization with anisaldehyde reagent under white light.



Tracks : 1-6, *H. gordonii*; 7, Std Mix-11; 8, P57; 9-11, *H. gordonii*

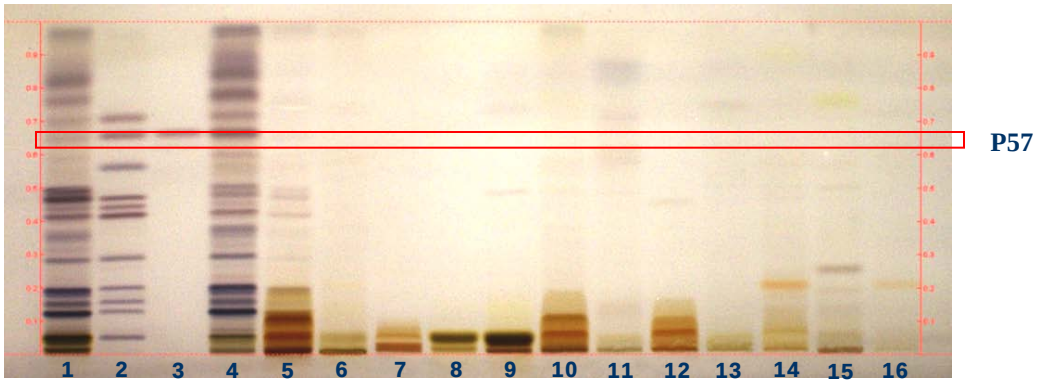


Tracks : 1, *H. gordonii*; 2, *H. currorii*; 3, Std Mix-11; 4, P57; 5, *H. ruschii*; 6, *H. parviflora*

C. S. Rumalla et al /J. Sep. Sci.31(2008) 3959-3964.

Method applied for dietary supplements claiming to contain *H. gordonii*

Image after derivatization with anisaldehyde reagent white light

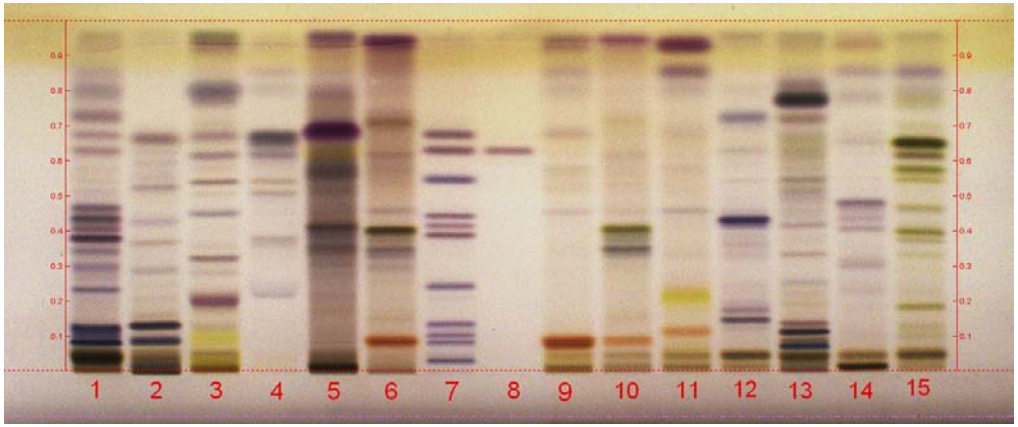


Tracks : 1, *H. gordonii*; 2, Std Mix-11; 3, P57; 7, P57; 4-16, dietary supplements

C. S. Rumalla et al /J. Sep. Sci.31(2008) 3959-3964.

Method applied for *Hoodia gordonii* with other related genera

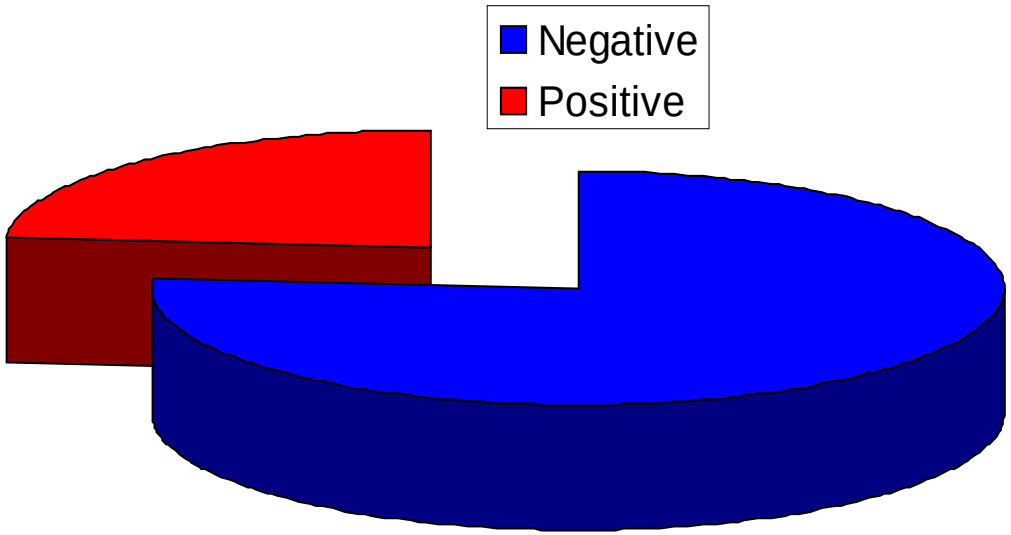
Image after derivatization with anisaldehyde reagent white light



Tracks : 1, *H. gordonii*; 2, *Caralluma fimbriata*; 3, *Opuntia ficus-indicus*; 4, *Orbea variegata*; 5, *Cynanchum stratum*; 6, *Gonolobus cundurango*; 7, Std Mix-11; 8, P57; 9, *Gymnema sylvestris*; 10, *Marsdemia cundurango*; 11, *Asdepias labriformis*; 12, *Huernia recondita*; 13, *Tridentea choanantha*; 14, *Ceropegia dichtoma*; 15, *Stapelia flavirostris*

C. S. Rumalla et al /J. Sep. Sci.31(2008) 3959-3964.

Number of sample analyzed for the Presence of P57



Total Dietary Samples = 151
Positive Dietary Samples = 37
Negative Dietary Samples = 114

Conclusions

The method is suitable for rapid, decisive authentication, and comparison of the subtle differences among samples from various plant sources.

The chemical fingerprint method developed in this study was able to verify the presence of *Hoodia* species in raw materials and commercial products.

HPTLC Identification of Chamomile

Roman Chamomile

Latin name: *Anthemis nobilis* syn. *Chamaemelum nobile*

Family: Astéracées
Part Used: Flowerheads

anthemis.nl

German Chamomile

Species: *Matricaria recutita* L.

Synonym(s): *Chamomilla recutita* (L .) Rauschert, Hungarian Chamomile, *Matricaria chamomilla* L., Matricaria Flowers, Wild Chamomile

Family: Asteraceae/Compositae
Part Used: Flowerheads

http://natureasmedicine.wordpress.com

Sample Preparation: Dry plant samples (0.5 g) or an adequate amount of powdered products extracted with 10 mL methanol. The supernatant was used for analyses.

Standards: Rutin; chlorogenic acid; hypersoside; apigenin -7-*O*-glucoside; luteolin; caffeic acid; daidzein; kaempferol; apigenin; umbelliferon; biochanin A; herniarin

Stationary phase : HPTLC Glass plates with silica gel 60F254 (Merck, Darmstadt, Germany)

Mobile phase: CH_2Cl_2: MEOH: Formic Acid: Acetic Acid:: 11.0 : 1.5 : 1.25 :1.25

Sample application: All samples were applied according to the following settings: 8 mm from the bottom of the plate, band width 8 mm; distance between bands 10 mm; application volume 3 µL

Derivatization reagent :1% methanolic diphenylboric acid-β-ethylamino ester reagent.

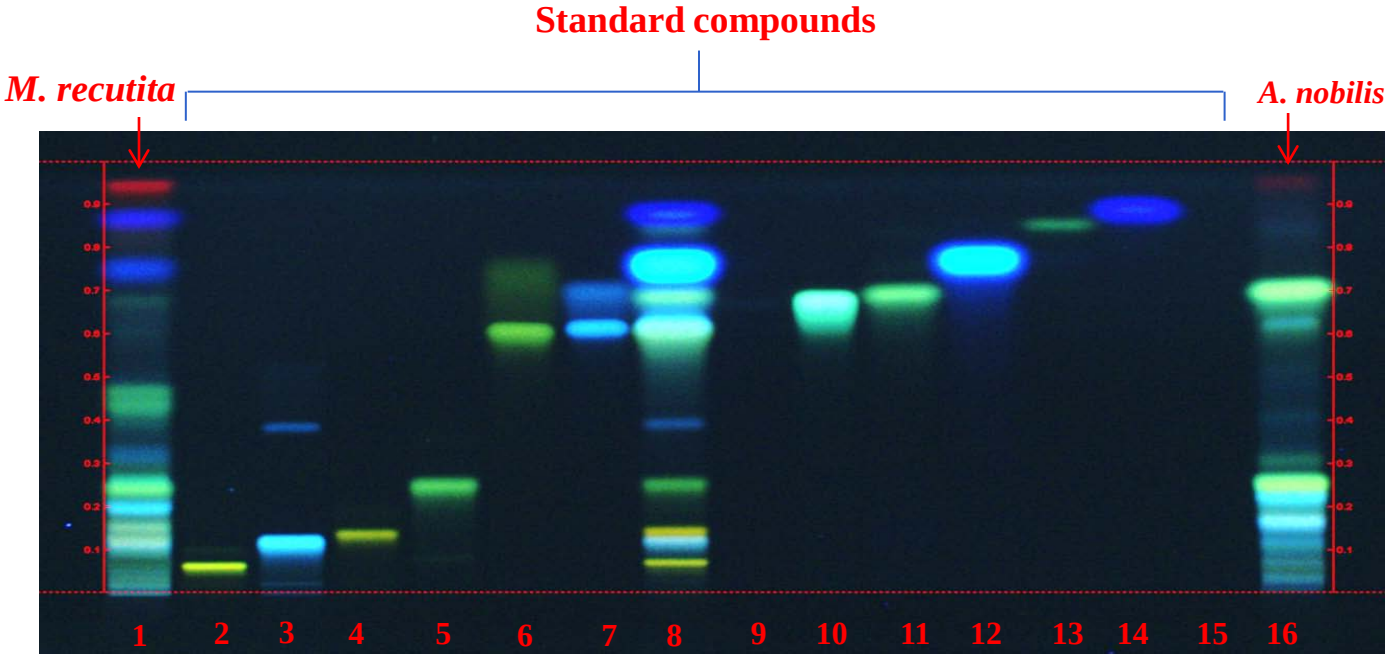
Chemical Constituents:

German chamomile flowers contains up to 8% flavone glycosides (apigenin-7-glycoside and its 6'-acetylated derivative) and flavonols (luteolin glucosides, quercetin glycosides, and isohamnetin)

Roman chamomile flowers contains 0.5 % flavonoids mainly in the glycosidic form (Anthemoside (apigenin-2,3-dihydorycinnamoyl acid 7-*O*-β-D-glucose), cosmosiosid (apigenin-7-*O*-β-D-glucose), apiin (apigenin-7-*O*-β-D-apiosylglucoside) and chamaemeloside [apigenin-7-*O*-β-D-glucose-6″-(3‴-hydroxy-3‴-methyl-glutarate)], luteolin-7-*O*-β-D-glucose, quercetin-3-*O*-α-L-rhamnoside and kaempferol; coumarins, anthemic acid

Results

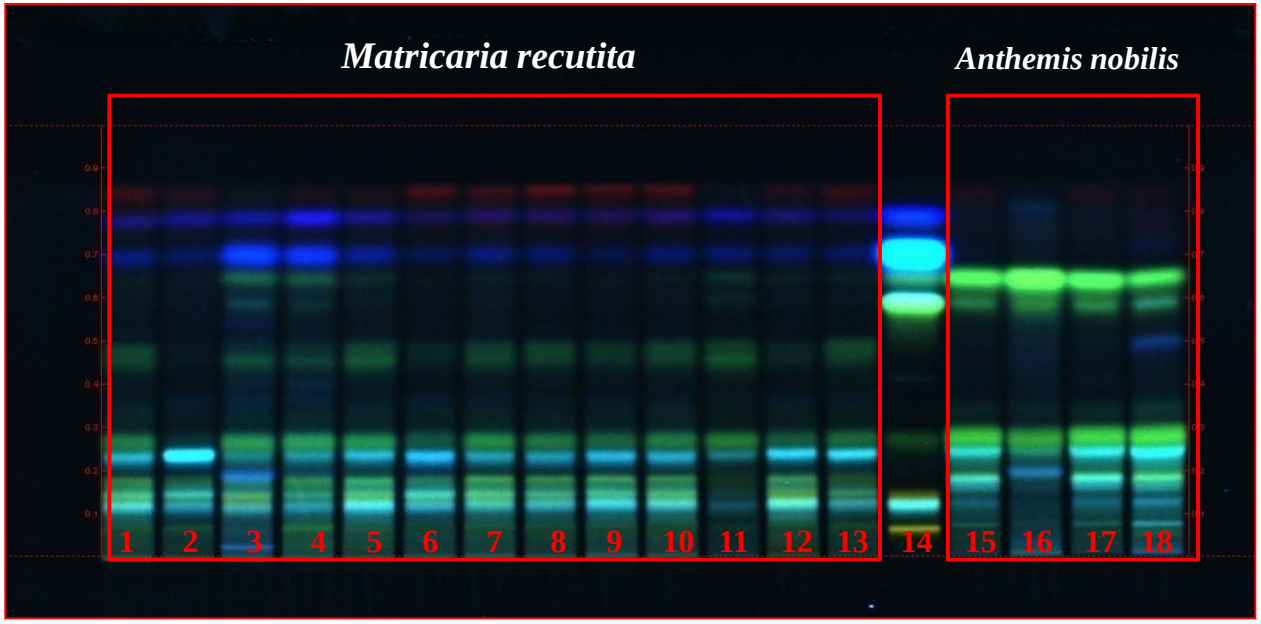
Method developed for standard compounds and Image after derivatization with 1% methanolic diphenylboric acid-β-ethylamino ester reagent under 366 nm



Track-1: 9172: *Matricaria recutita*; track-2: rutin; track-3: chlorogenic acid; track-4:hypersoside; track-5: apigenin -7-*O*-glucoside; track-6: luteolin; track-7: caffeic acid; track-8: Std. Mix-13; track-9: daidzein; track-10: kaempferol; track-11: apigenin; track-12: umbelliferon; track-13: biochanin A; track-14: herniarin; track-15: 2-bisabolol; track-16: 9254: *Anthemis nobilis* under 366 nm after derivatization with % methanolic diphenylboric acid –β-ethylamino ester reagent

Method applied for different populations of *M. recutita* and *A. nobilis*

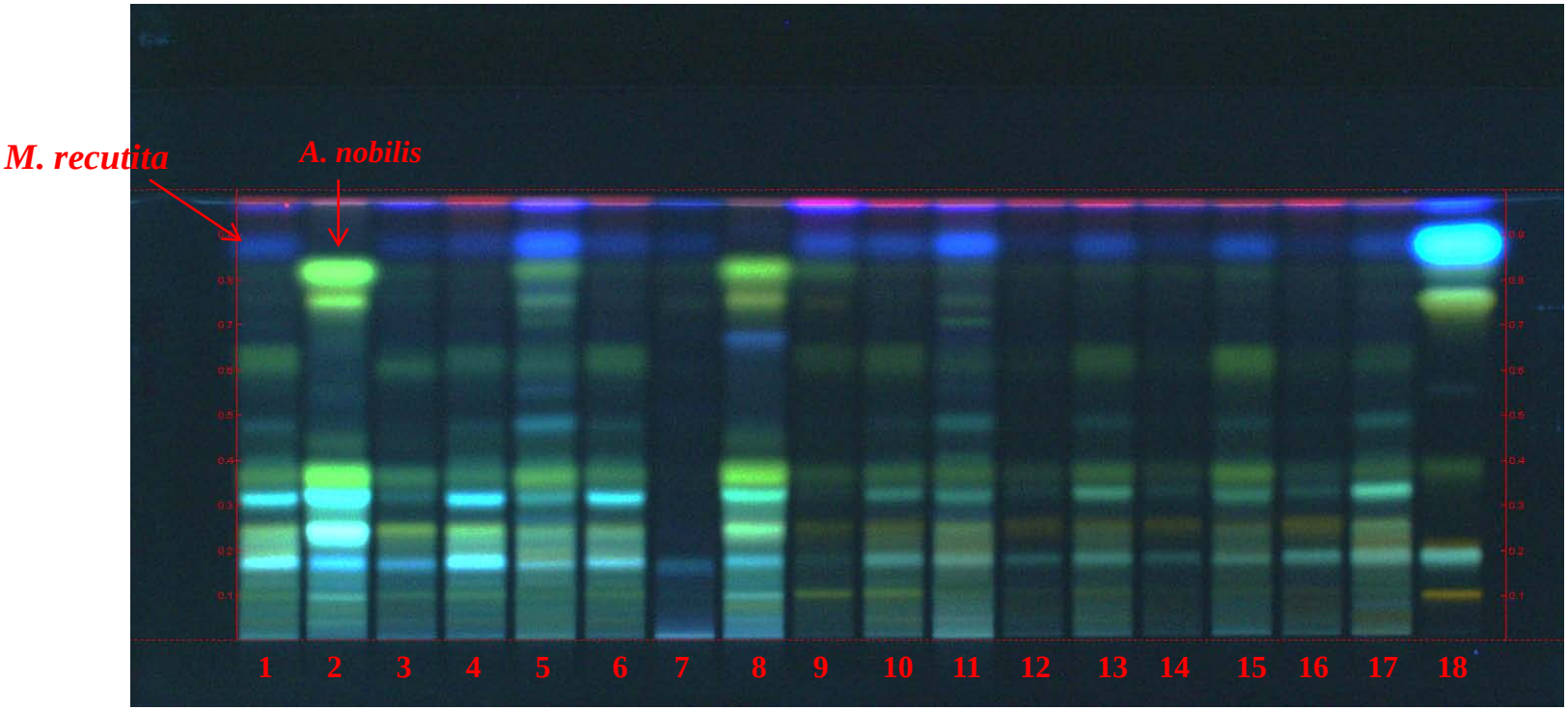
Image after derivatization with 1% methanolic diphenylboric acid –β-ethylamino ester reagent under 366 nm



Comparison of Chamomile samples: Tracks : 1. 9172: *Matricaria recutita* (V); 2. 9334: *Matricaria chamomilla* (CS); 3. 7539: *Matricaria chamomilla* (V); 4. 259: *Matricaria recutita* (V); 5. 2802: *Matricaria recutita* (V); 6. 9362: *Matricaria recutita* (A); 7. 7390: *Matricaria recutita* (CS); 8. 9364: *Matricaria recutita* (A); 9. 9359: *Matricaria recutita* (A); 10. 9172: *Matricaria recutita* (V); 11. 4903: *Matricaria recutita* (CS); 12. 9357: *Matricaria* spp (CP); 13. 9387: *Matricaria recutita* (CP); 14. Std. Mix; 15. 9254: *Anthemis nobilis* (CS); 16. 2061: *Anthemis nobilis* (V); 17. 9254: *Chamaemelum nobile* (CS); 18. 3074: *Chamaemelum nobile* (CS).

Application for dietary supplements claiming to contain *chamomile*

Image after derivatization with 1% methanolic diphenylboric acid –β-ethylamino ester reagent under 366 nm



Comparison of Chamomile samples: Tracks : 1. 9172: *Matricaria recutita*; 2. 9254: *Anthemis nobilis*; 3. 9367: *M. recutita* (CP); 4. 9365: *M. recutita* (A); 5. 5770: *M. recutita* (CS); 6. 9388: *M. recutita* (CP); 7. 3998: *M. chamomilla* (CS); 8. 3076: *A. nobilis* (CS); 9. 9361: *M. recutita* (CP); 10. 9390: *M. recutita* (CP); 11. 3670: *M. recutita* (CS); 12. 9384: *Matricaria* spp (CP); 13 9391: *M. recutita* (CP); 14. 9386: *Matricaria* spp (CP); 15. 9385: *M. recutita* (CP); 16. 9389: *M. recutita* (CP); 17. 9357: *Matricaria* spp (CP) ; 18. Std. Mix

Conclusions

The method is suitable for rapid, decisive authentication, and comparison of the subtle differences among samples from German chamomile and Roman chamomile

The chemical fingerprint method developed in this study was able to verify the presence of German chamomile and Roman Chamomile in products

***M. recutita* and *A. nobilis* samples showed different chemical fingerprinting as shown in previous figures.**

HPTLC fingerprint analysis of Blue cohosh

Scientific Name: ***Caulophyllum thalictroides***

Family: Berberidaceae

Origin: America

Major Class of Compounds: Triterpenoids

Use: To induce labor, regulate menstrual flow, suppress menstruation, and ease the pain and difficulty that accompany childbirth

http://en.wikipedia.org/wiki/Caulophyllum_thalictroides

Sample Preparation: Dry plant samples (0.3 g) or an adequate amount of powdered products extracted with 10 mL methanol. The supernatant was used for analyses.

Standards: Cauloside H (**1**), Cauloside G (**2**), Magniflorine (**3**), Leonticin D (**4**),Cauloside D (**5**), Cauloside C (**6**), saponin PE (**7**), Cauloside B (**8**) and Cauloside A (**9**)

Stationary phase : HPTLC Glass plates with silica gel 60F254 (Merck, Darmstadt, Germany)

Mobile phase: Chloroform: methanol: water (65:35:10.5 *v/v/v*)

Sample application: All samples were applied according to the following settings: 8 mm from the bottom of the plate, band width 8 mm; distance between bands 9.7 mm; application volume 1–4 µL

Derivatization reagent : Anisaldehyde reagent

Results

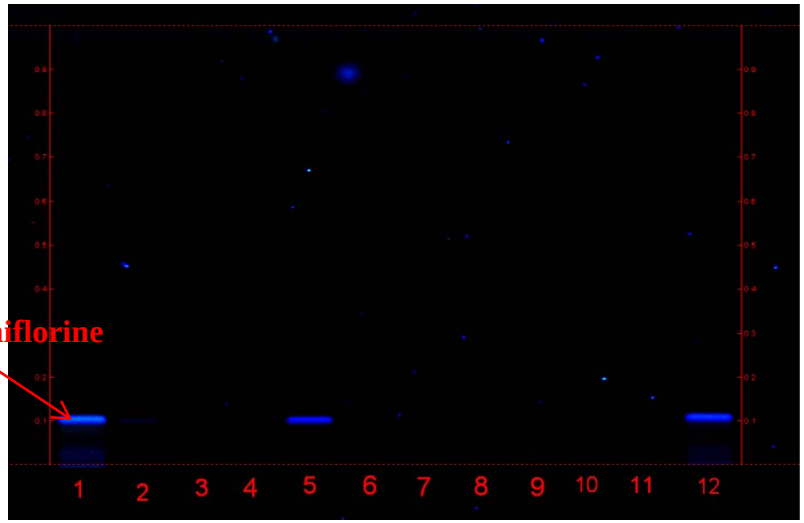
Method developed for standard compounds (Triterpenoids)

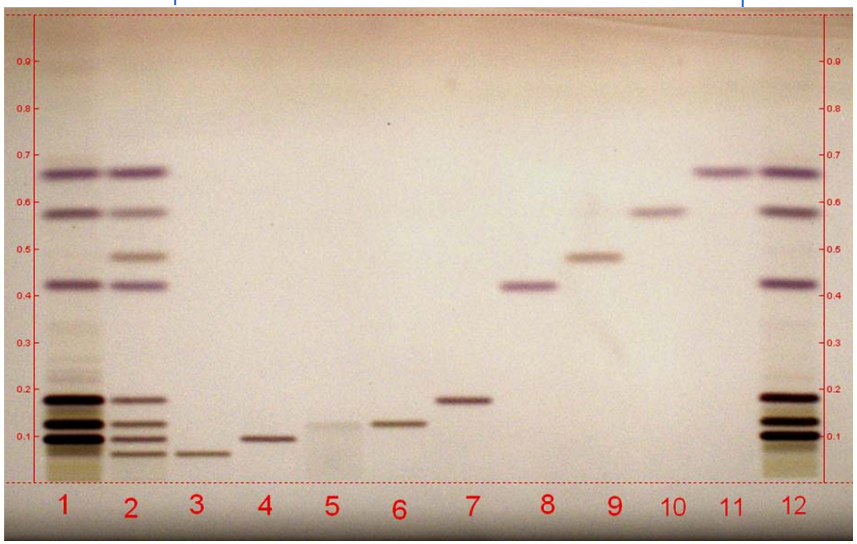
Image under 366 nm before derivatization

Image after derivatization with anisaldehyde reagent under white light.

Track 1& 12. Plant sample Blue cohsoh

Standard compounds



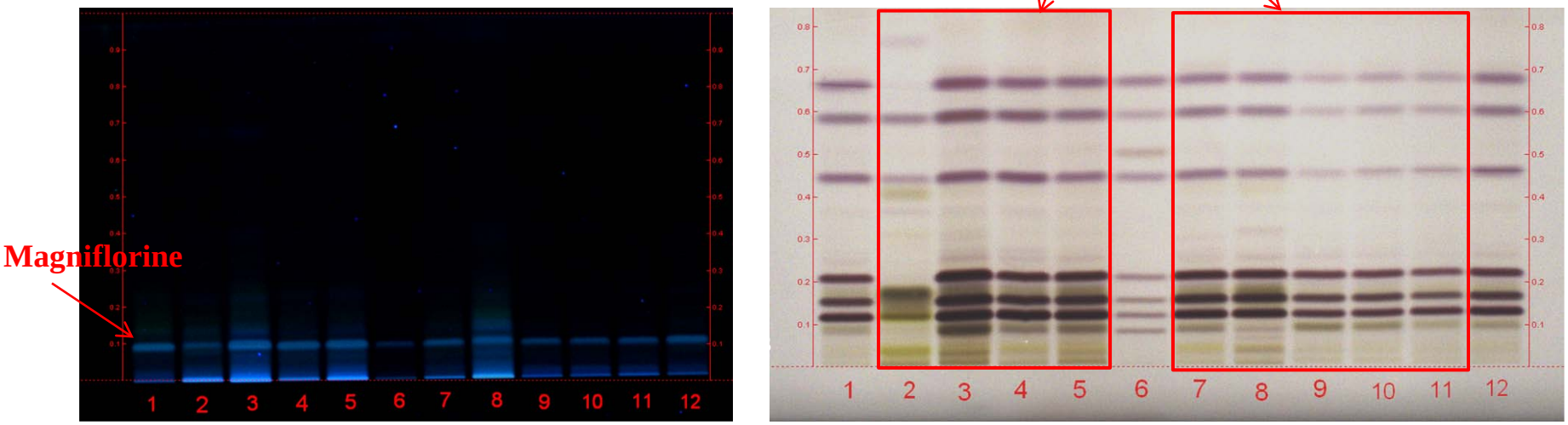


HPTLC fingerprint for standard compounds. Track assignment: 1& 12. Plant sample;
2. Standard mixture-9; 3 -11. Standard compounds (Cauloside H (**1**), Cauloside G (**2**), Magniflorine (**3**), Leonticin D (**4**),Cauloside D (**5**), Cauloside C (**6**), saponin PE (**7**), Cauloside B (**8**) and Cauloside A (**9**)

Method developed for dietary supplements claiming to contain blue cohsoh

Image under 366 nm before derivatization

Image after derivatization with anisaldehyde reagent under white light.



HPTLC fingerprint of (a. Before derivatization for magniflorine at 366 nm; b. After derivatization with anisaldehyde reagent for triterpenoids) various products of Blue cohosh samples. Track assignment: 1 & 12. Plant sample; 2 - 5 & 7 - 11; 6. Standard mixture 9 [increasing R_f values of Cauloside H (1), Cauloside G (2), Magniflorine (3), Leonticin D (4), Cauloside D (5), Cauloside C (6), saponin PE (7), Cauloside B (8) and Cauloside A (9)]

Conclusions

The results showed, well resolved spots for targeted compounds.

The analysis of nine dietary products showed variation in the relative intensities of the separated zones, but the profile was found to be same in comparison with the plant samples.

The developed method is useful for the authentication of *Caulophyllum thalictroides* and screening of blue cohosh products.

Analysis of Anthocyanins from Acai (*Euterpe oleracea* Mart.) Berries and commercial products using HPTLC

Scientific Name: ***Euterpe oleracea***

Family: Arecaceae

Origin: Central and South America

Major Class of Compounds: Anthocyanins and Anthocyanidins

Use: Food or beverage

http://www.free-trial-acai-berry.com/wp-content/uploads/2009/10/acai-berry-tree.jpg&imgrefurl

Sample Preparation: Dry plant samples (0.5 g) or an adequate amount of powdered products extracted with 10 mL [methanol: water (9: 1)] The supernatant was used for analyses.

Standards: : cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside

Stationary phase : HPTLC Glass plates with silica gel 60F254 (Merck, Darmstadt, Germany)

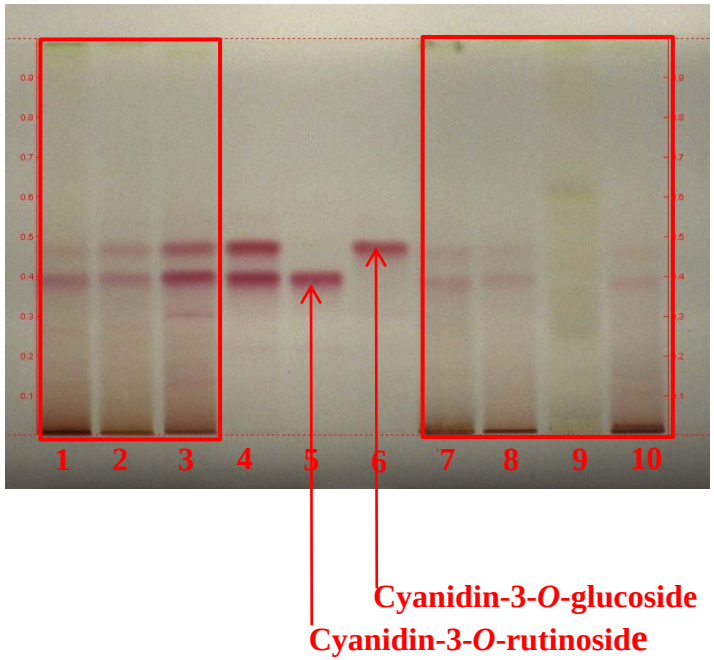
Mobile phase: Ethyl acetate: formic acid: acetic acid: water (10:1.1:1.1:2.6 *v/v/v/v*)

Sample application: All samples were applied according to the following settings: 8 mm from the bottom of the plate, band width 8 mm; distance between bands 9.7 mm; application volume 1–4 µL

Method developed for commercial products claiming to contain Acai berries

Image under white light.

Acai berries track-1-3



commercial products

Track-1: berries of Acai; track-2-3: commercial samples of berries; Track-4: standard mix-2;track-5: cyanidin-3-*O*-rutinoside; track-6: cyanidin-3-*O*-glucoside; track 7-10: commercial products claiming to contain acai under white light.

CONCLUSIONS

HPTLC method was successfully used for the determination of both cyanidin-2-*O*-rutinoside (1) and cyanidin-2-*O*-gulcoside (2) in various commercial products.

The method was validated for precision, accuracy and repeatability.

The method was applied to the six dietary supplements claiming to contain *E. oleracea* and the percentage content varied from 0.015 to 0.30% and 0.014 to 0.26% of compounds 1-2 respectively for four products. Two dietary products did not show for the presence of two major compounds.

Developed method is very simple, precise, and rapid. It can be used for routine analysis for anthocyanins in acai containing commercial products.

CONCLUSIONS

HPTLC provides the ability to display the fingerprint of several unknown samples compared to that of reference/authenticated samples. This procedure can be applied for raw materials, extracts and finished products.

HPTLC fingerprints based on multiple detection of the same plate can provide a very characteristic visual impression to help to identify samples.

While HPTLC usually shows less resolution as compared to GC, HPLC or UPLC chromatograms, such fingerprints are still sensitive to minor differences between samples.

HPTLC can be utilized to effectively identify a large number of samples in parallel affording rapid results.

Collaborations

- The NCNPR has formal agreements with several international academic and governmental institutions including:
  - Pakistan
  - India (FRLHT)
  - South Africa
  - Central/South America
  - Vietnam
  - Empresa Brasileira de Pesquisa Agropecuá (EMBRAPA)
  - Sino-US TCM Research Center in 2007
  - Indo-US Center for Research in Indian Systems of Medicine (CRISM - www.CRISM.net) in 2008

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UNIVERSITY, MISSISSIPPI

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Society for Medicinal Plant and Natural Product Research (GA),
American Society of Pharmacognosy (ASP)
Ministry of Indigenous Medicine, Sri Lanka
The Korean Society of Pharmacognosy

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U.S. Dept. of Defense
State of Mississippi
Research Institute of Pharmaceutical Sciences
National Institute of Undersea Technology
Center for Water and Wetlands Resources
Department of AYUSH and CSIR (IIIM), India


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