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Simple Densitometric-TLC Analysis of Non-Chromophore Containing Bioactive Constituents in Medicinal Plant Extracts

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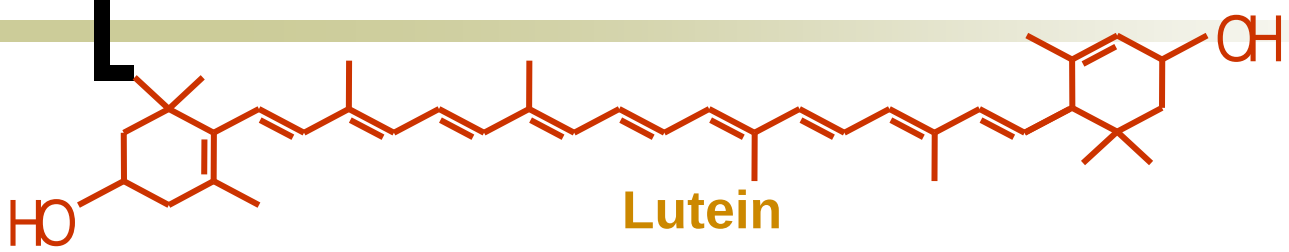
Simple Densitometric-TLC Analysis of Non-Chromophore Containing Bioactive Constituents in Medicinal Plant Extracts

Densitometric TLC technique has been used successfully in Thailand for the analysis of active constituents in medicinal plants and heath products:

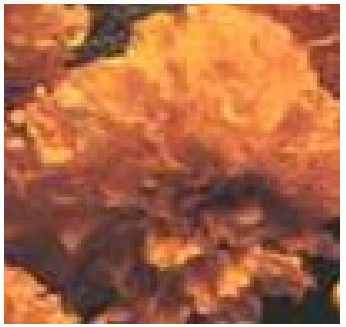
- Plaunotol (an acyclic diterpenoid) in *Croton stellatopilosus* leaves
- Curcuminoids in various *Curcuma* species
- Alliin (a sulfur-containing amino acid) in garlic cloves and products
- Lutein (a xanthophyll) in marigold (*Tagetes erecta*) flowers
- Artemisinin (a sesquiterpene lactone) in *Artemisia annua* leaves
- Asiaticoside (a triterpene glycoside) in *Centella asiatica leaves*
- Etc.

Densitometric TLC for Xanthophyll Analysis in Marigold Flower Extracts



Lutein

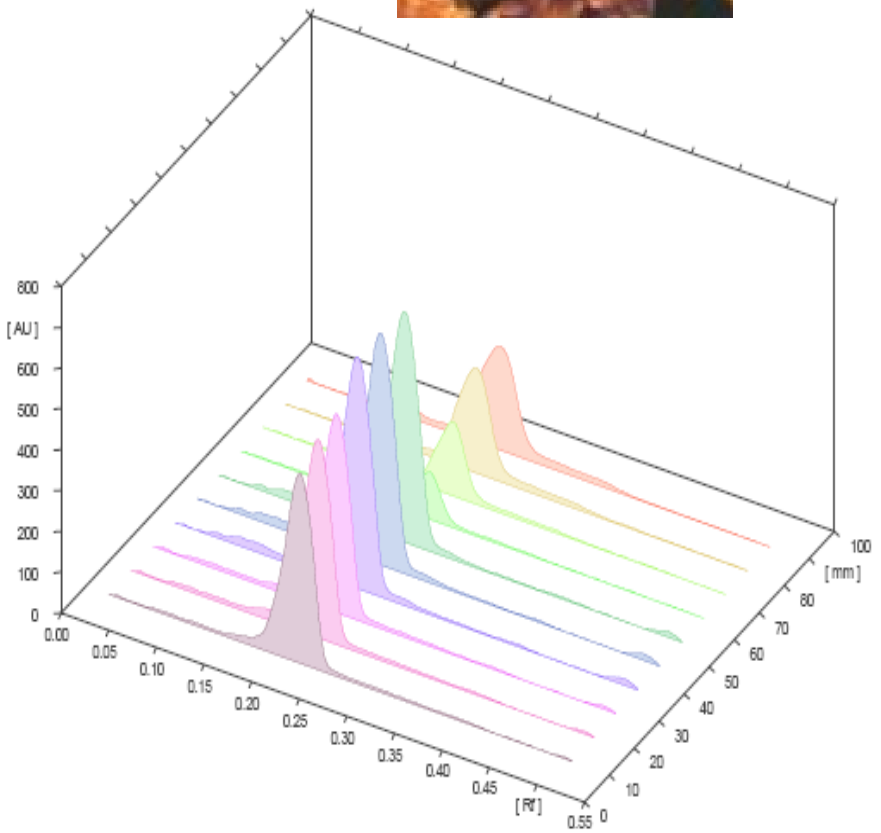
a xanthophyll in marigold (*Tegetes erecta*) flowers



Sample: marigold extracts (hexane : acetone : ethanol = 10:7:6)
Mobile Phase:
Hexane : ethyl acetate : methanol = 10:3:1
Stationary Phase: silica gel
Scan Wavelength: 434 nm

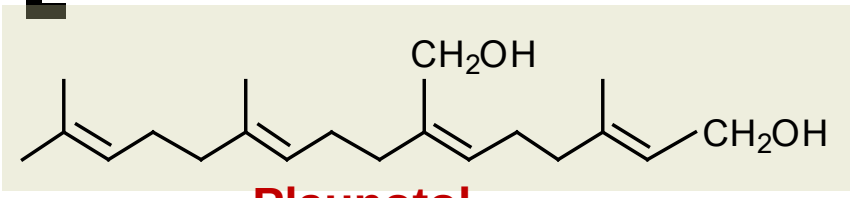
Samples

Lutein standard solutions



Production of Marigold Powder and Extracts in Thailand

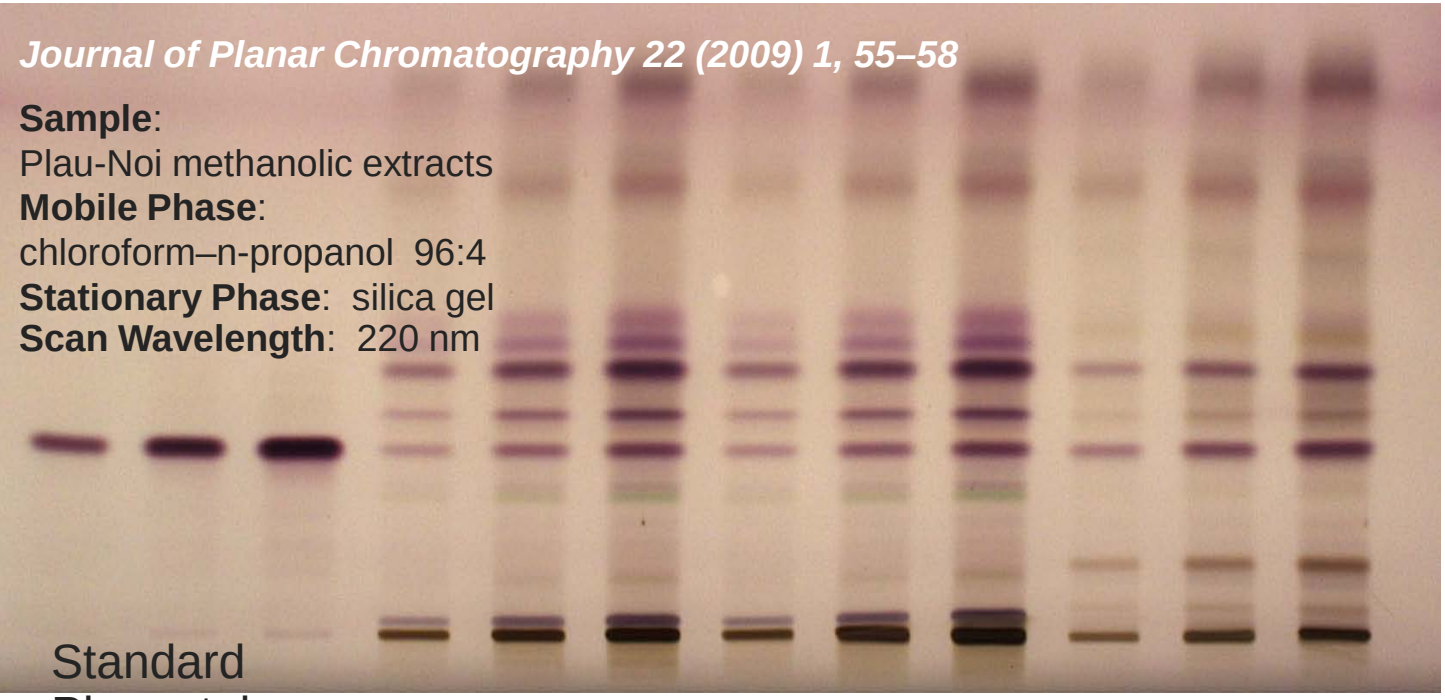
Densitometric TLC for Plaunotol Analysis in *Croton stellatopilosus* Extracts



Plaunotol

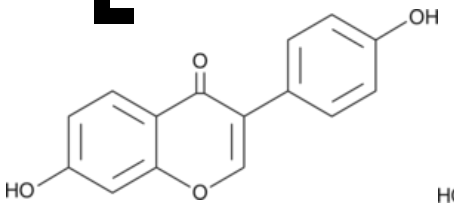
acyclic diterpene alcohol in
Croton stellatopilosus (Plau-Noi) leaves

Kelnac for Antipeptic Ulcer

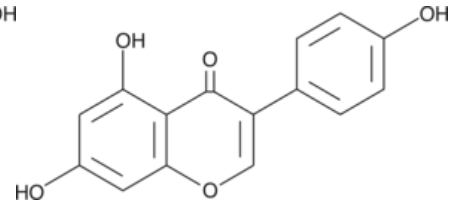


Croton stellatopilosus

Densitometric TLC for Standardization of *Pueraria minifica* Extracts

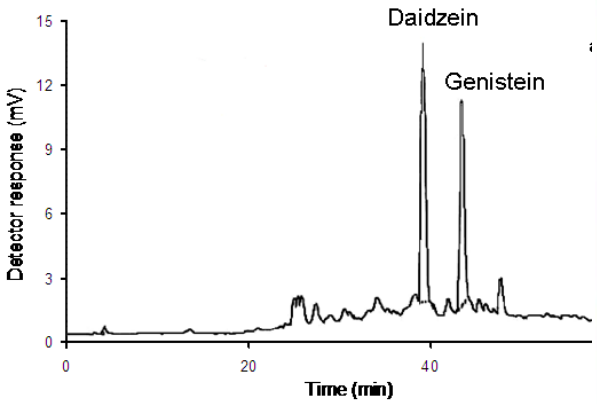


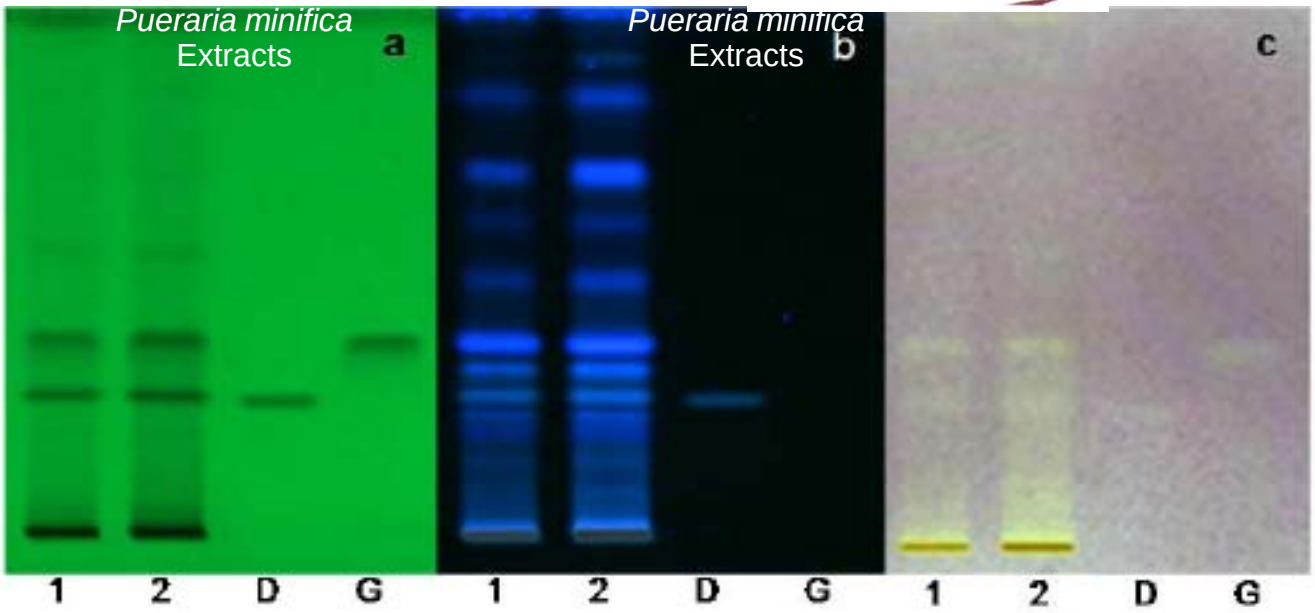
Daidzein (D)



Genistein (G)

Pueraria minifica





Sample:
ethyl acetate-ethanol extracts
Mobile Phase:
chloroform: methanol (9.2: 0.8)
Stationary Phase: silica gel
Scan Wavelength: 254 nm

Wavelength 254 nm

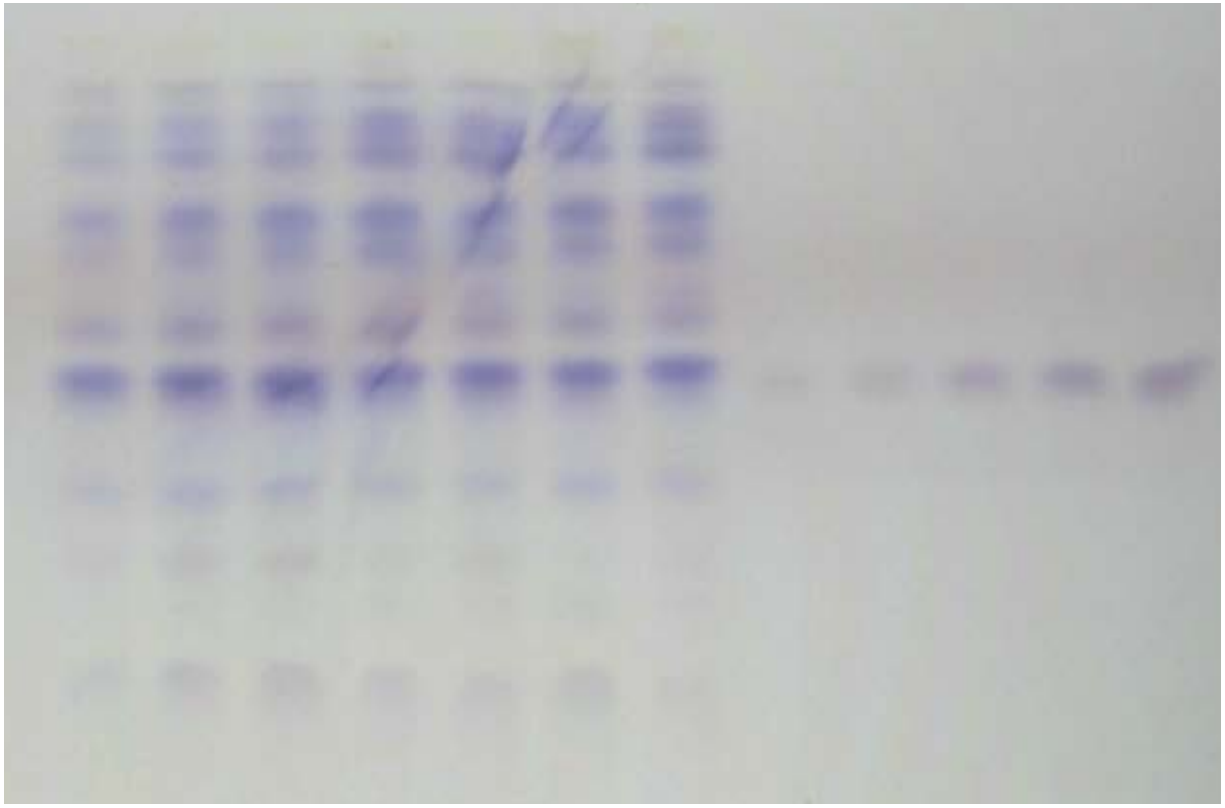
Wavelength 330 nm

Sprayed with DPPH, visible light

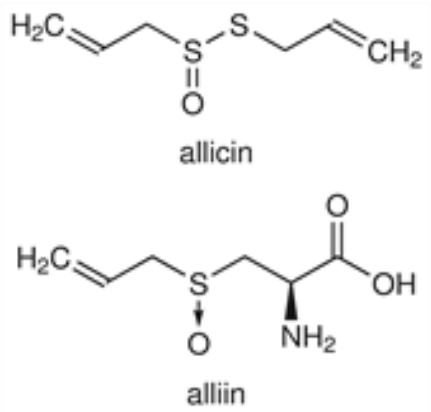
J. Natural Medicines,, 65 (2011), 1, 1-8

Densitometric TLC for Alliin Analysis in Garlic Extracts

Sample: Sprayed-dry garlic juice
Mobile Phase: n-butanol-methanol-water-glacial acetic acid = 15:3:6:1) **Stationary Phase**: cellulose, **Detection::**dipped in 0.4%ninhydrin, **Scan wavelength**: 418 nm

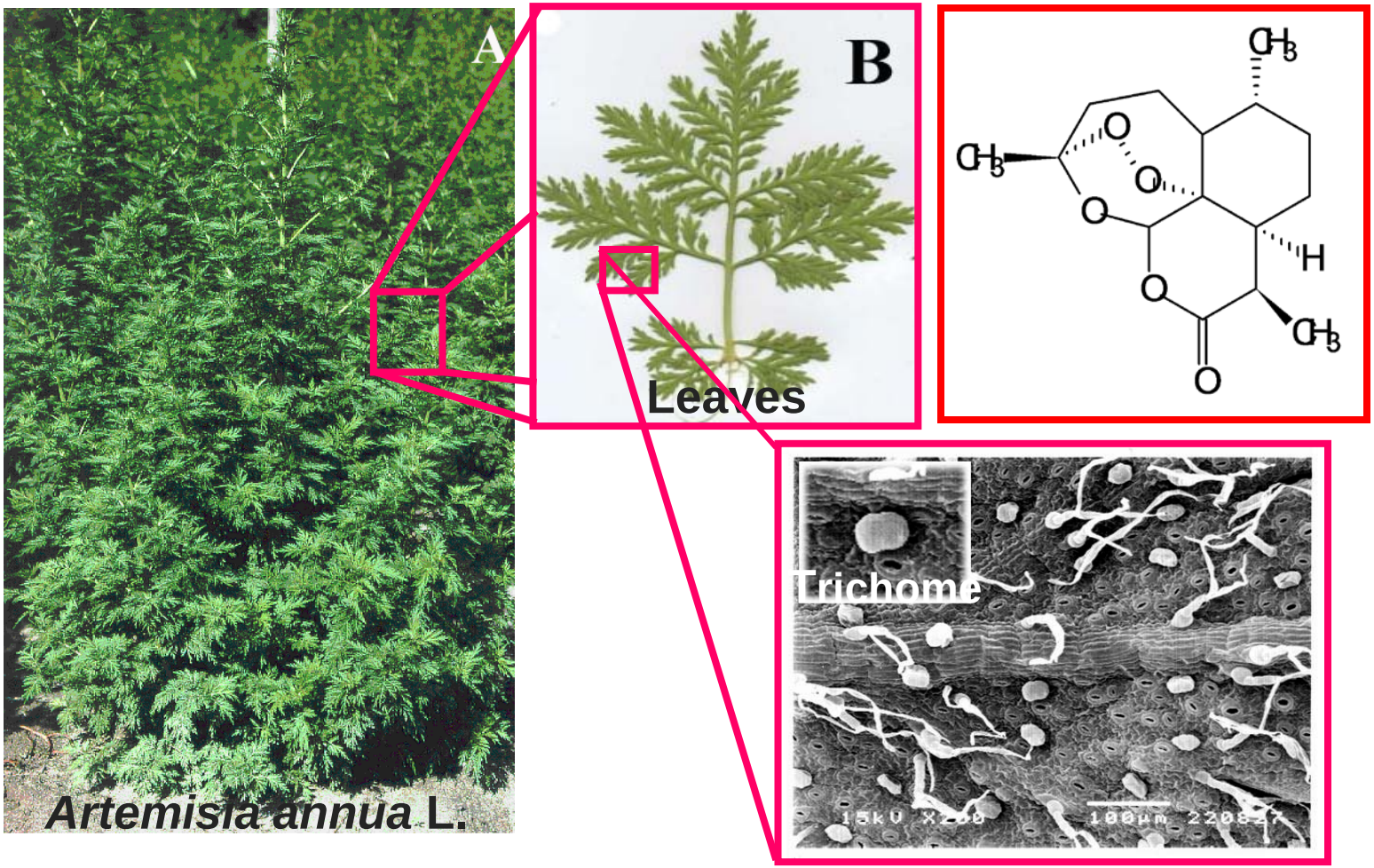


Garlic Extract Samples Alliin Standard

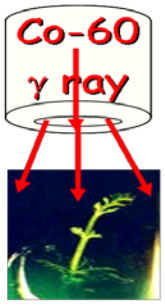


Immunitop's Garlic Extract

Densitometric-TLC Analysis of Non-Chromophore Containing Artemisinin in Artemesia annua Extracts



Effect of gamma irradiation on the survival and artemeimin content of *in vitro* plantlets of *A. annua*



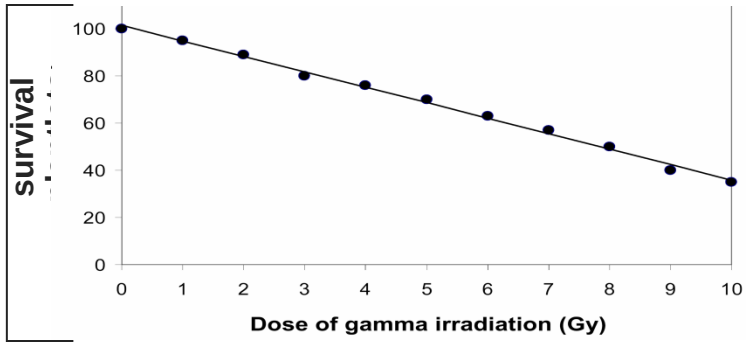
Gamma irradiation

***In vitro* plantlets**

Transfer

***Ex vitro* plant**

***In vitro* plantlets**



Dose-response (survival) curve

Artemisinin Analysis

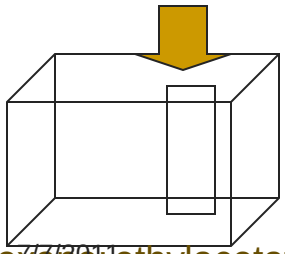
In vitro plantlets

Dried leaves

Refluxed with hexane

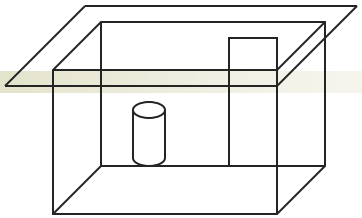
Artemisinin extracts

sample application onto TLC plate

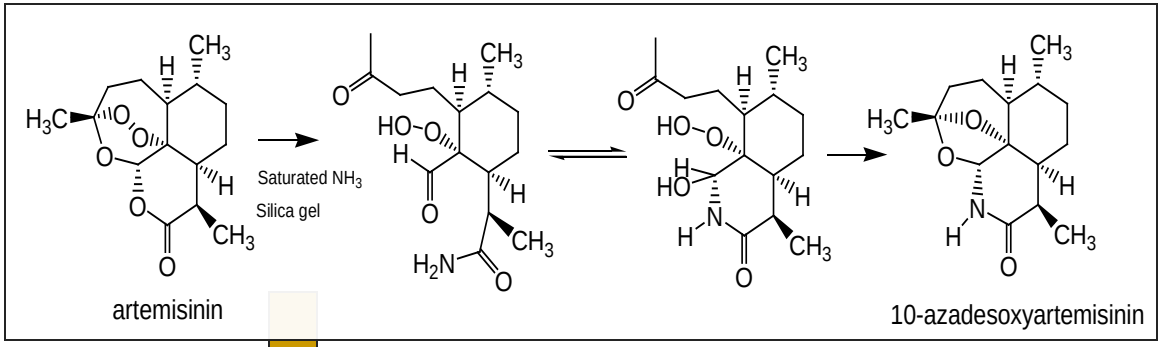


Developed in a solvent system

Hexane: ethylacetate: acteone, 16:1:1



Exposed with saturated ammonia vapour

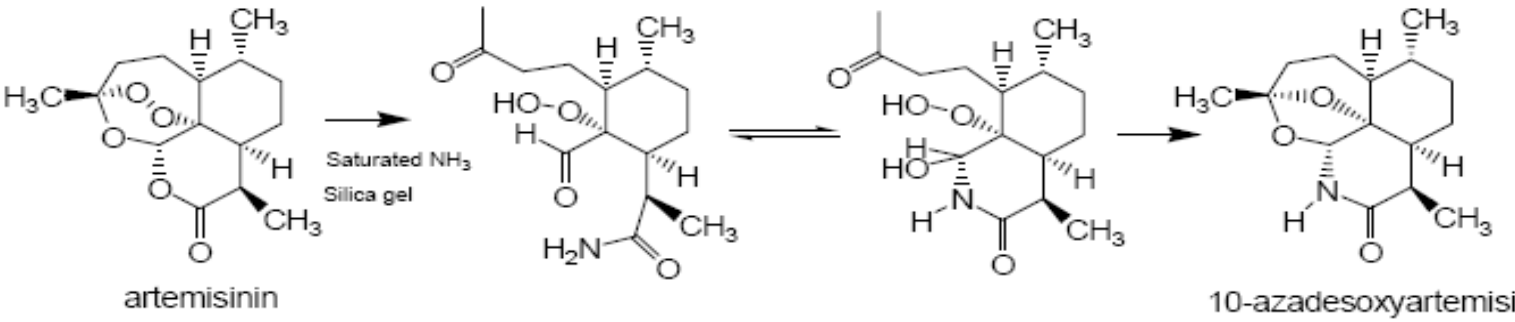


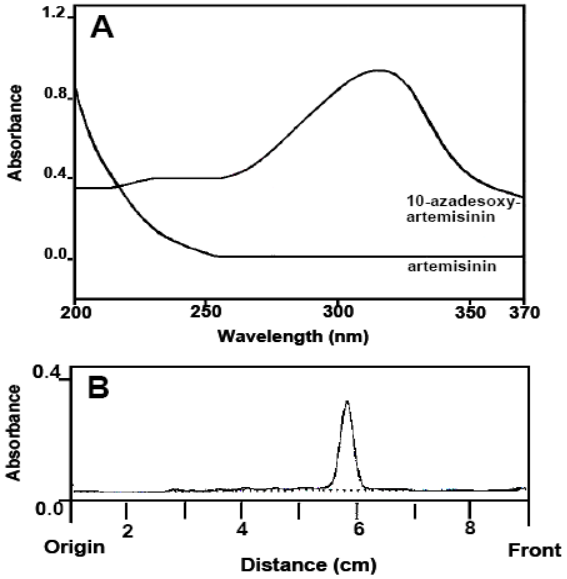
Densitometric scan at 320 nm

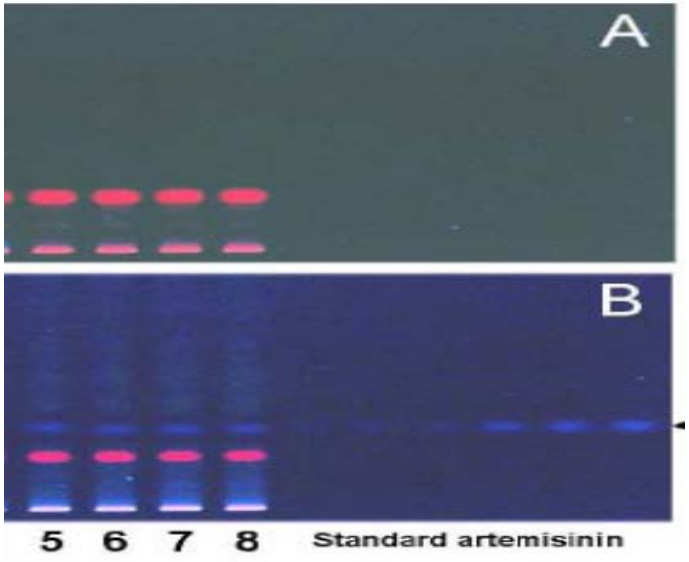
Limit of detection: 5 ng artemisinin

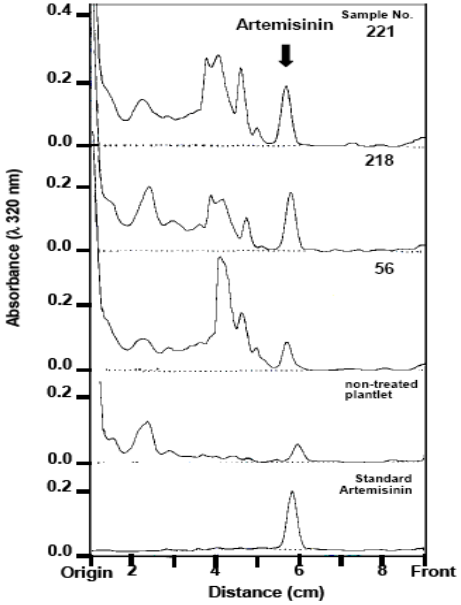
(Koobkokkruad et al., *Phytochemical Analysis* 2007)

Densitometric TLC for Artemisinin Analysis

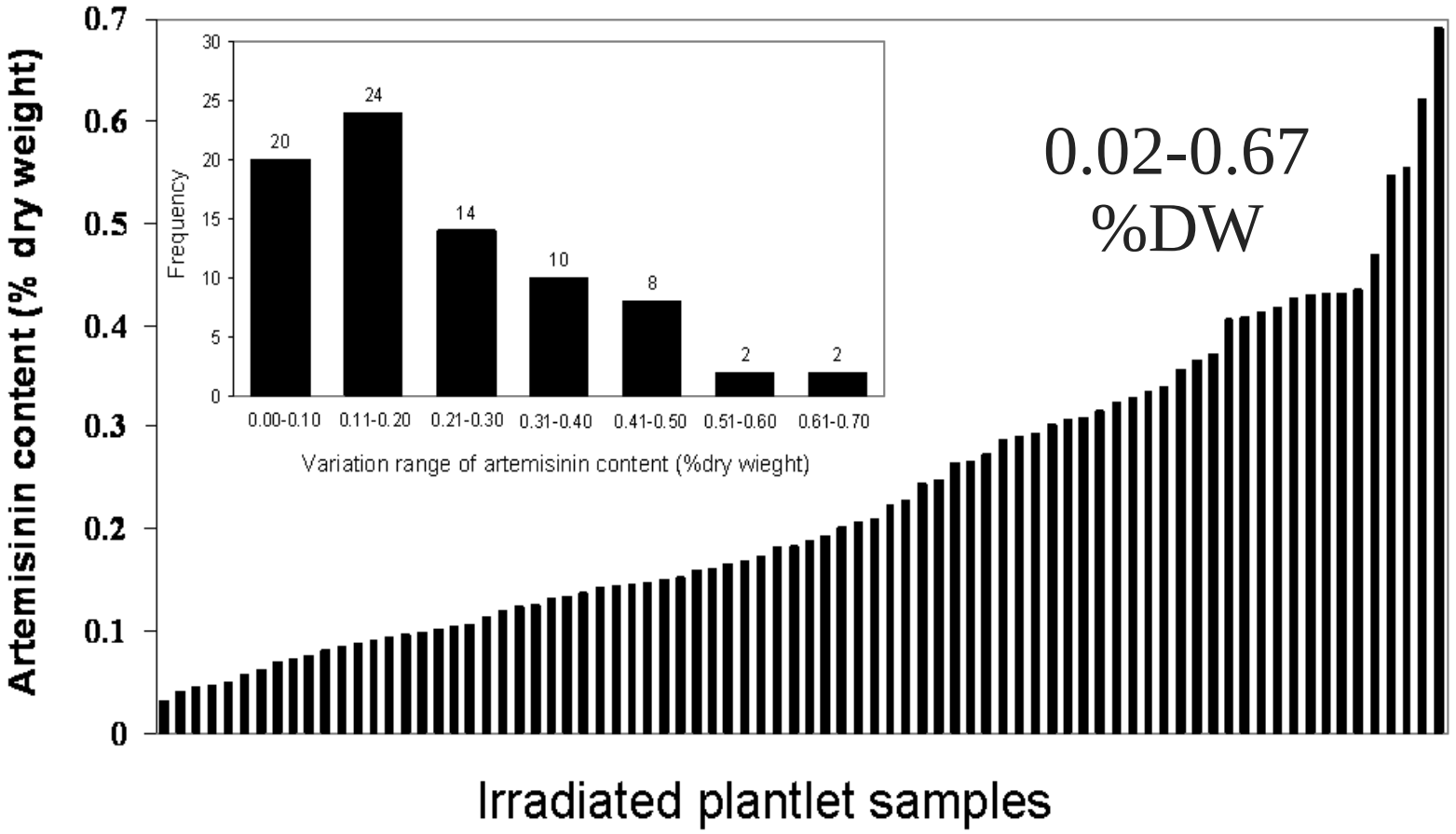








Variation of artemisinin content in various plantlets of *A. annua*

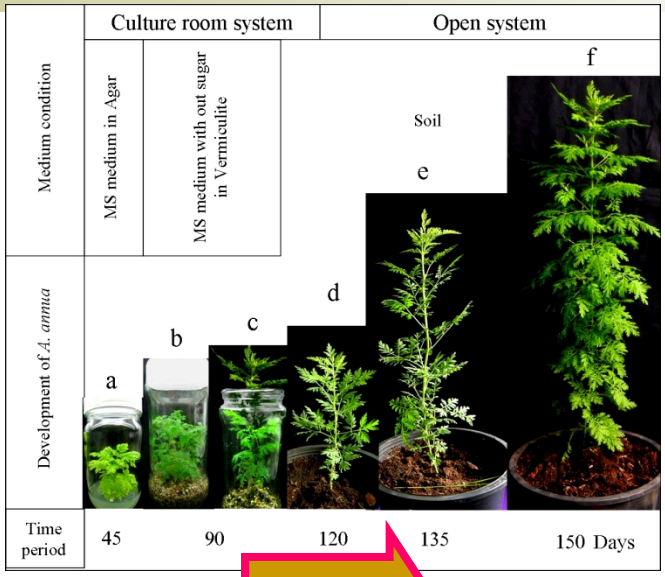


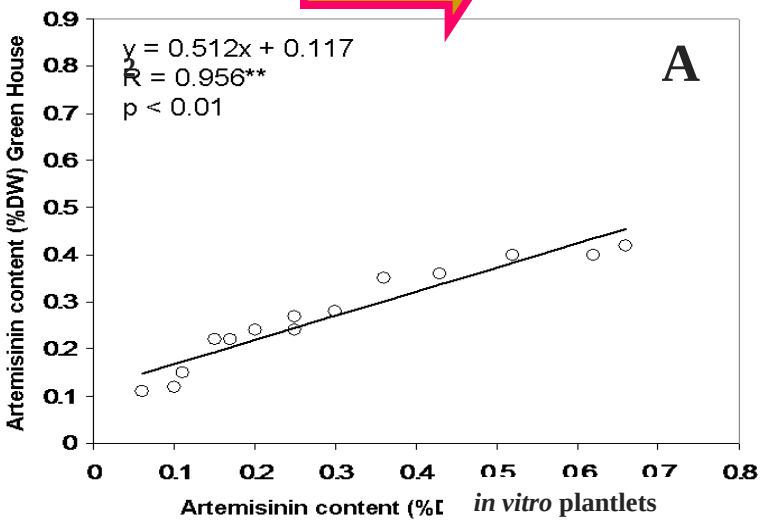
(**Koobkokkruad et al., *Inter. J. Radiat. Biol*. 2008**.

Artemisinin Content:

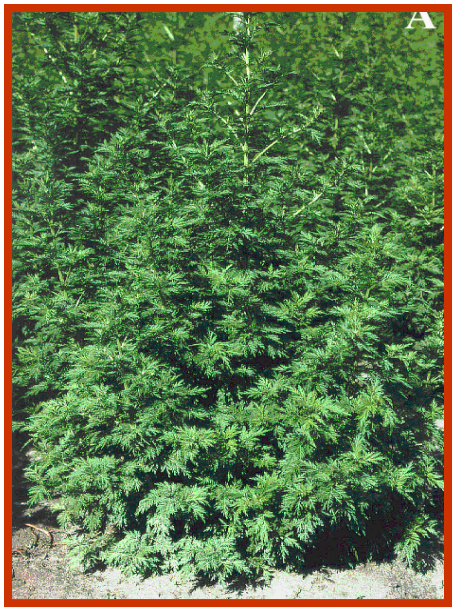
Comparison between the *in vitro* and *ex vitro plants*

In vitro Plantlets

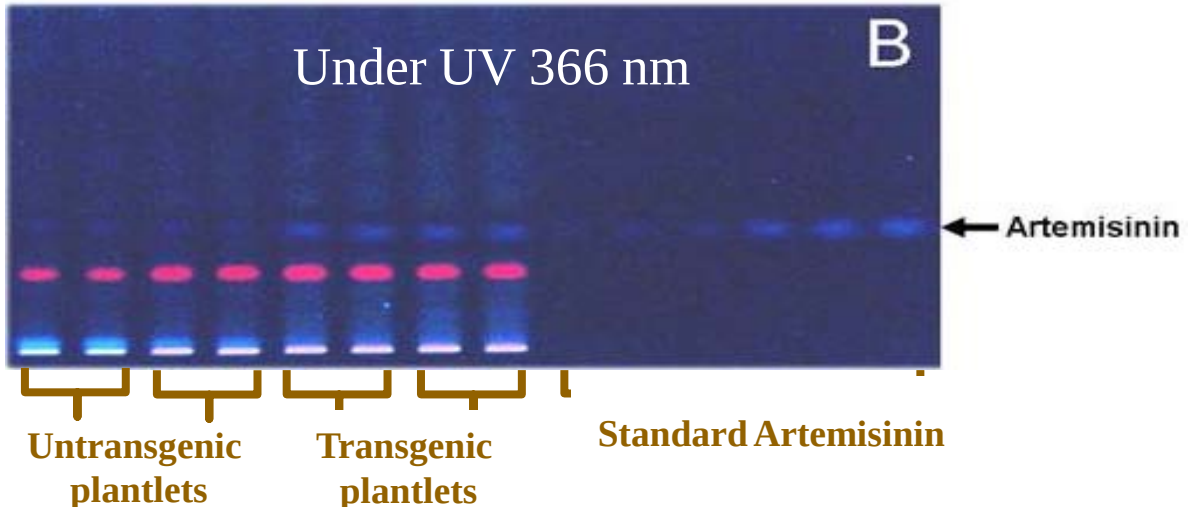


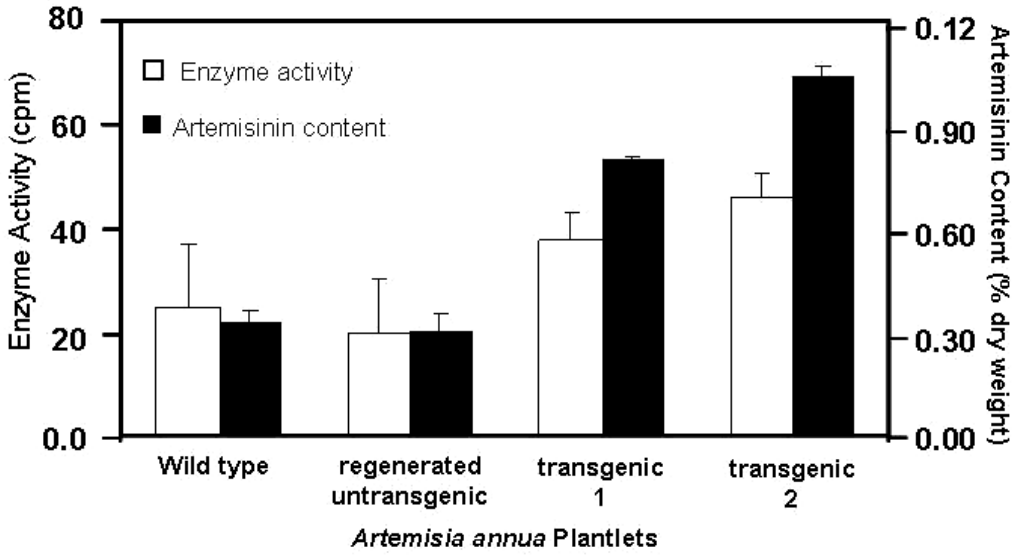


Ex vitro Plants



Compare of TLC pattern of some crude extracts of untransgenic and transgenic of *A. annua*





Densitometric-TLC Analysis of Non-Chromophore Containing Asiaticoside in *Centella asiatica* L. Extracts

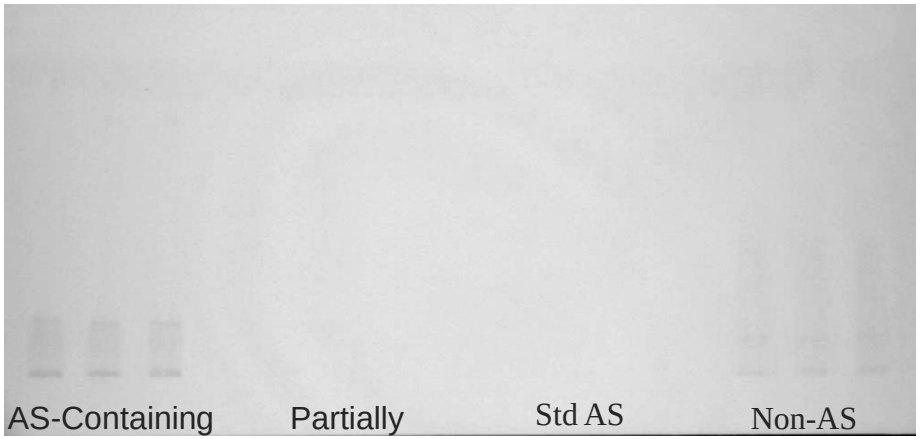
Centella asiatica Linn. (Gotu Kola)

-used to treat various disorders in traditional eastern medicine: syphilis, hepatitis, epilepsy, diarrhea, fever and asthma

-Western herbalists use it for disorders that cause connective tissue swelling: scleroderma, psoriatic arthritis and rheumatoid arthritis

-The plant contains triterpenoid glycosides, madecassoside (MS), asiaticoside (AS) and their aglycones, madecassic acid (MA), and asiatic acid (AA)

Asiaticoside (AS)



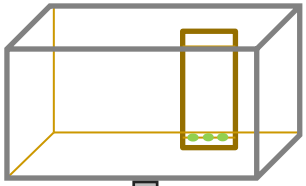
Densitometric-TLC Analysis of Non-Chromophore Containing Asiaticoside in *Centella asiatica* L. Extracts

Ground dried leaves

Refluxed with 80% methanol for 2 hours

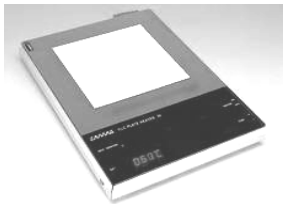
Crude Extract

Spotted into a TLC plate



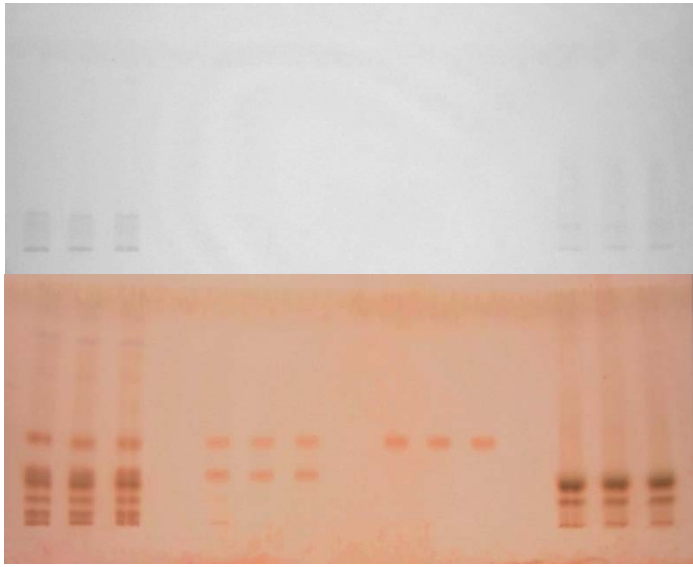
Developed in Chloroform-methanol-water; 30:15:1.2

Dipped in 2-naphthol reagent



Heat plate at 120ºC for 5 min

Scanned with TLC densitometric scanner at 530 nm.



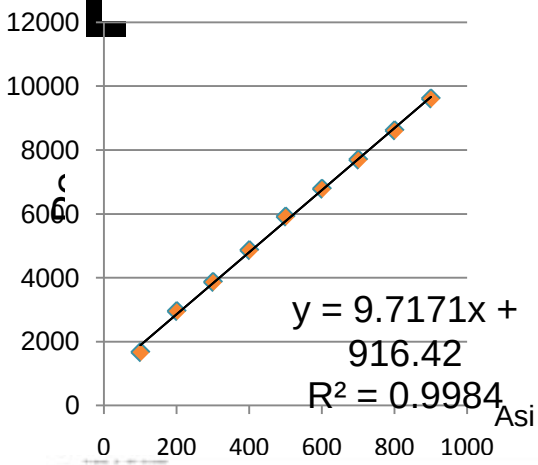
AS-Containing Crude Ext.

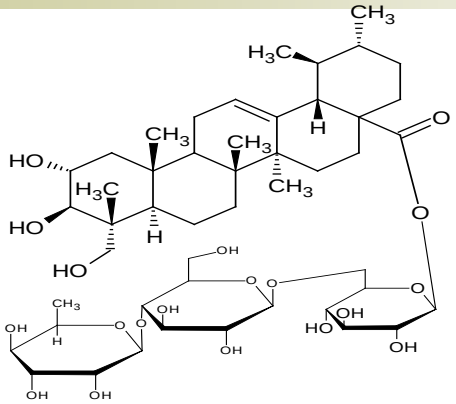
Partially Purified Crude Ext

Std AS

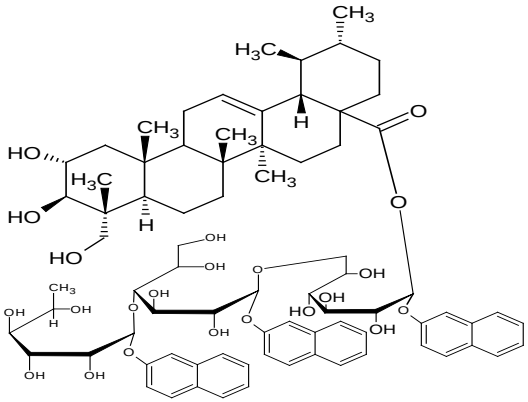
Non-AS Containing Crude Ext.

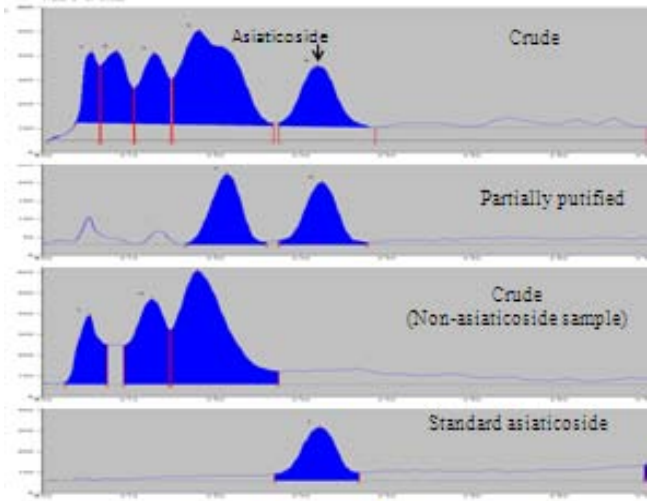
Densitometric-TLC Analysis of Non-Chromophore Containing Asiaticoside in *Centella asiatica* L. Extracts

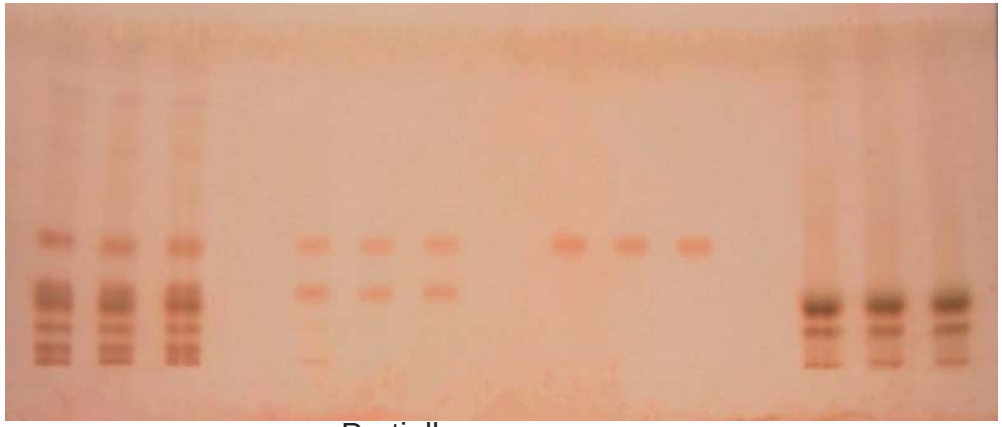




2-naphthol







AS-Containing Crude Ext.

Partially Purified Crude Ext

Std AS

Non-AS Containing Crude Ext

Conclusions

- Densitometic TLC techniques have been used successfully in Thailand for the analysis of active constituents both chromophore- and non-chromophore- containing in medicinal plant extracts and heath products
- The non-chromophore-containing artemisinin can be rearranged to 10-azadesoxyartemisini n by ammonia vapour
- The non-chromophore-containing asiaticoside can be derivatized with 2-naphthol for sensitive detection and densitometric scanning
- Densitometic TLC is the technique of choices for simple standardization of plant raw materials and finished products

Acknowledgements

- Dr. Thongchai Koobkokkruad

  Artemisinin detection

- Mr. Ariya Chaisawadi

  Asiaticoside detection

- Thailand National Center for Genetic Engineering and Biotechnology

  for financial support

- *CAMAC* for supporting my accommodation during the HPTLC 2011 Symposium

A Proposal from Bangkok

For the Next HPTLC Symposium

How about "Bangkok 2013" ? !