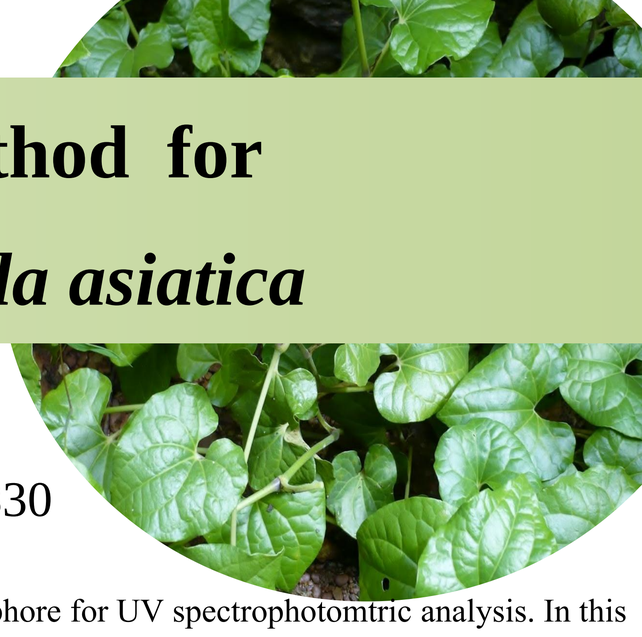
Development of a New Densitometric-TLC Method for Determination of Asiaticoside Content in *Centella asiatica*

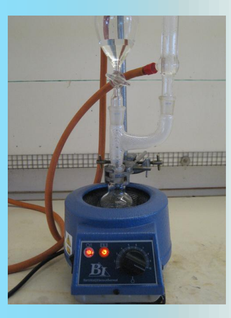
Ariya Chaisawadi, Wanchai De-Eknamkul
Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, 10330



Asiaticoside (Fig.1), a major active compound in *Centella asiatica* L., is an ursane-type triterpene glycoside with no particular chromophore for UV spectrophotomtric analysis. In this study, derivatization of asiaticoside with 2-naphthol on a TLC silica gel plate was developed for direct determination of the triterpenoid glycoside in *C. asiatica* crude extracts using the wavelength of UV–visible range. The developed densitometric TLC appeared to be simple, accurate and rapid.

In practice, crude extracts of *C. asiatica* are prepared from the plant materials under reflux with 80% methanol and followed by a step of solvent extraction using dichloromethane and butanol. The partially purified extract is then separated on a silica gel 60 F_{254} TLC plate (20x10cm.) using chloroform/methanol/water system as the mobile phase. The plate is then dipped into the solution of 2-naphthol sulfuric acid reagent to obtain a brownish band of the glycoside. After the 2-naphthol derivatization, the plate is scanned under the wavelength of 530 nm. To obtain the TLC chromatograms (Fig.3,4) of various samples. This new method has good sensitivity and selectivity, with the linear range for the analysis from 100-1000 ng/band (fig.5) ($r^2 \geq 0.99$) and good precision and accuracy (1.15- 1.9 %RSD, 98.18-104.3%).

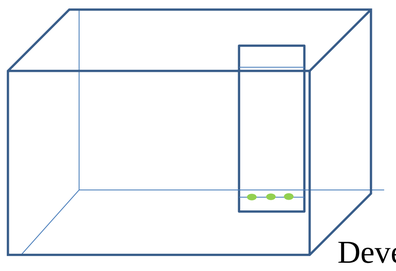
Ground dried leaves



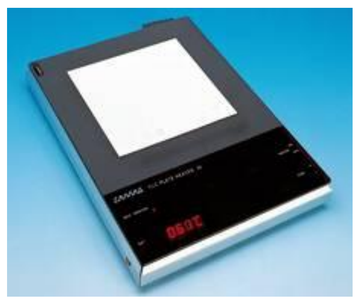
Reflux with 80% methanol for 2hours

Crude extract of *C. asiatica*

Spotted into a TLC plate



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



Dipped in 2-naphthal reagent then heat at 120ºC for 5 mins.

Scanned with TLC densitometric scanner at 530 nm.

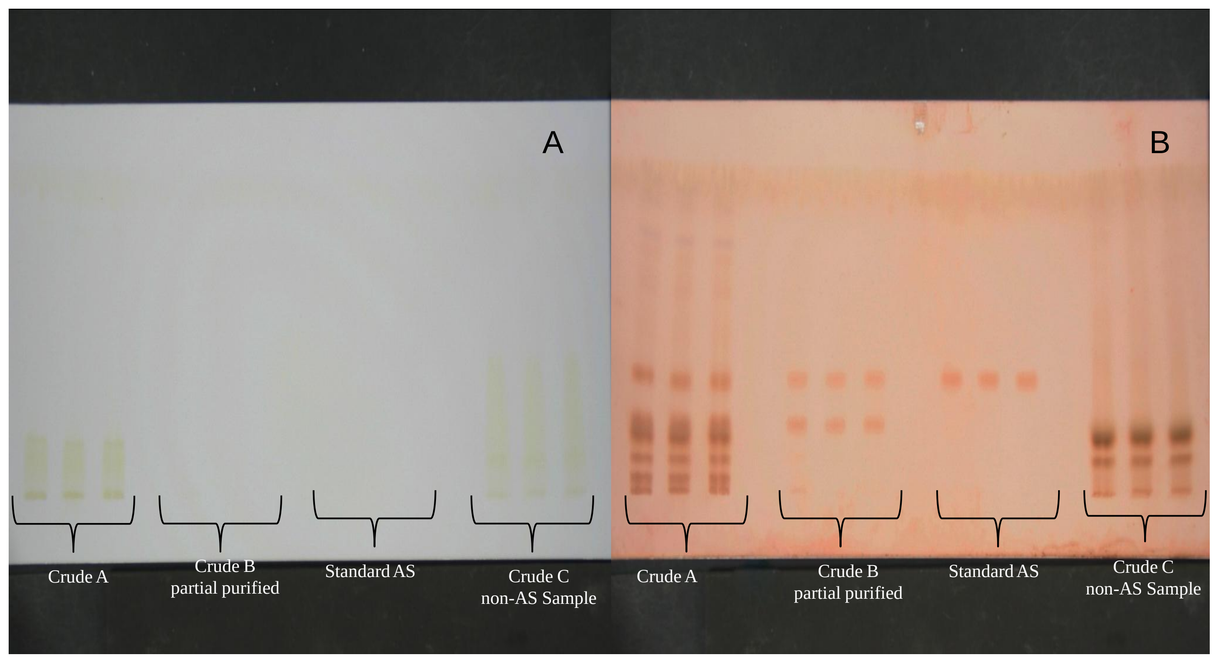
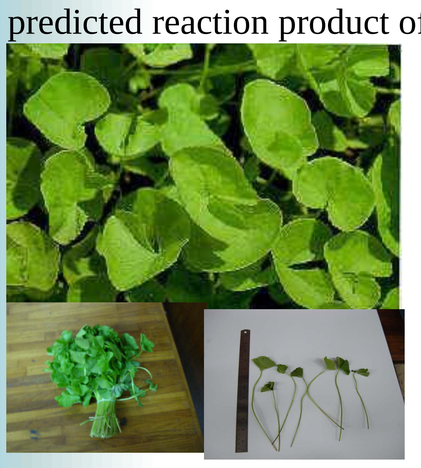


Fig. 3 TLC-patterns of some *C. asiatica* crude extracts observed under visible light before (A) and after (B) derivatization with 2-naphthol acid reagent.

TLC-Densitometric analysis. Crude extracts of *C. asiatica* samples (10 uL each) and asiatocoside standard solution (2uL) were spotted onto a pre-coated siliga gel (Siliga gel 60 F 254, 0.25 mm thickness). The TLC plate was then developed for 9-10 cm. from the origin using the solvent system of chloroform : methanol : DI water (30:15:1.2). The plate was dried, dipped into 2-naphthol sulfuric acid reagent, then heated with TLC plate heater at 120ºC for 5 minutes for completing reaction. After the derivatization, the TLC plate was scanned under the wavelength of 530nm. The predicted reaction product of the derivatization is shown in Fig.6.



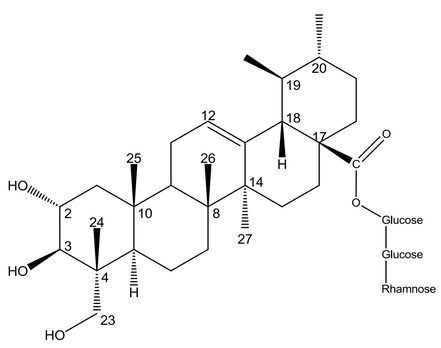


Fig. 1: *Centella asiatica* L. and its major active compound, asiaticoside.

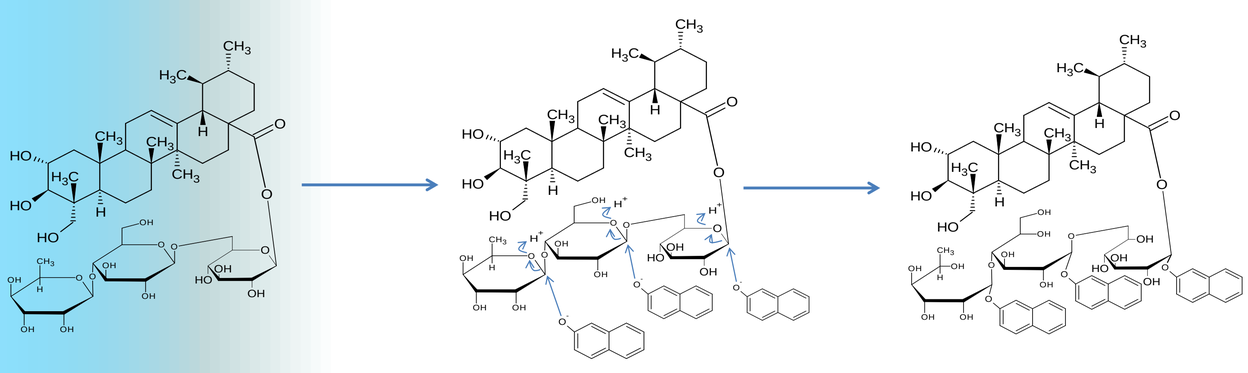


Fig. 6: Predicted mechanism of asiaticoside derivetization

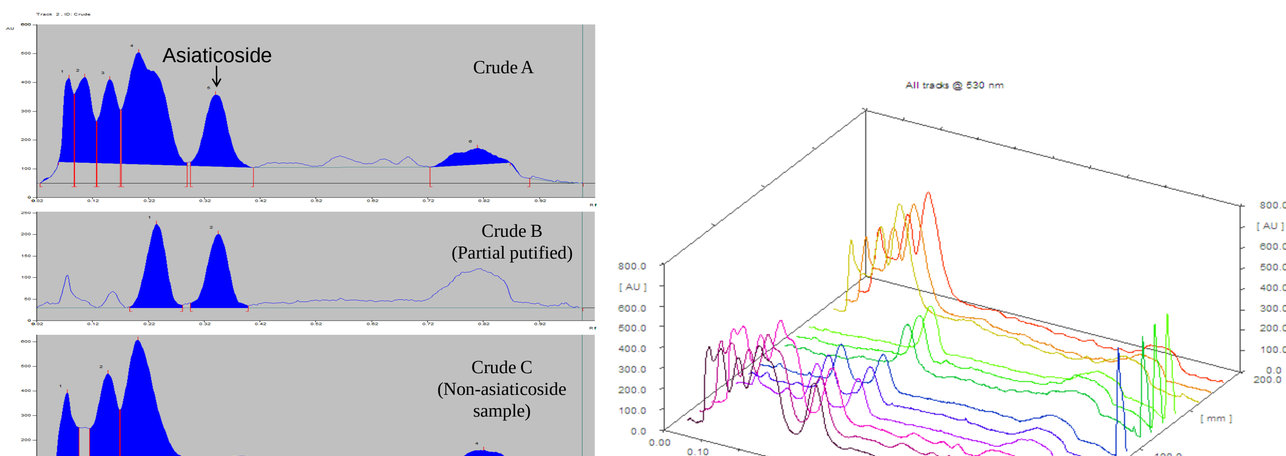


Fig. 4 TLC-densitometric chromatograms of some *C. asiatica* crude extracts by using solvent system of chloroform-methanol-water; 30:15:1.2

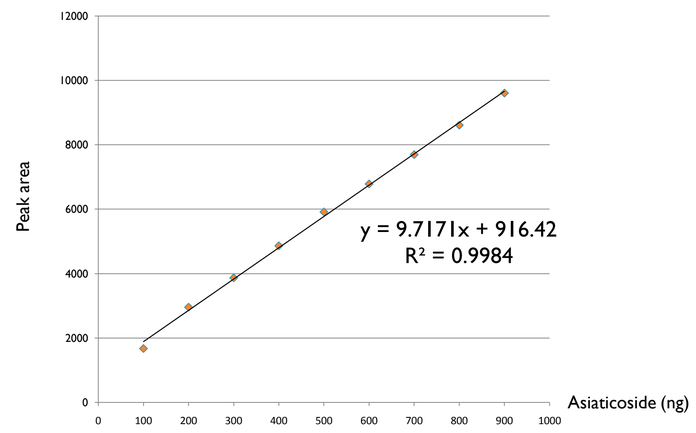


Fig. 5 Calibration curve of standard asiaticoside obtained using the new TLC-densitonetric method

In conclusion, we have shown a simple, accurate, method of TLC-densitometric analysis of asiaticoside. This new method consists of 2 steps. First, asisticoside within the crude extracts of *C. asiatica* is separated from other components on a siliga gel plate by a normal thin layer chromatography. Second, the band of asiaticoside on the plate is derivatized with 2-naphthol acid reagent to add the chromophore of 2-naphthol to its structure witch can be quantitated directly from its TLC-densitometry chromatogram (λ530). The method showed good sensitivity and selectivity and appears to be comparable to the UV-HPLC method witch is standard method.

Zheng, C., and Qin, L. 2007. Review: Chemical component of *Centella asiatica* and their bioactivities. Journal of Chinese Integrative Medicine. 5: 348-351. Oleszek, W.A. 2002. Chromatographic determination of plant saponins.. Journal of Chromatography A. 967: 147-162.

Malinowska, E., Krzyczkowski, W., Lapienis, G., and Herold, F. 2010. Densitometric determination of carbohydrate: application to purification and molecular weight determination of polysaccharide from *Hericium erinaceum* mushroom. Food research International. 43: 988-995.