CHAPTER III

EXPERIMENTAL

Part I : Phytochemical Study

Source of Plant Material

The stem bark of *Derris reticulata* Craib was obtained from its habitat in the area of Erawan waterfall, Kanchanaburi, Thailand, in August, 1993. The plant material was authenticated by comparison with herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperative. A voucher specimen was deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

General Techniques

1. Analytical Thin-Layer Chromatography

Technique : One way, ascending

Adsorbent : Silica gel 60 GF_{254} (E. Merck) precoated plate

Layer thickness : 0.2 cm.

Distance : 5.0 cm.

Temperature : Laboratory temperature (30-35°C)

Detection :

1. Visual detection under ultraviolet light at the wavelength of 254 and 365 nm
2. 10% Sulphuric acid in ethanol (10 ml sulphuric acid was dissolved in 90 ml ethanol)

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3. Anisaldehyde-sulphuric acid spraying reagent (0.5% ethanolic solution of anisaldehyde with 5% sulphuric acid)

4. Vanillin-HCl spraying reagent (5% Vanillin in ethanol with concentrated hydrochloric acid in the ratio 4:1, just prior to use)

2. Column Chromatography (CC)

Adsorbent : Silica gel 60 (No. 9385) particle size 0.040 -0.063 mm

Packing method : Wet packing and dry packing

Sample loading : **Wet packing**

A portion of crude extract was dissolved in a small amount of organic solvent then added gently on the top of the column.

Dry packing

A portion of crude extract was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, then dried, triturated and added gently on the column.

Examination of eluates : Fractions were examined by TLC using visual detection under ultraviolet light at wavelength of 254 and 365 nm and sprayed with 10% sulphuric acid in ethanol, anisaldehyde-sulphuric acid and vanillin-HCl spraying reagents.

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3. Gel Filtration Chromatography

Gel filter : Sephadex LH-20 (Pharmazia)

Packing method : Gel filter was suspended in the eluent and left standing to swell prior to use for 24 hours. Then poured it into the column and allowed it to be settled tightly.

Sample loading : The sample was dissolved in a small volume of eluent and put on the top of column.

Examination of eluates : Fractions were examined in the same manner as described in the section 2.1.

4. Spectroscopy

4.1 Ultraviolet (UV) Absorption Spectra

Ultraviolet absorption spectra were obtained with a Lambda Series PECSS UV spectrophotometer.

4.2 Infrared (IR) Absorption Spectroscopy

IR absorption spectra were obtained with a Shimadsu IR-440 infrared spectrophotometer, using potassium bromide disc.

4.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

All the ^{1}H NMR and ^{13}C NMR spectra were obtained with a JEOL alpha FT NMR spectrometry by operating at 400, 500 MHz and 100, 125 MHz respectively and taken by using acetone- d_6 as a solvent. The chemical shifts were reported in ppm scale.

4.4 Mass Spectroscopy (MS)

Electron-impact mass spectra (EIMS) were obtained by operating at 70ev with a Hitachi M-60 and with a Fisons VG Trio 2,000 mass spectrometer (using a direct inlet system) and the high resolution mass spectra (HRMS) were measured with a Hitachi RMU-7M mass spectrometer. The spectrum type is regular (MF-Linear).

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5. Solvents

Throughout this work, all organic solvents were commercial grade and had to be redistilled prior to use.

Extraction

The fresh stem bark of *Derris reticulata* Craib (5.5 kg) were marcerated twice for three-day period, with 95% ethanol and then filtered. The filtrate of each

maceration were pooled and concentrated to remove ethanol under reduced pressure to yield 801 g (14.56%) of brown syrupy mass.

Isolation of Chemical Substances

The crude ethanol extract (210 g) was dissolved in water and then partitioned with ethyl acetate. The ethyl acetate extract was separated and evaporated to dryness under reduced pressure to yield 65.5 g of brown syrupy mass and named DR-EA. The water extract was evaporated on water bath to yield 118.0 g of redish-brown syrupy mass and named DR-W.

The DR-EA syrupy mass was divided into eleven equal portions. Each portion (5.95 g) was purified by using a column of sephadex LH-20 (4 x 70 cm) with methanol as an eluent. The elulates were collected depend on the colour bands (approximately 20 ml per fraction). The fractions were collected and examined by thin layer chromatography (TLC). Fraction containing similar pattern were combined together as shown in Table 20.

Table 20 The Combined Fractions from DR-EA Crude Extract

Fraction	Number of Eluate	Remark
F-01	1-10	yellowish-brown solution
F-02	11-20	deep green solution
F-03	21-50	orange solution
F-04	51-71	pale yellow solution
F-05	72-86	pale brown solution

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1. Isolation of DR-1

The combined fraction (eleven equal portions), F-03 (30 g), was divided into six portions. Each portion (5 g) was dissolved in a small quantity of chloroform, titurated with silica gel G and dried under reduced pressure. It was fractionated by the column chromatographic technique using a column of silica gel (4 x 70 cm) with a gradient system that shown below.

a) Chloroform

b) 10-80% Ethyl acetate in chloroform

c) Ethyl acetate

d) 10-90% Methanol in ethyl acetate

Twenty ml fractions were collected based on the colour band. The eluates were examined by TLC using chloroform : ethyl acetate (6:4) as developing solvent. The fractions showing the same pattern were combined together as shown in Table 21.

Table 21 Solvent Systems Used in Column Chromatography of Fraction F-03

Fraction	Eluent	Remark
1-30 (afford Fraction A)	Chloroform	yellowish-brown solution
31-39 (afford Fraction B)	gradient of ethyl acetate in chloroform (0%, 10%)	pale yellow solution
40-92 (afford Fraction C)	gradient of ethyl acetate in chloroform (20%, 30%, 40%)	yellow solution
93-137 (afford Fraction D)	gradient of ethyl acetate in chloroform (50%, 60%, 70%, 80%)	pale yellow solution
138-162 (afford Fraction E)	ethyl acetate ; gradient of methanol in ethyl acetate (10%, 20%, 30%)	pale yellow to colorless solution
163-220 (afford Fraction F)	gradient of methanol in ethyl acetate (40%, 50%, 60%, 70%, 80%, 90%)	reddish-brown to brown solution

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Fraction C gave interesting spots on TLC after treated with spraying reagents. The combined fraction was evaporated under reduced pressure and rechromatographed over silica gel column with chloroform : ethyl acetate (8:2) as an eluents. The fractions which showed a major spot on TLC were combined together and then evaporated to dryness. The residue was dissolved in small amount of chloroform and hexane was

added dropwise to yield of 29.98 g (0.55%) of yellow needle crystals. It was designated as DR-1, and was identified as lupinifolin.

2. Isolation of DR-2

Fraction E (1.6 g) was dissolved in a small volume of ethyl acetate. It was fractionated by wet packing column chromatography over silica gel 60 (3 x 60 cm) with petroleum ether : ethyl acetate : methanol (7:3:1) as an eluent. Fraction of fifteen ml were collected. The eluates were examined by TLC using chloroform : acetone : petroleum ether (7:3:2) as developing solvent. The fractions showing the same pattem were combined. The combined fraction (0.80 g) was purified using a column of sephadex LH-20 (2 x 70 cm) with methanol as an eluent. The eluates were collected depend on the color bands (approximately 10 ml per fraction) that shown in Table 22. The eluates were examined by TLC using chloroform : ethyl acetate : methanol (7:2.5:0.5) as developing solvent. The fractions showing the same pattern were combined.

Table 22 The Combined Fractions from DR-4 that was Purified Using a Column of Sephadex LH-20.

Fraction	Number of eluate	Remark
DR4-01	1-8	pale yellow solution
DR4-02	9-20	colourless solution
DR4-03	21-39	yellow solution

A white colourless amorphous powder was obtained from fraction DR4-02 and yielded 10.12 mg (1.84×10^{-4}%). It was named as DR-4, and was identified as 8-Prenylgenistein (Lupiwighteone).

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Physical and Spectroscopic Data of the Isolated Compounds

1. Physical and Spectroscopic Data of DR-1

DR-1 was obtained as yellow needle crystals. It is soluble in chloroform and ethyl acetate.

$[\alpha]_D^{24}$; - 8.7° (c 1.15 in $CHCl_3$)

m.p. ; 115-117 °C

EIMS ; *m/z* (% relative intensity) ; Figure 27
406 (72), 391 (100), 363 (6), 351 (5), 335 (4), 285 (7), 271 (22), 243 (12), 215 (34), 189 (4), 187 (3), 147 (4), 120 (9), 91 (9), 77 (4), 43 (5)

IR ; υ-cm^{-1}, KBr disc ; Figure 28
3250, 2980, 2910, 1645, 1620, 1600

UV ; λ_{max} nm in chloroform ; Figure 29
268 sh, 275 sh, 304 sh, 318 sh

^{1}H-NMR ; δ ppm, 400 MHz, in acetone-d_6 ; Figure 30 - 30.2
1.43 (3H, s), 1.44 (3H, S), 1.61 (3H,br s), 1.64 (3H, br s), 2.87 (H, dd, *J*=15.0, 3.0 Hz), 3.14 (H, dd, *J*=15.0, 12.7 Hz) 3.19 (2H, br d, *J*=7.82), 5.15 (H, t, *J*=7.33), 5.44 (H, dd, *J*=12.7, 3.0 Hz), 5.60 (H, d, *J*=10.0 Hz), 6.59 (H, d, *J*=10 Hz), 6.90 (2H, d, *J*=8.3 Hz), 7.39 (2H, br d, *J*=8.3 Hz), 8.43 (H, s), 12.43 (H,s)

^{13}C-NMR ; δ ppm, 100 MHz, in acetone-d_6 ; Figure 31 - 31.1
18.18, 22.25, 26.09, 28.50, 43.64, 79.01, 80.00, 103.46, 103.52, 109.26, 116.39, 116.52, 123.75, 127.13, 128.98, 131.04, 131.37, 160.30, 160.69, 157.52, 158.79, 198.09

2. Physical and Spectroscopic Data of DR-2

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DR-2 was obtained as white amorphous powders.

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EIMS ; *m/z* (% relative intensity) ; Figure 35
338 (83), 323 (100), 295 (5), 283 (34), 270 (39), 254 (5), 231 (2), 203 (3), 165 (11), 150 (12), 118 (8), 81 (5), 55 (7)

IR ; υ cm^{-1}, KBr disc ; Figure 36
3400, 3200, 1660, 1580, 1260, 1415, 880, 840, 820

^{1}H-NMR ; δ ppm, 400 MHz, in acetone -d_6 ; Figure 37-37.1

1.64 (br s), 1.76 (br s), 3.43 (br d, J=6.0 Hz), 5.17 (t), 6.36 (S), 6.92 (d, J=8.0 Hz), 7.64 (d, J=8.0 Hz), 8.24 (S), 9.44 (br s), 12.88 (S)

^{13}C-NMR ; δ ppm, 100 MHz, in acetone -d_6 ; Figure 38-38.1
17.90, 22.00, 25.80, 99.50, 106.30, 107.20, 116.00, 123.10, 123.30, 123.70, 131.20, 132.00, 154.30, 156.30, 158.40, 161.60, 162.20, 182.00

Part II : Pharmacognostic Specification

Material and Method Used in Microscopic Investigation

Stem and mature leaves of *Derris reticulata* Craib were collected from its habitat in the area of Erawan waterfall in Kanchanaburi Province, Thailand, in August 1993, and stem and mature leaves of *Derris scandens* Benth. were collected from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, in September, 1993. These plant materials were authenticated by comparison with herbarium specimens in the Botanical Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperative.

1. Experimental Procedure

Examination and illustration of the external characters of the plants were performed by hand drawing and photography. The dried powder, sifted through a sieve no. 60, of the stem and mature leaves of each plant was studied microscopically. For quantitative microscopy, the leaf fragments of certain regions were cleared by gently warming in concentrated solution of chloral hydrate and 30 readings were taken for each value. The data were analysed statistically and the standard deviation was given in parenthesis in each case.

2. Method of Palisade Ratio Determination (Trease and Evans, 1978)

Pieces of leaf about 5 x 5 mm were cleared by warming with chloral hydrate solution, mounted and examined under the microscope. A drawing apparatus was arranged so that the epidermal cells and the palisade cells lying below them may be traced. First, four epidermal cells were traced and their outlines linked in to make them

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more conspicuous. The palisade cells lying beneath were then focused and traced. The palisade cells in each group were counted, those being included in the count which were more than half covered by the epidermal cells ; the figure obtained divided by four gave the palisade ratio of that group.

3. Method of Stomatal Number and Stomatal Index Determination (Trease and Evans, 1978)

Fragments of leaf other than extreme margin or midrib were cleared with chloral hydrate solution. The lower surface of the clarified leaf strips were examined under the microscope. The epidermal cells together with stomata were traced with the aid of a drawing apparatus. The diameter of field of view was measured by means of a stage micrometer. Calculated the area in square milimetres.

Counts were made of the number of ordinary epidermal cells and of stomata. A trichome was also counted as an ordinary epidermal cell. The stoma, consisting of the two guard cells and osteole, was counted as one unit. Count the epidermal cells and stomatal apparatus in the field of view ; incomplete part of the cells in one semicircle were inclusive, while those in the other were exclusive.

The average number of stomata per square millimetre, known as "stomatal number", was calculated by dividing the number of stomata by the area of the field of view. The results should be recorded for each surface of the leaf and the ratio between the two surfaces.

Calculated the stomatal index by the formula of Salisbury. If the leaf bears stomata on both surfaces, the stomatal index may be determined for each surface.

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4. Method of Vein-Islet Number Determination (Trease and Evans, 1978)

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Pieces of mature leaf bladed approximately 5 x 5 mm^2 were cut from the central part of the lamina, midway between the margin and midrib, cleared by gently warming in concentrated solution of chloral hydrate.

A microprojector drawing apparatus was set up and by means of a stage micrometer an enlarged square corresponding to 2 mm x 2 mm was drawn on the paper. The stage micrometer was then replaced by the cleared preparation and the veins were traced within this 4 mm square area.

When counting, it was convenient to number each vein-islet on the tracing. Each numbered area was be completely enclosed by veins, and those which were incomplete were included if cut by a two adjacent sides but excluded if cut by the other two sides. The total number of vein-islets divided by 4 gave the average number of vein-islets per square millimetre, or "vein-islet number".

5. Method of Veinlet Termination Number Determination (Trease and Evans, 1978)

The number of free vein-tips, or veinlet terminations, in 4 square millimetre of the above tracing of vein-islets were counted. The total number divided by 4 gave the average number of veinlet terminations per square millimetre of leaf surface.

Material and Technique Used in Determining the Thin-layer Chromatographic Pattern of Chemical Constituent

1. Materials

Stem of *Derris reticulata* Craib, *D. scandens* Benth. were collected and dried in the oven at 60°C.

2. The Technique for Analytical Thin-layer Chromatography (TLC)

The conditions used for the analytical TLC used in this work are as follows :

Technique : Two-dimensional TLC

Adsorbent : Silica gel 60 F_{254} (E. Merck) precoated plate

Solvent system : Three 2-Dimension solvent systems as shown in Table 23

Layer thickness : 0.2 mm

Distance : 15.0 cm

Temperature : Laboratory temperature (30-35°C)

The detail of each step is decribed below.

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2.1 Method of Extraction

Ten grams of dried coarsely powdered stem of each plant were macerated separately in 100 ml of 70% ethanol for three days. After being filtered through a Whatman filter paper No. 1, the filtrates were evaporated under vacuum at 60-70°C to nearly dryness. The syrupy mass of the crude extracts obtained were then kept in small well-closed containers and stored in a refrigerator.

2.2 Solvent System for Two-Dimensional TLC

In the present work, three thin-layer chromatogram patterns of chemical constituents were illustrated for each of the plants. Three different pairs of solvent systems, as listed in Table 23, were used for developing the chromatograms.

For better separation of various components of the crude extracts, the process of double development in the same solvent system was applied to the development in dimension 1. Freshly prepared solvent was used in each development in order to improve the reproducibility of R_f values.

Table 23 Developing Solvent System for Two-Dimensional Thin-Layer Chromatography

Pattern	Dimension	Solvent System
A	1	Petroleum spirit : ethyl acetate (7:3)
	2	n-Hexane : ethyl acetate (3:2)
B	1	Toluene : ethyl acetate (7:3)
	2	Chloroform : acetone (3:2)
C	1	Chloroform
	2	Toluene : acetone : dichloromethane (40:25:35)

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2.3 Method of Chamber Saturation

Care should be taken to saturate the chromatographic chamber with solvent vapour as completely as possible before use since it strongly influences R_f values (Bobbitt, 1963 ; Randerath, 1968).

In order to accomplish uniform saturation, the solvent system (100 ml) was introduced into the chamber, of which the walls were lined as completely as possible with filter paper, 30 minutes before the introduction of the chromatographic plate. The vessel was then swirled round so that the filter paper was soaked with solvent.

2.4 Method of Sample Application

An amount of sample to be applied is chosen which is large enough to be effectively visualized to show trace substances, and small enough to give discrete spots with a minimum of tailing.

It should be noted that the lower limits of detection are higher for two-dimensional chromatograms, since diffusion effects cause greater dilution of the substance in the longer development time of the two-dimensional method (Randerath, 1968). Thus, higher concentrations of sample solution are required for the preparation of two-dimensional chromatograms.

The extracts (0.25 g) were dissolved in 70% ethanol (2 ml). Spotting was made by means of a filled capillary tube (1 mm in diamter) onto the starting point at a corner of the plate, 3 cm from the edges. Five to six applications (about 0.3-0.4 ml) with solvent evaporation after each were required. The spot was kept small (not exceeding 0.5 cm in diameter) by using an air blower to produce warm current air for rapid evaporation of the solvent. The solvent was removed as completely as possible before commencing chromatography.

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2.5 Method of Development of the Chromatogram

When the sample solution had already been applied to the starting point and allowed to dry, the plate was placed in a vertical position in a closed, saturated chromatographic chamber such that the bottom of the layer dipped into the developing solvent. The chromatogram was then developed. The distance for development was

15 cm, marking by the finishing line which was drawn by means of a spotting template and a sharp object. Laboratory temperature was about 30-35°C.

First Dimension : The spotted TLC plate was developed firstly in direction 1 with solvent 1. After the solvent ascended 15 cm, the plate was removed from the tank and allowed to dry in the air for 2 hours. If solvent 1 was chloroform the plate was developed two times in the first dimension. Complete removal of the solvent from the plate was then obtained.

Re-developed the air-dried plate in the same solvent system, using fresh solvent. The plate was allowed to dry in the air overnight before commencing the development in the second direction.

Second Dimension : The plate from the first dimension development was now placed in a second solvent and developed in the direction perpendicular to the first dimension. After the solvent ascended 15 cm, the plate was removed and allowed to dry in the air overnight.

It is essential to be noted that in order to improve the reproducibility of R_f values the plate must be removed from the tank as soon as the solvent reaches the finishing line. If several plates are being chromatographed simultaneously in a single container, all the plates have to be removed at the same time.

The process of double development in the first dimension provided considerable advantages in better separations of various components of the sample such that discrete spots with a minimum of tailing were obtained.

2.6 Method of Detection

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Special methods were used to detect compounds which could not be directly distinguishable by their own colours. Many compounds became visible when the chromatogram was viewed in short and long wave ultraviolet light. Some of them had to be visualized by spraying with special detection reagents.

Treatments and detection reagents applied were as follows :

2.6.1 Colour in Daylight.

In order to provide a uniform light intensity over the whole chromatograms, a 20 Watts (Daylight) Fluorescence lamp was used.

2.6.2 Fluorescence

The chromatograms were examined in 254 nm and 365 nm ultraviolet light.

2.6.3 10% Sulphuric acid in ethanol (reagents for general detection)

Reagent : Sulphuric Acid (10 ml) was dissolved in ethanol (90 ml).

Procedure : The chromatogram was sprayed with the reagent and allowed to dry in the air for 15 minutes, then heated at 100°C for 5-10 min until the colour developed to its maximum.

Detection : Sterols, steroids, bile acids and gibberellins.

2.6.4 Anisaldehyde-sulphuric acid reagent (AS)

Reagent : 0.5 ml Anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order.

The reagent has only limited stability, and is no longer useable when the colour has turned to red-violet.

Procedure : The TLC plate is sprayed with about 10 ml, heated at 100°C for 5-10 min, then evaluated under UV-365 nm.

Detection : Essential oil, pungent principles, bitter principles, saponins, etc.

2.6.5 Natural products-polyethylenglycol reagent (NP/PEG)

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Reagent : 1% Methanolic diphenylboric acid-β-ethylamino ester (= diphenylboryloxyethylamine) (NP) and 5% ethanolic polyethyleneglycol 4000 (PEG).

Procedure : The plate is sprayed with 1% methanolic diphenylboric acid-β-ethylamino ester (= diphenylboryloxyethylamine) (NP), followed by 5% ethanolic polyethyleneglycol 4000 (PEG) (10 ml and 8 ml, respectively).

Intense fluorescences is produced immediately or after 15 min under UV 365 nm, PEG increases the sensitivity (from 10 µg to 2.5 µg). The fluorescence behaviour is structure-dependent.

Detection : Flavonoids, aloin.

2.6.6 Potassium hexacyanoferrate (III) - ferric chloride reagent

Reagent : 1% Potassium hexacyanoferrate and 1% ferric chloride.

Procedure : Equal volumes of 1% potassium hexacyanoferrate and 1% ferric chloride are mixed before used 2M HCl increases the sensitivity.

Detection : Phenols, tannins, flavonoids.

2.7 Recording of R_f Value and Colour

The locations and colours of the spots were recorded after each treatment. R_f values were determined from the mean of a series of independent observations undertaken on three chromatograms of the same solvent system development.

$$R_f \text{ value} = \frac{\text{Distance of spot moving from starting point}}{\text{Distance of solvent front from starting point}}$$

Concomitant use of more than one compatible chromatographic sprays, revealed at times more than one compound in some areas of the chromatogram and made it possible to mark each. Samples of the chromatograms are given. An R_f grid was used for convenience in recording the values. The R_f values were coded as follows :

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00 (zero zero)	corresponds to R_f	.000 to .075
08	"	.075 to .125
10	"	.125 to .175
18	"	.175 to .225
20	"	.225 to .275
28	"	.275 to .325
30	"	.325 to .375
38	"	.375 to .425
40	"	.425 to .475
48	"	.475 to .525
50	"	.525 to .575
58	"	.575 to .625
60	"	.625 to .675
68	"	.675 to .725
70	"	.725 to .775
78	"	.775 to .825
80	"	.825 to .875
88	"	.875 to .925
90	"	.925 to .975
98	"	.975 to 1.000

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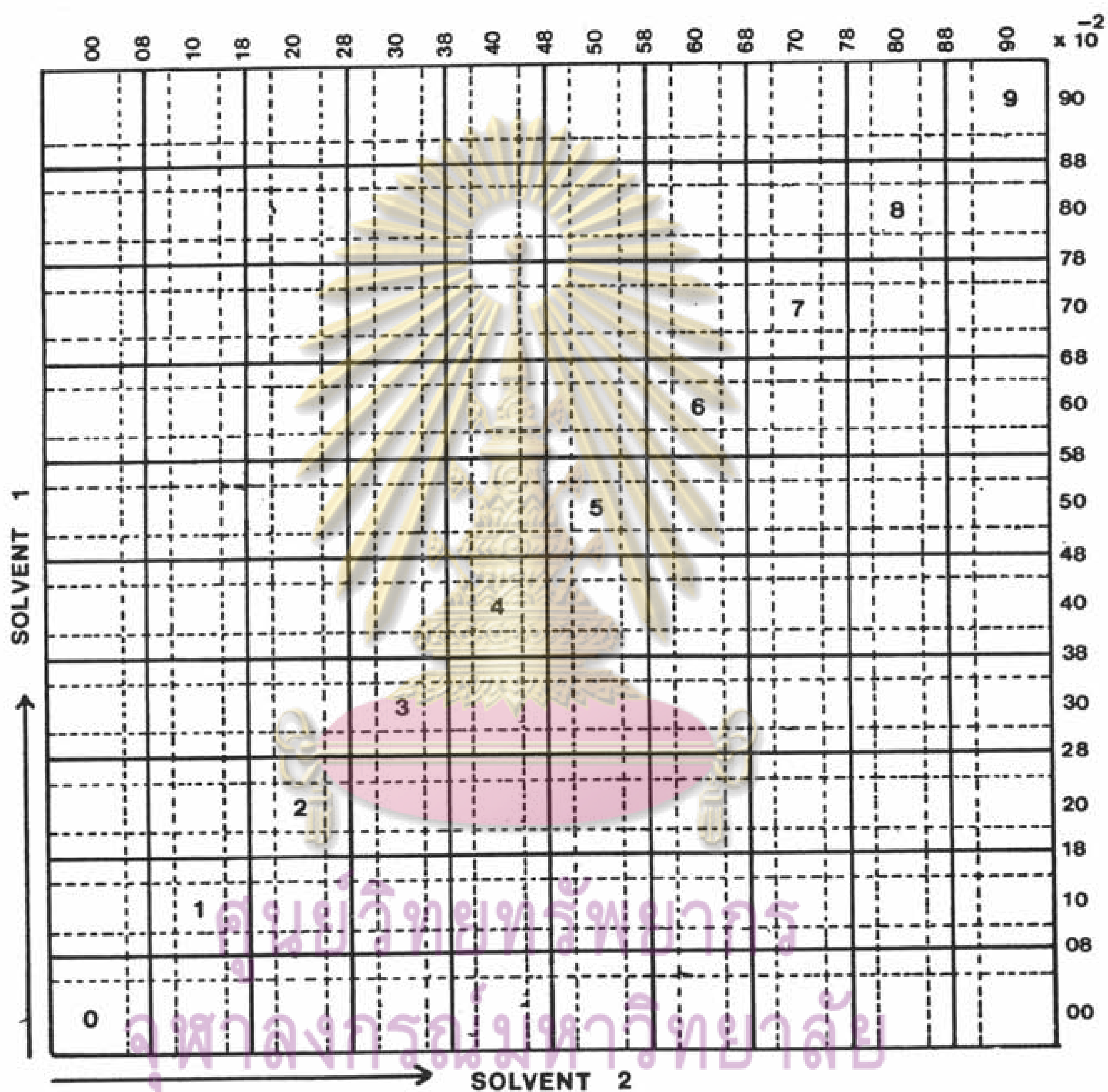

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Figure 3 R_f GRID
(Scale 1: 1)

2.8 Coding of the Data

The chromatographic data were coded for making the table of basic plant data.

The following categories were considered for coding of each spot. 1. Source (plant identification, or name of plant) 2. Treatments and/or detection reagents 3. Solvent systems 4. R_f values and 5. Colours of spots by treatment given (coding of colours)

2.8.1 Source (Plant identification) (10 columns)

Phylum or Division	is under	"PHY"	2	Spermatophyta (Fernald, 1950)
Class or group	" "	"CL"	2	Dicotyledoneae (Fernald, 1950)
Order	" "	"ORD"	32	Rosales (Fernald, 1950)
Family	" "	"FAM"	83	Leguminosae (Fernald, 1950)
Genus	" "	"GEN"	01	*Derris*
Species	" "	"SP"	01	*D. reticulata* Craib
			02	*D.seandens* Benth.

2.8.2 Treatments and/or Detection Reagent

The treatments and/or detection reagents are under "TRT" (2 columns) and they are numbered as follows :

Code Number	Treatment, Detection Reagent
01	Colours in daylight
02	Fluorescence under 254 nm
03	Fluorescence under 365 nm
04	10% Sulphuric acid in ethanol reagent
05	Anisaldehyde-sulphuric acid reagent (AS)
06	Natural products-polyethylenglycol reagent (NP/PEG) (Observed under visible light)
07	Natural products-polyethylenglycol reagent (NP/PEG) (Observed under UV-365 nm)
08	Potassium hexacyanoferrate (III)-ferric chloride reagent.

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2.8.3 Solvent System

Pattern A	R_f values in direction	1	is under	"A1"
	" "	2	"	"A2"
Pattern B	" "	1	"	"B1"
	" "	2	"	"B2"
Pattern C	" "	1	"	"C1"
	" "	2	"	"C2"

A1	refers to	Petroleum spirit : ethyl acetate (7:3)
A2	"	n-Hexane : ethyl acetate (3:2)
B1	"	Toluene : ethyl acetate (7:3)
B2	"	Chloroform : acetone (3:2)
C1	"	Chloroform
C2	"	Toluene : acetone : dichloromethane (40:25:35)

2.8.4 R_f Value

By using the R_f grid, the R_f values were coded. In coding work, when informations become truly voluminous the two digits of coding R_f values may easily be replaced by a one-digit system by shifting the 08, 18, 38, etc. to the higher whole number , thus 1, 2, 3, 4, etc.

2.8.5 Colour of Spot by Treatment Given (Coding of Colours).

The colours obtained with the various reagents were recorded in numerical system under "C" (2 columns). They are arranged in a continuous disc system according to the natural solar spectrum colours No.1 to 7 are used (Vichiara Jirawongse, 1964).

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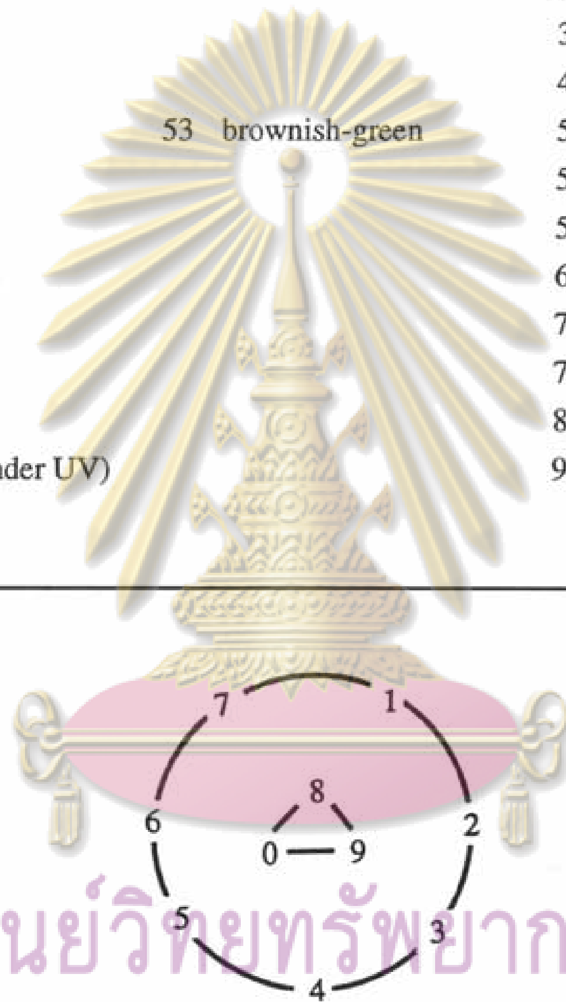
In the coding system used, the first digit stands for the basic colour itself, the second digit 0 stands for light or pale shade, and 5 for darker. For combination of colours, two first digits of the basic colours were used to indicate the various shades of colours. thus :

10 pink	13 purplish-red	15 red
	21 pinkish-orange	
20 pale orange		25 orange
	24 yellowish-orange	
		28 reddish-brown
30 brown		35 dark brown
		38 yellowish-brown
40 pale yellow		45 yellow
50 pale green	53 brownish-green	55 green
		56 bluish-green
		58 greyish-green
60 prussian-blue		65 blue
70 purple		75 violet
		78 pinkish-grey
80 grey		85 black
90 quenching (under UV)		95 strong quenching (under UV)
00 nil		



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Colour Designation

It is difficult to indicate colour with precision (e.g. shade of pink and pale purple), but moderate errors will not invalidate the present system. It is possible to allow wide tolerance (with, of course, less precision) in the computer analysis by including preceding and following number so that moderate error in recording of colour will not discriminate against identifying spot.

Material and Technique Used in Determining the Thin-layer Chromatographic Pattern of Cha-aem Thai from Various Local Traditional Drug Distributor.

1. Material

The samples of crude drug, Cha-aem Thai, for the specification analysis were secured from various local traditional drug distributors. Identification for the corrected species from each source was made by comparison of Thin-layer chromatography pattern of the extract with those of the authentic plant (*Derris reticulata* Craib, Cha-aem nuea).

Thirteen samples of Cha-aem Thai used in this study were shown below.

1 = sample from Vej-ja-pong, Bangkok.
Purchased date 7/7/1994.

2 = sample from Chao-krom-peur, Bangkok.
Purchased date 21/11/1993.

3 = sample from Kruang-yaa-thai, Bangkok.
Purchased date 19/1/1994.

4 = sample from Buan-sae, Bangkok.
Purchased date 22/8/1994.

5 = sample from Bho-pra-dit, Bangkok.
Purchased date 21/12/1994.

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6 = sample from Cha-roen-o-sot, Bangkok.
Purchased date 7/7/1994.

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7 = sample from Gra-ding-thong, Bangkok.
Purchased date 5/6/1994.

8 = sample from Heng-chun-tung, Nakhon Pathom.
Purchased date 11/11/1994.

9 = sample from U-thong-sa-moon-pli, Suphan Buri.
Purchased date 19/4/1994.

10 = sample from Mor-wing-o-sot, Chanthaburi.
Purchased date 18/8/1994.

11 = sample from Tian-chee-tung, Phrae.
Purchased date 16/9/1994.

12 = sample from Sri-ra-ja-o-sot, Chol Buri.
Purchased date 14/7/1994.

13 = sample from Sri-ri-vej-o-sot, Kanchanaburi.
Purchased date 5/3/1994.

2. Extraction

The samples of Cha-aem Thai were grounded to powder. Five grams of powder of Cha-aem Thai from each local traditional drug distributor was macerated in 15 ml of 95% ethanol for three days. The whole solution was filtered and the filtrate was evaporated under reduced pressure until a 5 ml solution was obtained. Four to five applications (about 0.2-0.3 ml) were required. The spot was kept small by using an air blower to produce warm current air for rapid evaporation of the solvent. The solvent was removed as completely as possible before commencing chromatography.

3. The Technique for Analytical Thin-layer Chromatography

Technique : One-dimension TLC

Adsorbent : Silica gel 60 F_{254} (E. Merck) precoated plate

Layer thickness : 0.2 mm

Distance : 15.0 cm

Temperature : Laboratory temperature (30-35°C)

Developing solvents :
1. Ethyl acetate : formic acid : glacial acetic acid : water (11:11:11:27)
2. Chloroform : ethyl acetate (6:4)

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Detection	:	1. Visual detection under ultraviolet light at the wavelength of 254 nm 2. 10% Sulphuric acid in ethanol 3. Anisaldehyde-sulphuric acid spraying reagent 4. Natural products-polyethylenglycol reagent (NP-PEG) (1% Methanolic diphenylboric acid-β-ethylamino ester (=diphenylboryloxyethylamine) (NP) and 5% ethanolic polyethyleneglycol 4000 (PEG)

Material and Technique Used in Determining the Thin-layer Chromatographic Pattern of Sugar

1. Material

The aqueous portion, DR-W, was determined by this technique in order to compare with authentic mono- and di-saccharides.

2. Sample Application

The aqueous portion, DR-W (1.0 g), was dissolved in 50% ethanol (5 ml). Four to five applications (about 0.2-0.3 ml) were required. The spot was kept small by using an air blower to produce warm current air for rapid evaporation of the solvent. The solvent was removed as completely as possible before commencing chromatography.

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3. The Technique for Analytical Thin-layer Chromatography (TLC) (Stahl, 1969)

Techinique	:	One-dimension TLC
Adsorbent	:	Cellulose (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	8.0 cm
Temperature	:	Laboratory Temperature (30-35°C)

Developing solvents : 1. Formic acid : n-butanol : tertiary-butanol : water (15:30:40:15)

2. Aqueous phenol (90%) : water +0.002% oxine (89:11)

Detection reagents : 1. 10% Sulphuric acid in ethanol, then heated at 100-150° for 5-10 minutes.

2. Anisaldehyde-sulphuric acid reagent (AS) is extremely sensitive (0.05 μg of sugar) and gives characteristic colours for the sugars as shown below.

Sugar	Colour reactions of sugars on thin-layers
D-Digitoxose	blue
L-Rhamnose	green
D-Ribose	blue
D-Xylose	grey
L-Arabinose	yellow to green
L-Sorbose	violet
D-Fructose	violet
D-Mannose	green
D-Glucose	light blue
D-Galactose	green to grey
Sucrose	violet
Maltose	violet
Lactose	greenish
D-Glucuronic acid	-
D-Galacturonic acid	-

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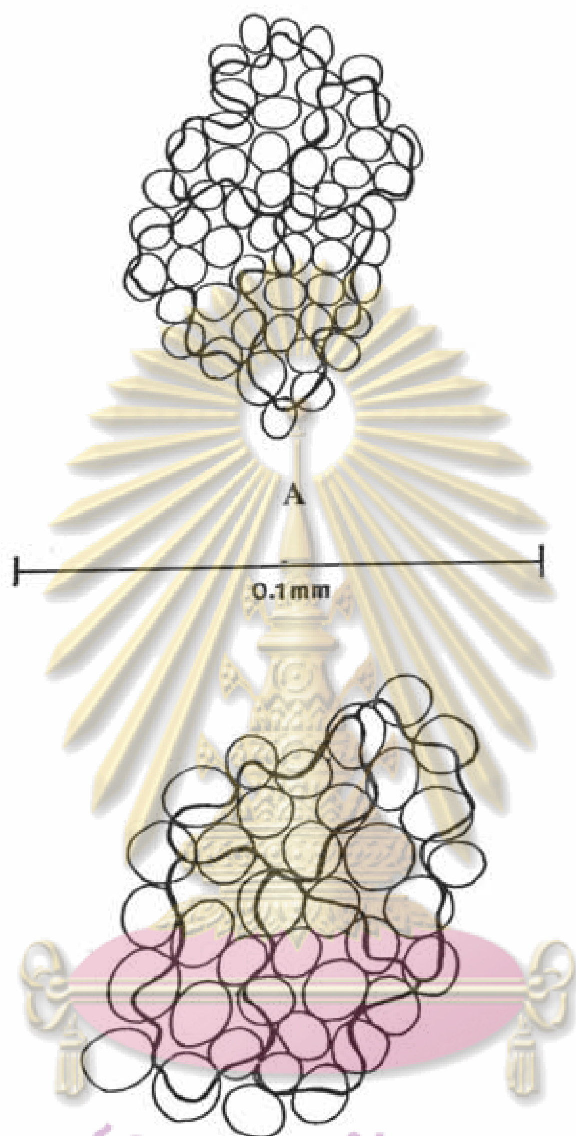

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Figure 4 Four upper epidermal cells with the underlying palisade cells in surface view.
A ; *Derris reticulata* Craib ,
B ; *Derris scandens* Benth.

Table 24 Data on Palisade Ratio Determination of *Derris reticulata* Craib

Number of Palisade cells to four epidermal cells	Palisade Ratio	D	D^2
35	8.75	-0.89	0.7921
36	9.00	-0.64	0.4096
36	9.00	-0.64	0.4096
35	8.75	-0.89	0.7921
42	10.50	0.86	0.7396
35	8.75	-0.89	0.7921
44	11.00	1.36	1.8496
38	9.50	-0.14	0.0196
38	9.50	-0.14	0.0196
38	9.50	-0.14	0.0196
41	10.25	0.61	0.3721
35	8.75	-0.89	0.7921
36	9.00	-0.64	0.4096
38	9.50	-0.14	0.0196
39	9.75	0.11	0.0121
43	10.75	1.11	1.2321
35	8.75	-0.89	0.7921
35	8.75	-0.89	0.7921
45	11.25	1.61	2.5921
39	9.75	0.11	0.0121
36	9.00	-0.64	0.4096
38	9.50	-0.14	0.0196
37	9.25	-0.39	0.1521
37	9.25	-0.39	0.1521
38	9.50	-0.14	0.0196
39	9.75	0.11	0.0121
41	10.25	0.61	0.3721
45	11.25	1.61	2.5921
41	10.25	0.61	0.3721
42	10.50	0.86	0.3796

Mean of Palisade Ratio = 9.64
Standard deviation = 0.768
D = Deviation from mean

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Table 25 Data on Palisade Ratio Determination of *Derris scandens* Benth.

Number of Palisade cells to four epidermal cells	Palisade Ratio	D	D^2
22	5.50	-0.49	0.2401
23	5.75	-0.24	0.0576
23	5.75	-0.24	0.0576
24	6.00	0.01	0.0001
24	6.00	0.01	0.0001
21	5.25	-0.74	0.5476
21	5.25	-0.74	0.5476
21	5.25	-0.74	0.5476
29	7.25	1.26	1.5876
28	7.00	1.01	1.0201
27	6.75	0.76	0.5776
26	6.50	0.51	0.2601
27	6.75	0.76	0.5776
20	5.00	-0.99	0.9801
21	5.25	-0.74	0.5476
21	5.25	-0.74	0.5476
22	5.50	-0.49	0.2401
22	5.50	-0.49	0.2401
22	5.50	-0.49	0.2401
25	6.25	0.26	0.0676
28	7.00	1.01	1.0201
29	7.25	1.26	1.5876
24	6.00	0.01	0.0001
24	6.00	0.01	0.0001
22	5.50	-0.49	0.2401
20	5.00	-0.99	0.9801
29	7.25	1.26	1.5876
29	7.25	1.26	1.5876
21	5.25	-0.74	0.5476
24	6.00	0.01	0.0001

Mean of Palisade Ratio = 5.99
Standard deviation = 0.740
D = Deviation from mean

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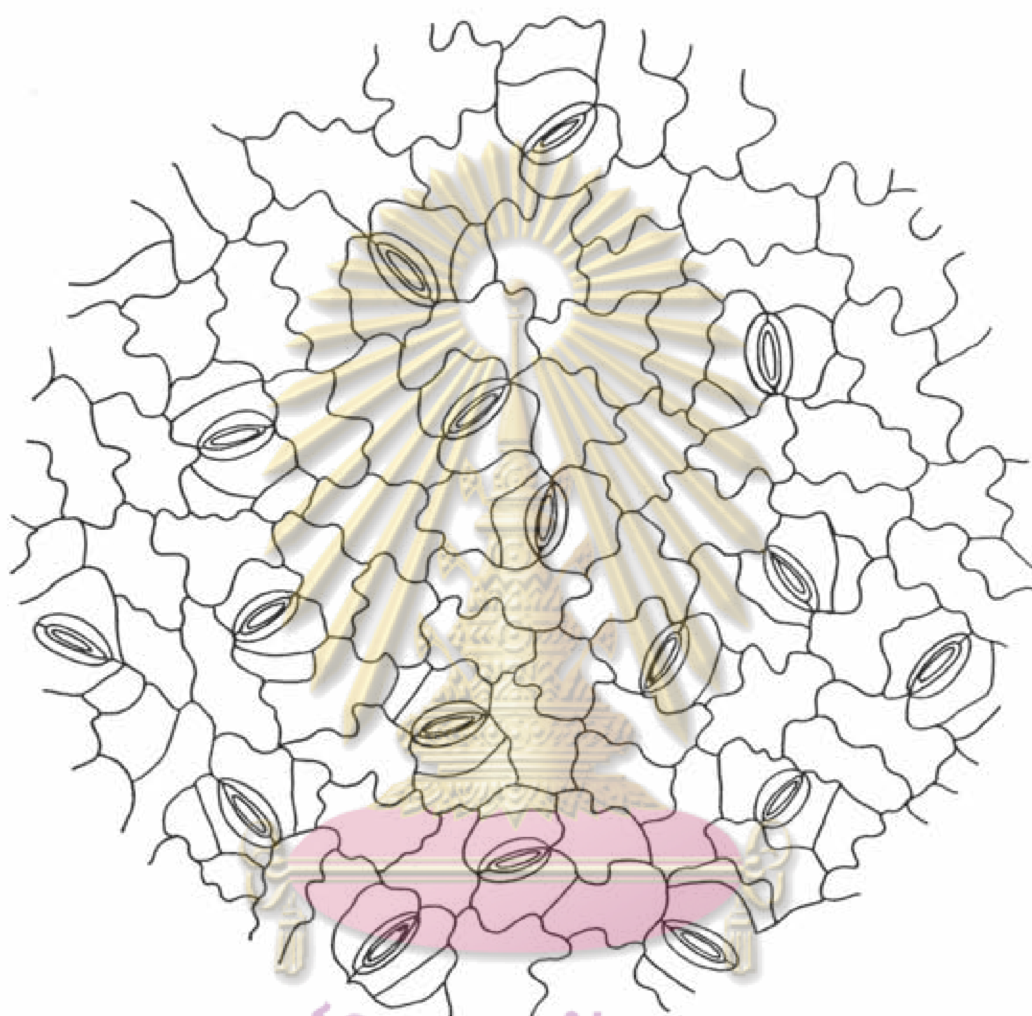

0.1 mm

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Figure 5 Lower epidermis of leaf of *Derris reticulata* Craib in surface view

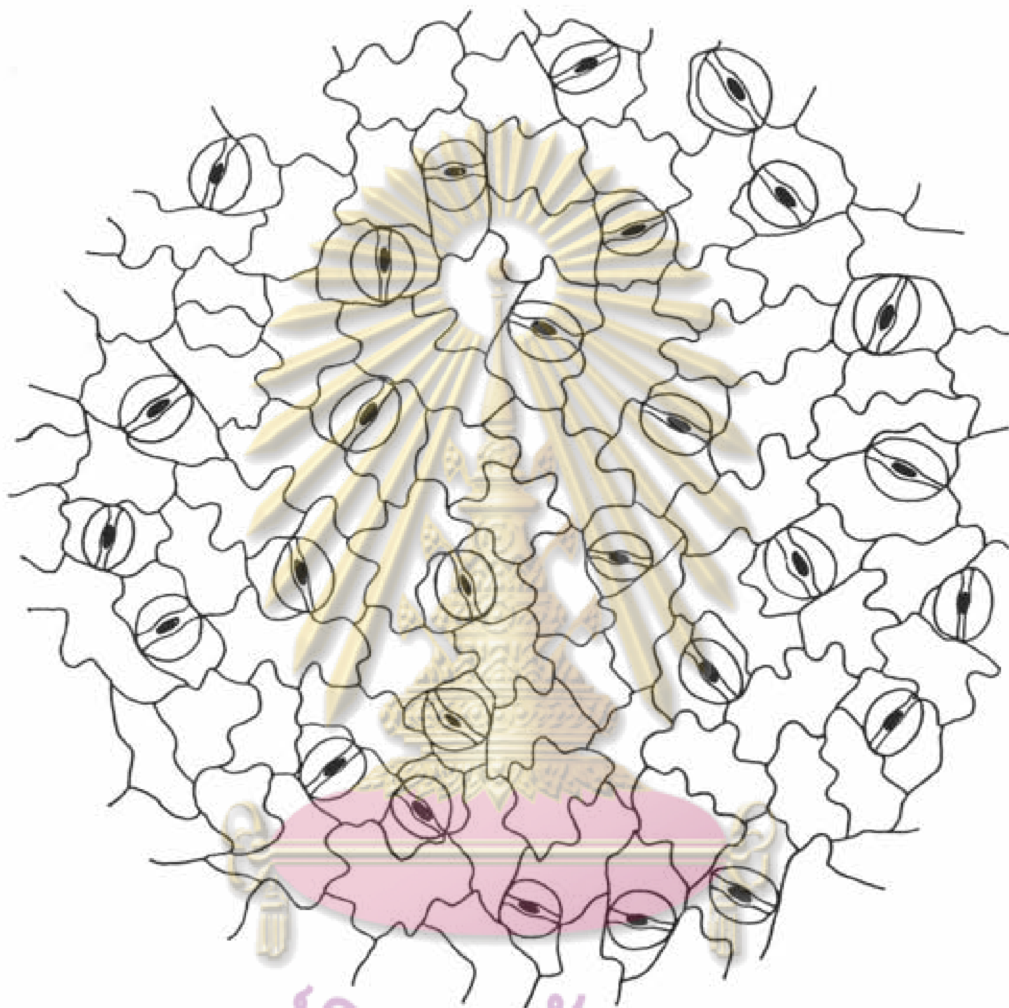

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Figure 6 Lower epidermis of leaf of *Derris scandens* Benth. in surface view

Table 26 Stomatal Number and Stomatal Index Determinations of *Derris reticulata* Craib

Number of Stomata	Number of Epidermal cells	Stomatal Number (Sn)	Stomatal Index (SI)	D (SI)	D^2 (SI)
13	104	342.11	11.11	-0.58	0.3364
14	106	368.42	11.67	-0.02	0.0004
15	111	394.74	11.81	0.12	0.0144
15	107	394.74	12.30	0.61	0.3721
15	107	394.74	12.30	0.61	0.3721
13	99	342.11	10.66	-1.03	1.0609
13	102	342.11	11.30	-0.39	0.1521
13	104	342.11	11.11	-0.58	0.3364
16	117	421.05	12.03	0.34	0.1156
13	104	342.11	11.11	-0.58	0.3364
13	109	342.11	10.66	-1.03	1.0609
14	104	368.42	11.86	0.17	0.0289
14	104	368.42	11.86	0.17	0.0289
14	105	368.42	11.76	0.07	0.0049
15	104	394.74	12.61	0.92	0.8464
13	111	342.11	10.48	-1.21	1.4641
14	105	368.42	11.76	0.07	0.0049
14	102	368.42	12.07	0.38	0.1444
16	119	421.05	11.85	0.16	0.0256
14	109	368.42	11.38	-0.31	0.0961
15	102	394.74	12.82	1.13	1.2769
15	115	394.74	11.54	-0.15	0.0225
13	112	342.11	10.40	-0.29	0.0841
14	116	368.42	10.77	-0.92	0.8464
14	120	368.42	10.45	-1.24	1.5376
15	108	394.74	12.20	0.51	0.2601
15	103	394.74	12.71	1.02	1.0404
15	104	394.74	12.61	0.92	0.8464
13	94	342.11	12.15	0.46	0.2116
15	98	394.74	13.27	1.58	2.4964

Area of Determination = 0.038 mm^2
Mean of Stomatal Number (lower epidermis) = 372.81
Mean of Stomatal Index (lower epidermis) = 11.69
Standard deviation of the stomatal Index = 0.753
D = Deviation from mean

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Table 27 Stomatal Number and Stomatal Index Determinations of *Derris scandens* Benth.

Number of Stomata	Number of Epidermal cells	Stomatal Number (Sn)	Stomatal Index (SI)	D (SI)	D^2 (SI)
23	87	605.26	23.00	-0.37	0.1369
26	90	684.21	22.41	-0.96	0.9216
30	99	789.47	23.26	-0.11	0.0121
30	98	789.47	23.44	0.07	0.0049
28	92	736.84	23.44	0.07	0.0049
29	93	763.16	23.77	0.40	0.1600
30	96	789.47	23.81	0.44	0.1936
30	91	789.47	25.00	1.63	2.6569
25	84	657.89	22.94	-0.43	0.1849
24	80	631.58	23.08	-0.29	0.0841
23	76	605.26	23.23	-0.14	0.0196
23	81	605.26	22.12	-1.25	1.5625
26	84	684.21	23.64	0.27	0.0729
27	89	710.53	23.28	-0.09	0.0081
28	109	736.84	20.44	-2.93	8.5849
25	80	657.89	23.81	0.44	0.1936
27	93	710.53	22.50	-0.87	0.7569
30	98	789.47	23.44	0.07	0.0049
30	103	789.47	22.57	-0.80	0.6400
29	85	763.16	25.44	2.07	4.2849
24	84	631.58	22.22	-1.15	1.3225
25	91	657.89	21.55	-1.82	3.3124
31	96	815.79	24.41	1.04	1.0816
28	96	736.84	22.58	-0.79	0.6241
29	89	763.16	24.58	1.21	1.4641
30	96	789.47	23.81	0.44	0.1936
27	88	710.53	23.48	0.11	0.0121
28	92	736.84	23.33	-0.04	0.0016
31	91	815.79	25.41	2.04	4.1616
29	86	763.16	25.22	1.85	3.4225

Area of Determination = 0.038 mm^2
Mean of Stomatal Number (lower epidermis) = 723.68
Mean of Stomatal Index (lower epidermis) = 23.37
Standard deviation of the stomatal Index = 1.097
D = Deviation from mean

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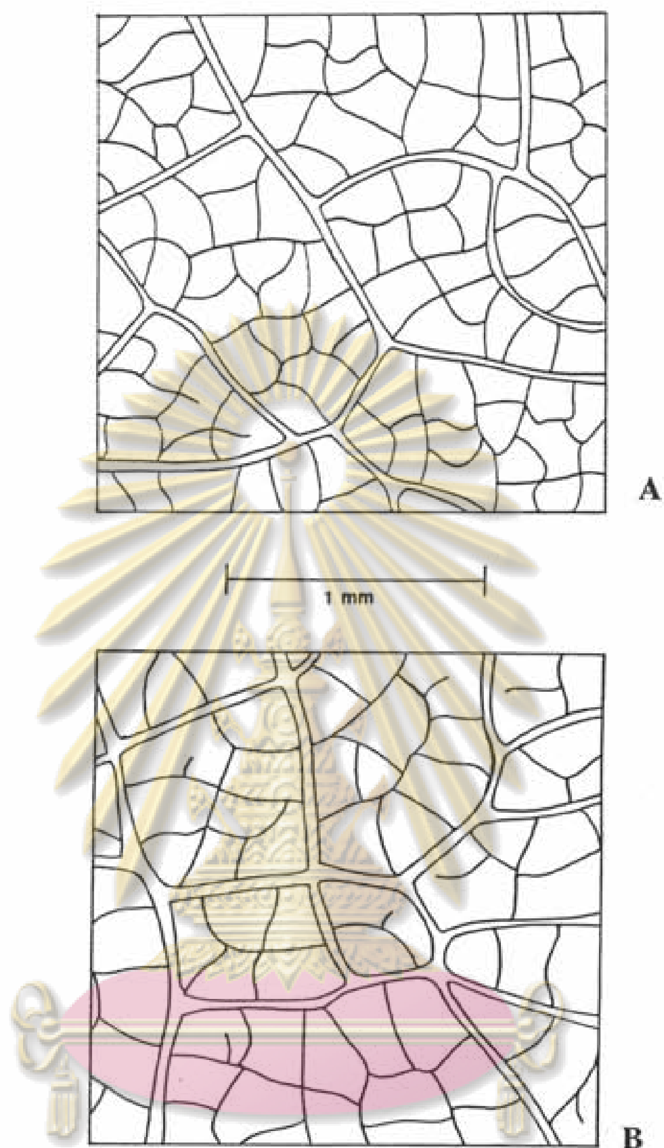

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Figure 7 Vein-islets and veinlet terminations of leaves in surface view
A; *Derris reticulata* Craib .
B; *Derris scandens* Benth.

Table 28 Vein-Islet Number Determination of *Derris reticulata* Craib

Number of Vein-Islets to 4 mm^2	Vein-Islet Number	D	D^2
75	18.75	-1.70	2.8900
80	20.00	0.45	0.2025
74	18.50	-1.95	3.8025
81	20.25	-0.20	0.0400
81	20.25	-0.20	0.0400
69	17.25	-3.20	10.2400
81	20.25	-0.20	0.0400
74	18.50	-1.95	3.8025
89	22.25	1.80	3.2400
90	22.50	2.05	4.2025
93	23.25	2.80	7.8420
89	22.25	1.80	3.2400
85	21.25	0.80	0.6400
82	20.50	0.05	0.0025
82	20.50	0.05	0.0025
70	17.50	-2.95	8.7025
74	18.50	-1.95	3.8025
75	18.75	-1.70	2.8900
83	20.75	0.30	0.0900
83	20.75	0.30	0.0900
85	21.25	0.80	0.6400
70	17.50	-2.95	8.7025
90	22.50	2.05	4.2025
90	22.50	2.05	4.2025
91	22.75	2.30	5.2900
79	19.75	-0.70	0.4900
76	19.00	-1.45	2.1025
77	19.25	-1.20	1.4400
93	23.25	2.80	7.8400
93	23.25	2.80	7.8400

Mean of Vein-Islet Number = 20.45
Standard deviation = 1.812
D = Deviation from mean

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Table 29 Vein-Islet Number Determination of *Derris scandens* Benth.

Number of Vein-Islets to 4 mm^2	Vein-Islet Number	D	D^2
60	15.00	0.05	0.0025
59	14.75	-0.20	0.0040
60	15.00	0.05	0.0025
59	14.75	-0.20	0.0040
59	14.75	-0.20	0.0040
63	15.75	0.80	0.6400
63	15.75	0.80	0.6400
67	16.75	1.80	3.2400
52	13.00	-1.95	3.8025
52	13.00	-1.95	3.8025
68	17.00	2.05	4.2025
64	16.00	1.05	1.1025
61	15.25	0.30	0.0900
61	15.25	0.30	0.0900
58	14.50	-0.45	0.2025
65	16.25	1.30	1.6900
61	15.25	0.30	0.0900
53	13.25	-1.70	2.8900
53	13.25	-1.70	2.8900
55	13.25	-1.20	1.4400
54	13.50	-1.45	2.1025
57	14.25	-0.70	0.4900
56	14.00	-0.95	0.9025
62	15.50	0.55	0.3025
55	13.75	-1.20	1.4400
54	13.50	-1.45	2.1025
71	17.75	2.80	7.8400
62	15.50	0.55	0.3025
71	17.75	2.80	7.8400
59	14.75	-0.20	0.0040

Mean of Vein-Islet Number = 14.95
Standard deviation = 1.295
D = Deviation from mean

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Table 30 Veinlet Termination Number Determination of *Derris reticulata* Craib

Number of Veinlet Terminations to 4 mm^2	Veinlet Termination Number	D	D^2
1	0.25	-0.4	0.1600
1	0.25	-0.4	0.1600
2	0.50	-0.15	0.0225
5	1.25	0.6	0.3600
1	0.25	-0.4	0.1600
2	0.50	-0.15	0.0225
2	0.50	-0.15	0.0225
3	0.75	0.1	0.0100
4	1.00	0.35	0.1225
1	0.25	-0.4	0.1600
2	0.50	-0.15	0.0225
1	0.25	-0.4	0.1600
1	0.25	-0.4	0.1600
3	0.75	0.1	0.0100
3	0.75	0.1	0.0100
2	0.50	-0.15	0.0225
2	0.50	-0.15	0.0225
2	0.50	-0.15	0.0225
4	1.00	0.35	0.1225
5	1.25	0.6	0.3600
4	1.00	0.35	0.1225
2	0.50	-0.15	0.0225
2	0.50	-0.15	0.0225
2	0.50	-0.15	0.0225
3	0.75	0.1	0.0100
3	0.75	0.1	0.0100
4	1.00	0.35	0.1225
4	1.00	0.35	0.1225
4	1.00	0.35	0.1225
3	0.75	0.1	0.0100

Mean of Veinlet Termination Number = 0.65

Standard deviation = 0.300

D = Deviation from mean

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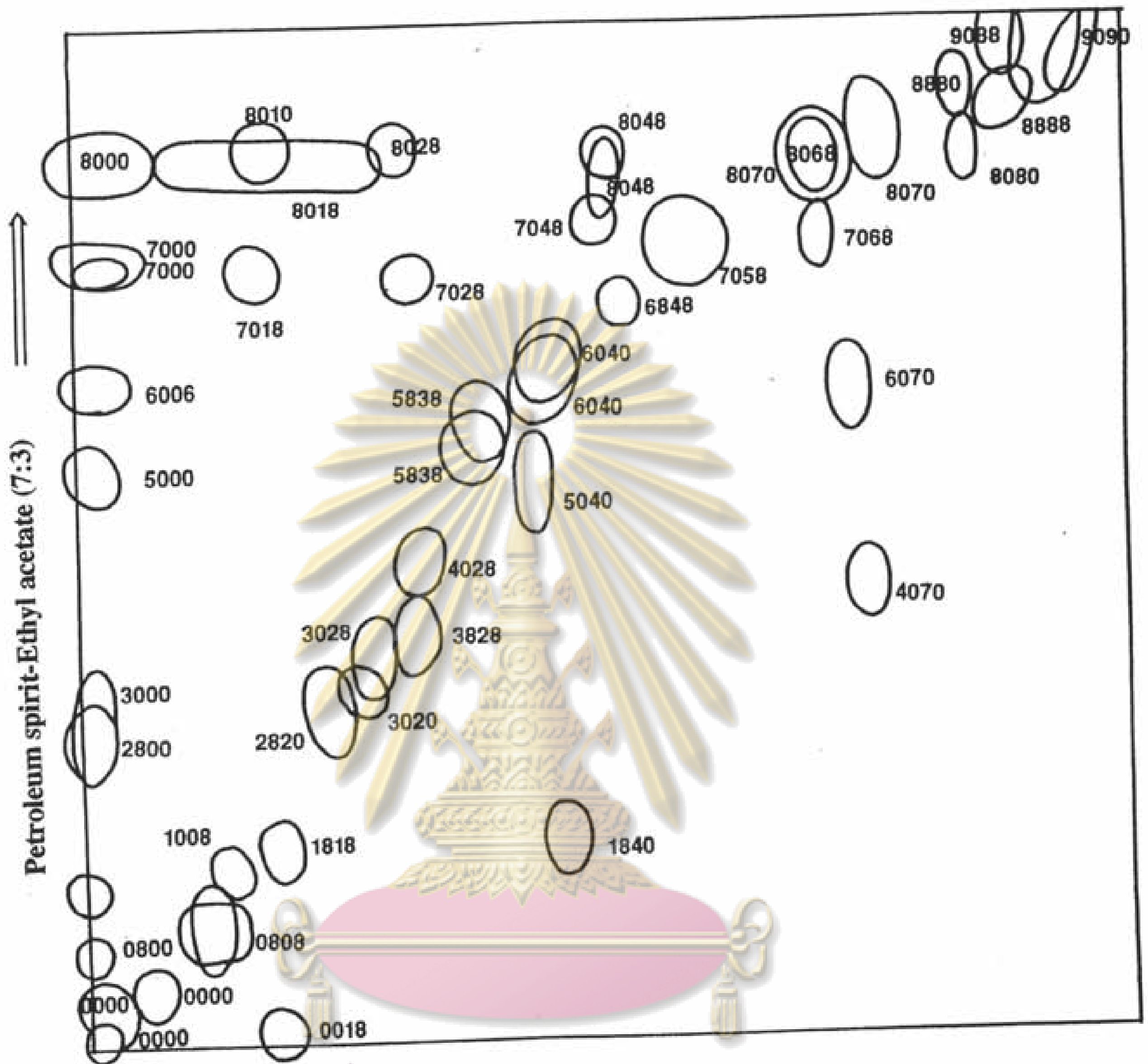
Table 31 Veinlet Termination Number Determination of *Derris scandens* Benth.

Number of Veinlet Terminations to 4 mm^2	Veinlet Termination Number	D	D^2
5	1.25	-0.43	0.1849
5	1.25	-0.43	0.1849
7	1.75	0.07	0.0049
6	1.50	-0.18	0.0324
6	1.50	-0.18	0.0324
7	1.75	0.07	0.0049
5	1.25	-0.43	0.1849
5	1.25	-0.43	0.1849
5	1.25	-0.43	0.1849
7	1.75	0.07	0.0049
7	1.75	0.07	0.0049
8	2.00	0.32	0.1024
6	1.50	-0.18	0.0324
6	1.50	-0.18	0.0324
6	1.50	-0.18	0.0324
6	1.50	-0.18	0.0324
7	1.75	0.07	0.0049
7	1.75	0.07	0.0049
7	1.75	0.07	0.0049
8	2.00	0.32	0.1024
8	2.00	0.32	0.1024
9	2.25	0.57	0.3249
5	1.25	-0.43	0.1849
6	1.50	-0.18	0.0324
6	1.50	-0.18	0.0324
9	2.25	0.57	0.3249
8	2.00	0.32	0.1024
9	2.25	0.57	0.3249
9	2.25	0.57	0.3249
6	1.50	-0.18	0.0324

Mean of Veinlet Termination Number = 1.675
Standard deviation = 0.3237
D = Deviation from mean

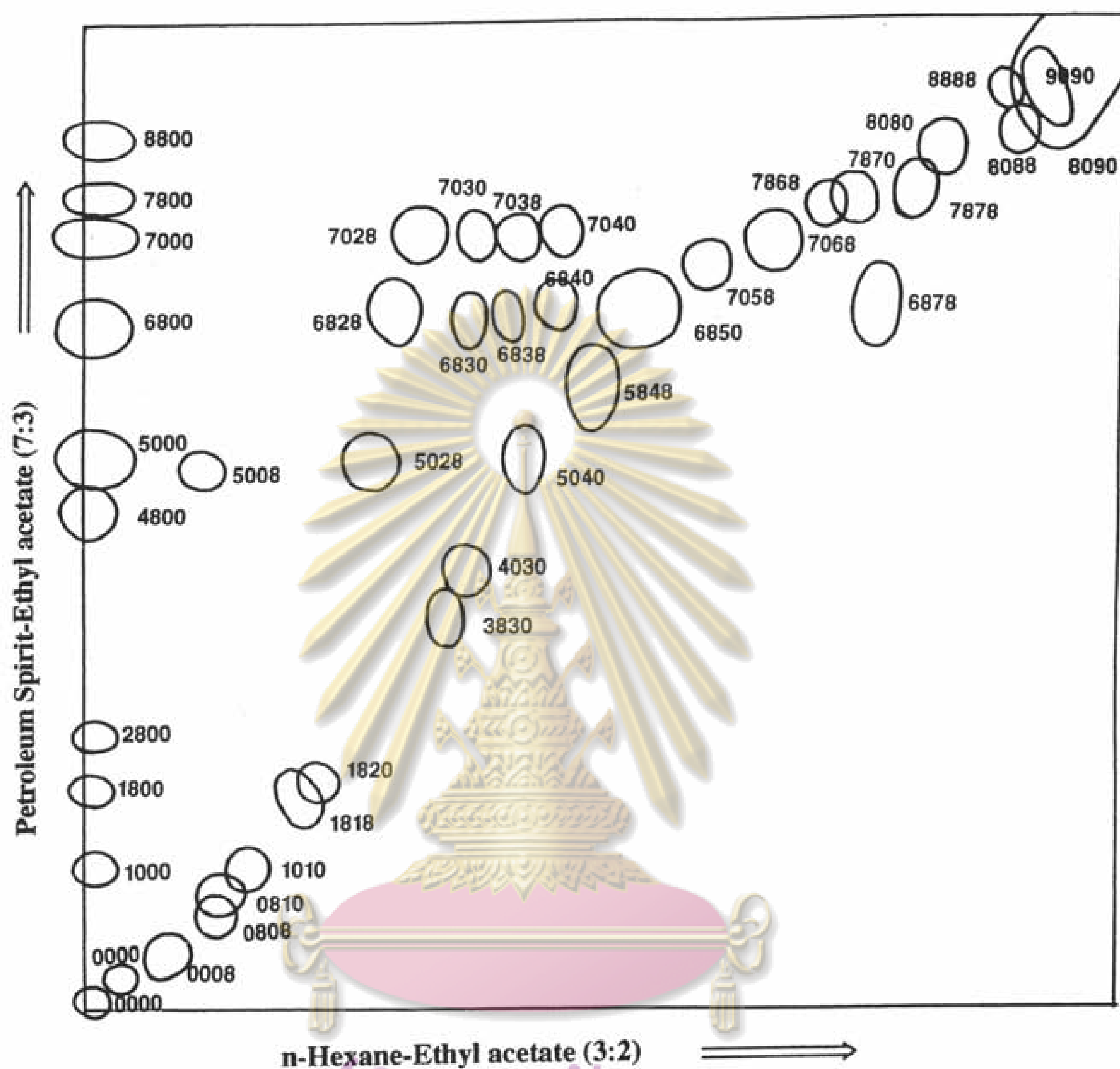
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Solvent system : A_1A_2 Treatment 01, 02, 03, 06, 07, 08

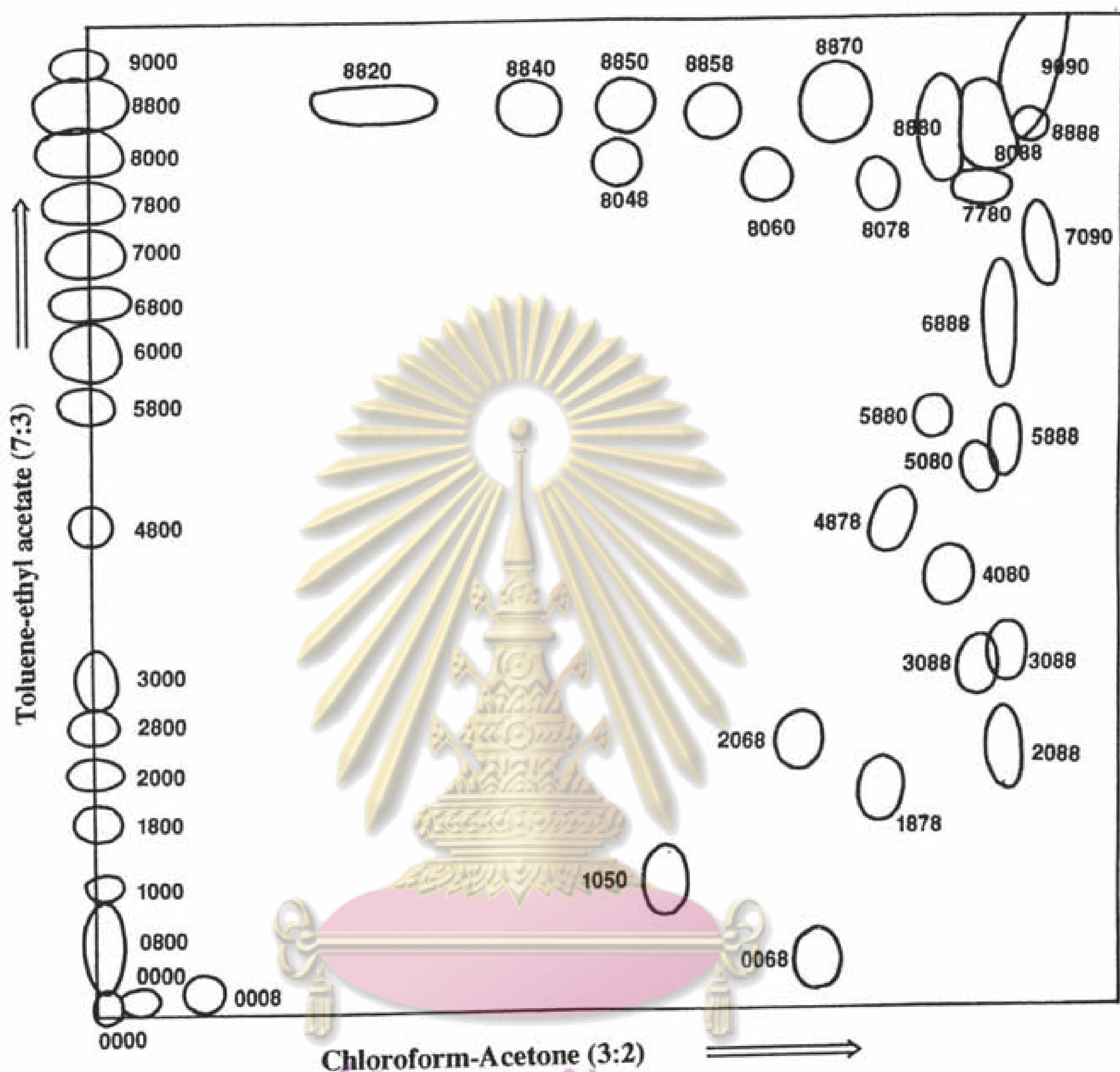
Figure 8 Two-dimensional thin-layer chromatogram of stem of *Derris reticulata* Craib



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Solvent system : A_1A_2 Treatment 01, 02, 03, 06, 07, 08

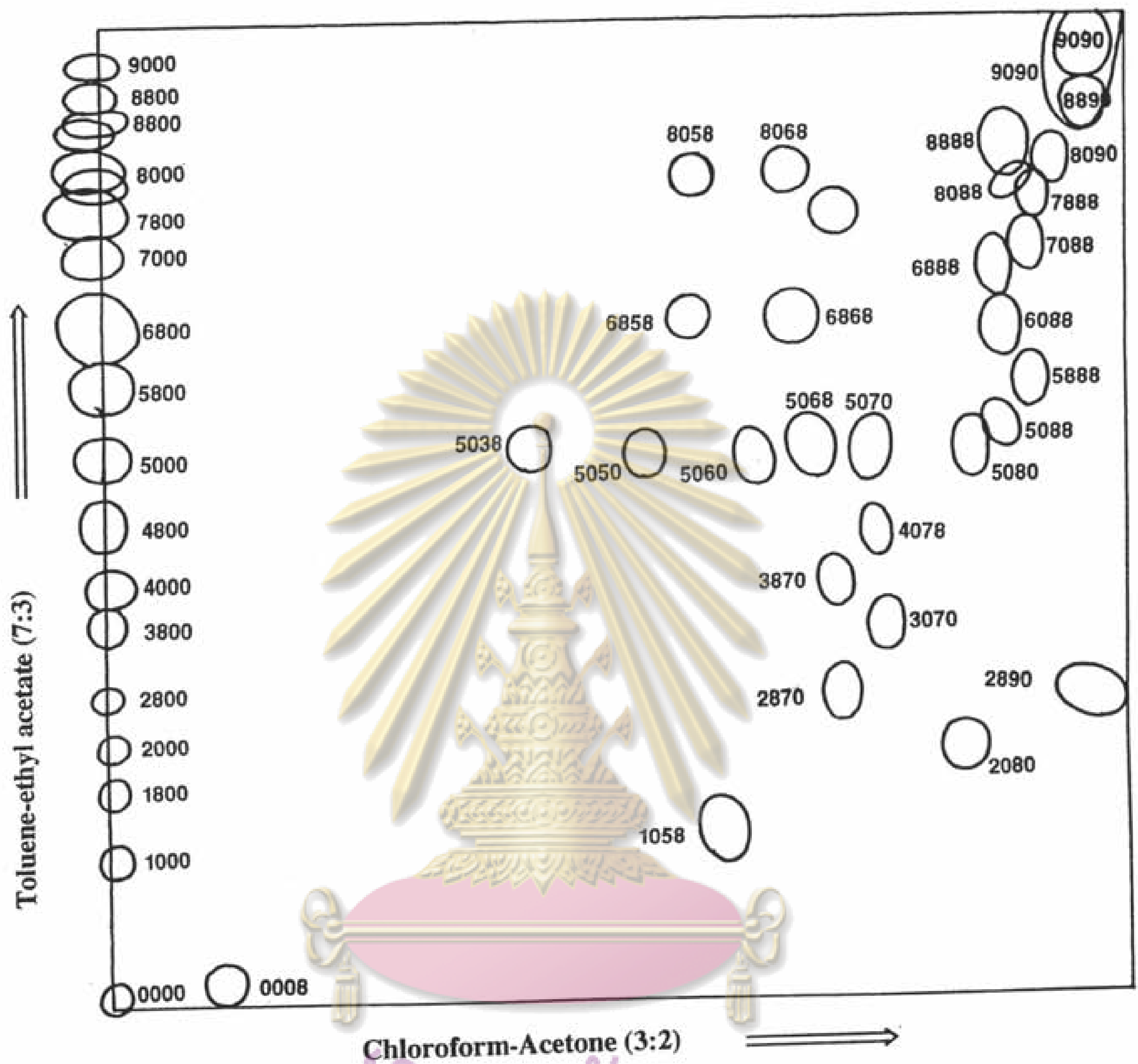
Figure 9 Two-dimensional thin-layer chromatogram of stem of *Derris scandens* Benth.



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Solvent system : B_1B_2 Treatment 01, 02, 03, 06, 07, 08

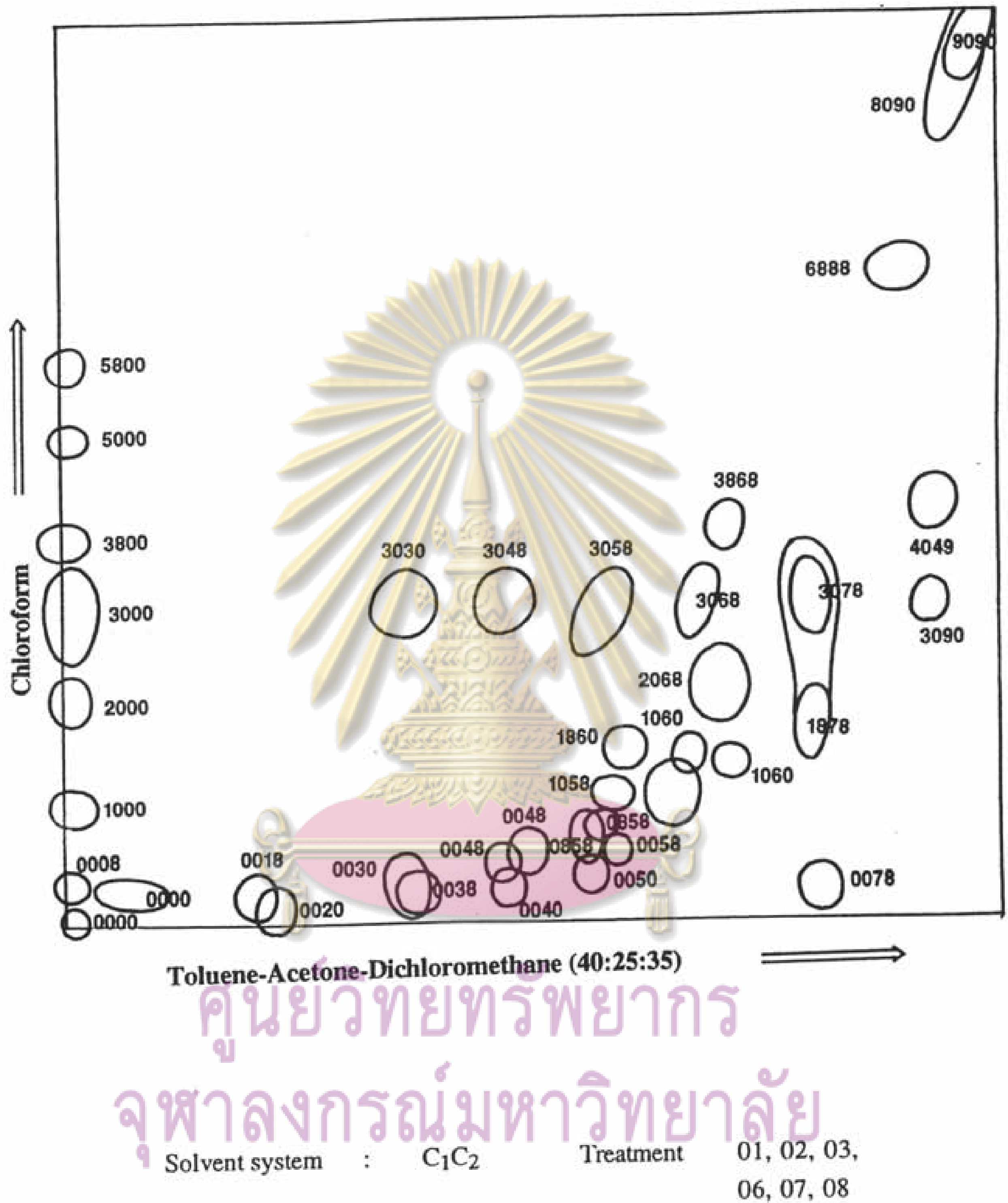
Figure 10 Two-dimensional thin-layer chromatogram of stem of *Derris reticulata* Craib



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

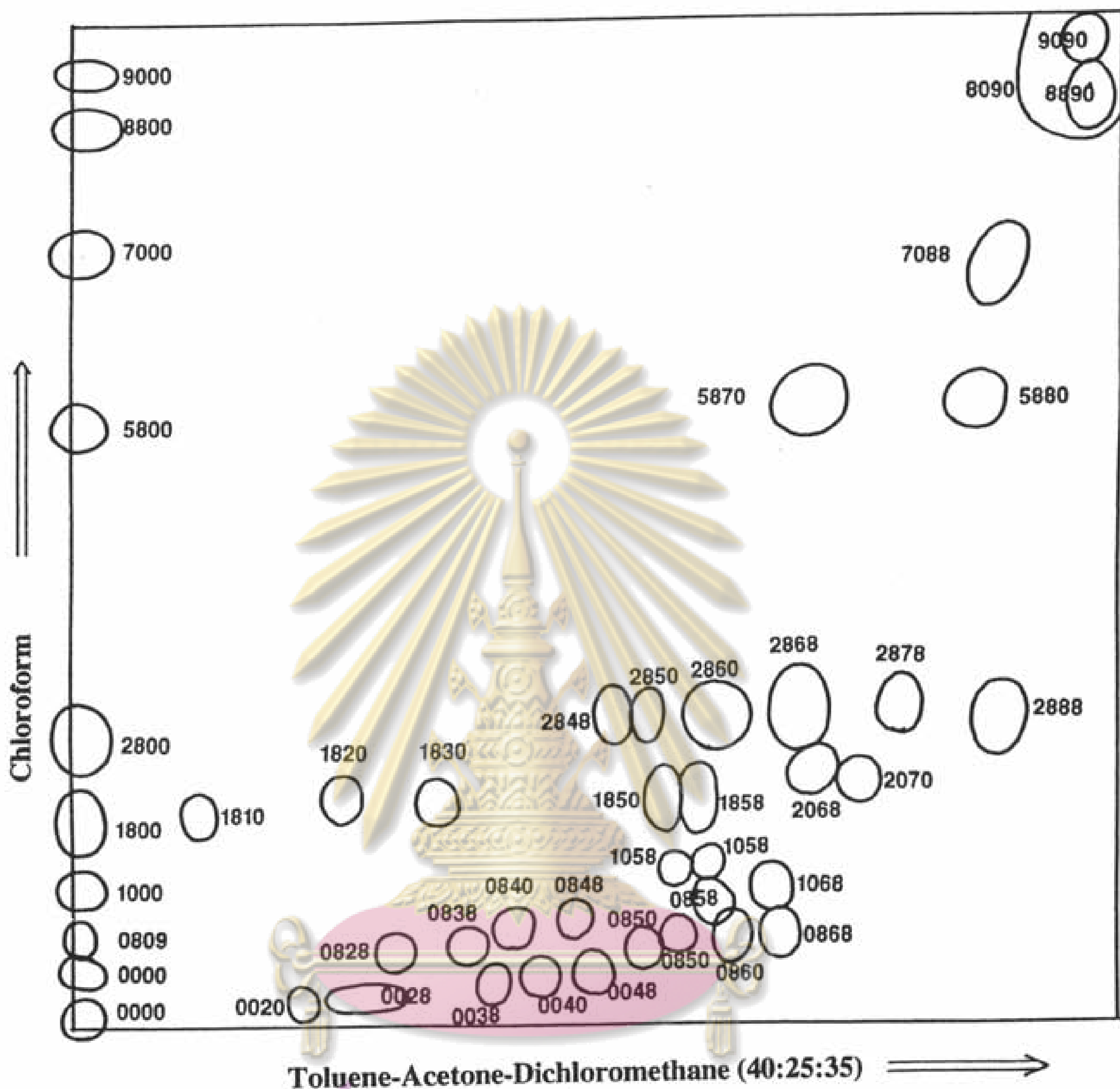
Solvent system : B_1B_2 Treatment 01, 02, 03, 06, 07, 08

Figure 11 Two-dimensional thin-layer chromatogram of stem of *Derris scandens* Benth.



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 12 Two-dimensional thin-layer chromatogram of stem of *Derris reticulata* Craib



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Solvent system : C_1C_2 Treatment 01, 02, 03, 06, 07, 08

Figure 13 Two-dimensional thin-layer chromatogram of stem of *Derris scandens* Benth.

Table 32 Basic Plant Data of stem of *Derris reticulata* Craib

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	01	01	000030	000030	000030
							000038	000038	200038
							705838	800038	206838
							807045	808845	307845
2	2	32	83	01	01	02	000095	000095	000090
							080090	100090	000095
							080890	180090	001890
							100090	187890	003090
							280090	200090	004090
							302890	206890	004890
							403090	280090	005090
							500090	308890	005890
							583890	408090	007890
							604065	480090	085890
							700090	487890	100090
							701890	508890	106090
							702890	580090	106865
							704890	588090	187890
							705890	600090	200090
							706890	688865	206890

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 32 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	01	02	800090	800090	300090
							801090	808895	303090
							802890	880090	304890
							804890	909090	305890
							807095		306890
							909095		307895
									909090
2	2	32	83	01	01	03	000015	000045	000045
							000045	000856	000065
							000075	006865	000815
							001865	080028	002075
							080865	105065	003865
							100855	208865	004875
							181865	300065	085845
							184075	308875	105865
							282065	487828	106075
							382865	508855	186045
							402850	588875	200045
							407065	588065	206845
							500040	600040	287838

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 32 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	01	03	504075	688875	300045
							583855	680065	303045
							600065	700065	304845
							604075	709065	305858
							607065	780065	306845
							700040	788055	307895
							700045	800045	309065
							705845	804838	386865
							800045	806065	409065
							801845	807865	500065
							806895	808895	580065
							807050	880050	688855
							807865	882038	909065
							808050	884038	
							888055	885038	
							888865	885838	
							908865	887038	
							909065	888038	
2	2	32	83	01	01	04	000030	000030	000030

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 32 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	01	04	000030	000030	100038
							280038	000830	187838
							500038	080038	200030
							700038	800030	206830
							705838	808030	300038
							800038	808838	307830
							804838	909030	909030
							807038		
							909030		
2	2	32	83	01	01	05	000015	000025	000015
							000024	000865	000024
							000075	080038	001065
							080865	084865	001890
							300055	105065	003065
							302840	408045	003875
							407040	487865	004865
							500055	505875	085856
							600055	588875	100065
							604075	680055	105040
							680055	688875	105875

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 32 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	01	05	684875	700075	106075
							700055	780075	186056
							705865	788045	200045
							800055	800055	206865
							801855	808090	287840
							806895	808895	300040
							807040	880040	303040
							908856	888855	304840
							909065		306840
									306875
									307895
									380075
									587040
									688856
									708040
									909065
2	2	32	83	01	01	06	000030	000030	000030
							000038	080038	187845
							705838	580040	200038
							800040	788038	206838

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 32 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	01	06	806845	800038	289845
							807045	80884	300040
								808845	304840
									305840
									306840
									307845
									909040
2	2	32	83	01	01	07	000055	000050	000015
							000075	000055	000055
							000080	080028	000065
							080856	105075	002075
							184075	187865	003865
							302015	206875	004015
							382865	300065	004865
							402865	308865	085855
							500040	308875	100075
							504075	480065	105865
							583855	487815	106075
							600065	508865	186050
								588875	187840

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 32 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	01	07	604075	600040	200045
							684865	680065	206845
							700045	688875	287845
							705845	700065	300056
							706865	780065	303045
							800040	788045	304845
							801865	800055	306845
							804865	808830	306858
							806830	880056	380075
							807050	887065	386865
							808056	888075	688856
							888865	888855	909065
2	2	32	83	01	01	08	680065	000865	000065
							700065	487865	003065
							705865	508865	004065
							800065	688865	005065
							801865	788065	005865
							804865	808865	085865
							806865	800045	206865
							807065		287865

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 32 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	01	08	909065		305865
									306865
									307865
									909065

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 33 Basic Plant Data of stem of *Derris scandens* Benth.

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	02	01	000038	000030	000030
							683840	789040	286840
							684040	909040	
							685040		
							703040		
							703840		
							704040		
							706040		
							787040		
							808040		
2	2	32	83	01	02	02	000095	000024	000085
							000890	100090	000095
							080890	105890	002895
							100090	180090	003895
							101090	200090	004095
							180090	280090	004890
							182090	287090	080095
							280090	307890	082890
							500090	380090	083895
							5000890	387090	084090

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 33 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	02	02	502890	400090	084890
							504090	407890	085090
							680095	480090	085890
							682890	500090	086095
							683090	505390	086890
							683890	505090	100095
							684090	506090	105895
							685095	506890	180095
							687895	507090	181090
							700090	508090	182090
							702890	580090	183090
							703090	588890	185095
							703890	680090	185895
							704090	685890	207095
							706895	686890	280095
							786895	688895	284890
							808093	700090	285090
							809090	708895	286090
							880090	787090	286895
								788895	287890
								800090	288890

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 33 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	02	02		805890	580090
								806890	587090
								809095	588090
								880090	700090
								888895	889095
								889090	909095
								900090	
2	2	32	83	01	02	03	000024	000038	000024
							000050	208075	002065
							000865	289028	085875
							081050	507080	105890
							181865	508080	106875
							383075	508875	205890
							403080	588850	207080
							480040	608875	206840
							503838	688895	280038
							584875	708895	286895
							600095	788895	288890
							683090	800050	708850
							683890	809095	809065

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 33 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	02	03	684090	888895	880040
							685095	909065	889065
							703090		900040
							703890		909065
							704090		
							705850		
							706095		
							786895		
							787865		
							888865		
							808875		
							880050		
							909065		
2	2	32	83	01	02	04	000030	000030	000030
							504038	708838	180038
							600038	788838	185038
							688338	809038	185838
							684038	909038	207838
							685038		280038
							703838		285038

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 33 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	02	04	704038		286838
							706838		809030
							787038		
							808038		
							809038		
2	2	32	83	01	02	05	000024	000024	000040
							000065	000840	002065
							086840	588890	086050
							100040	608865	106875
							204040	688890	185090
							383065	708828	185890
							504065	787090	206865
							582040	788828	207090
							584875	809028	286895
							683090	808890	287840
							684090	909065	288890
							685095		587065
							687890		588090
							703890		802050
							704090		880040

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 33 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	02	05	705865		
							706890		
							787890		
							788024		
							808865		
							809065		
							888865		
							909065		
2	2	32	83	01	02	06	000030	000030	000030
							684040	708845	185040
							685045	788845	185840
							704040	809045	207040
							706840	889040	286845
							787040	909040	
							808040		
2	2	32	83	01	02	07	000024	000025	000025
							504038	289025	086035
							584875	507065	105890

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 33 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	02	07	680024	508065	106875
							682865	508875	185090
							683090	588840	185890
							683890	608875	206875
							684090	688895	207065
							685028	708828	280024
							687890	787025	286828
							703090	788828	580040
							703890	809095	700040
							704090	808845	809065
							705865	880040	909065
							706890	888895	
							786865	909065	
							787880		
							800040		
							808075		
							888850		
							909075		
2	2	32	83	01	02.	08	504065	688865	185065
							684065	708865	185895

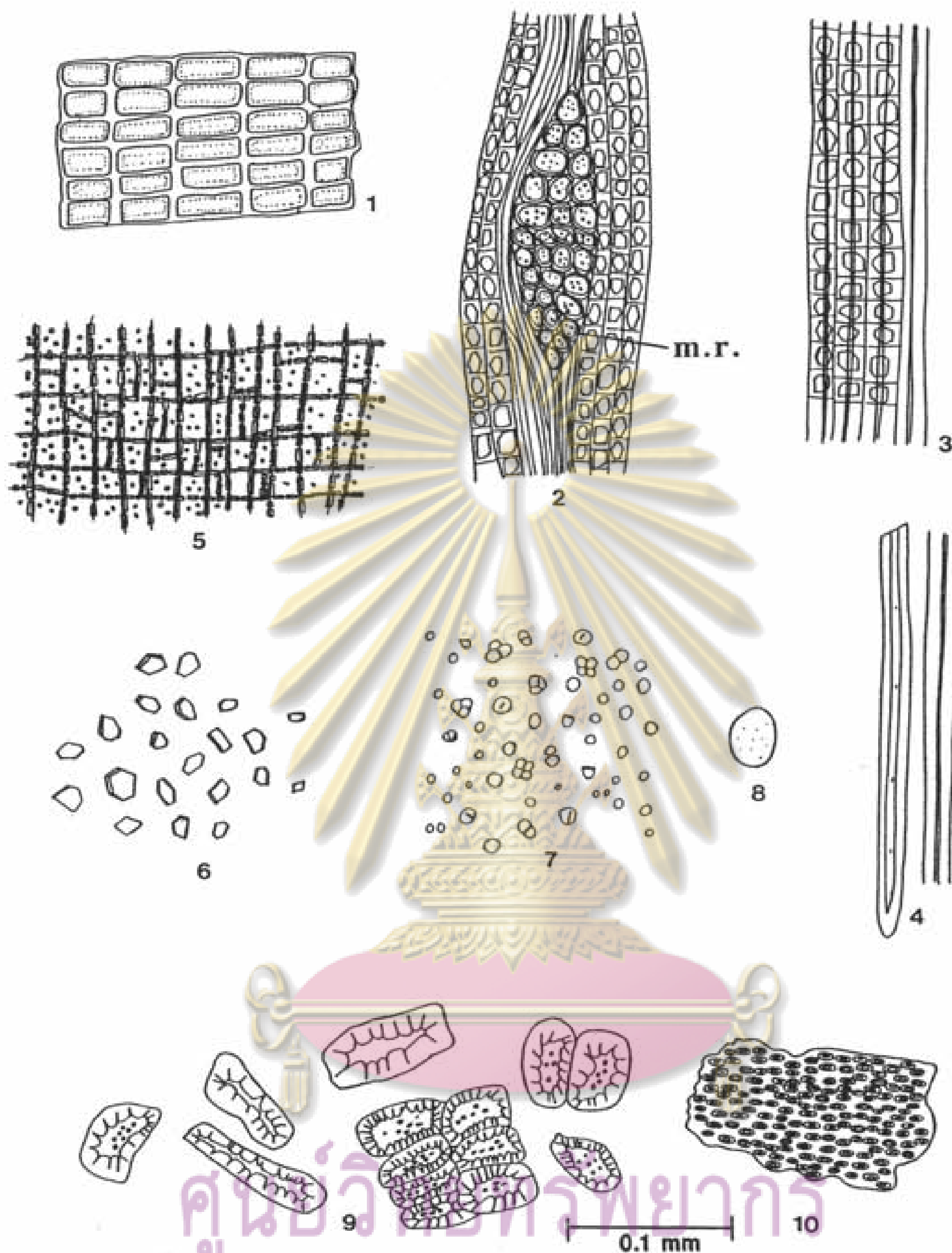
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 33 (continued)

Plant Identification						TRT	A_1A_2C	B_1B_2C	C_1C_2C
PHY	CL	ORD	FAM	GEN	SP				
2	2	32	83	01	02	08	685065	788865	207065
							700065	789065	286865
							704065	809065	288865
							706865	880065	889065
							787065	888865	909065
							787865		

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

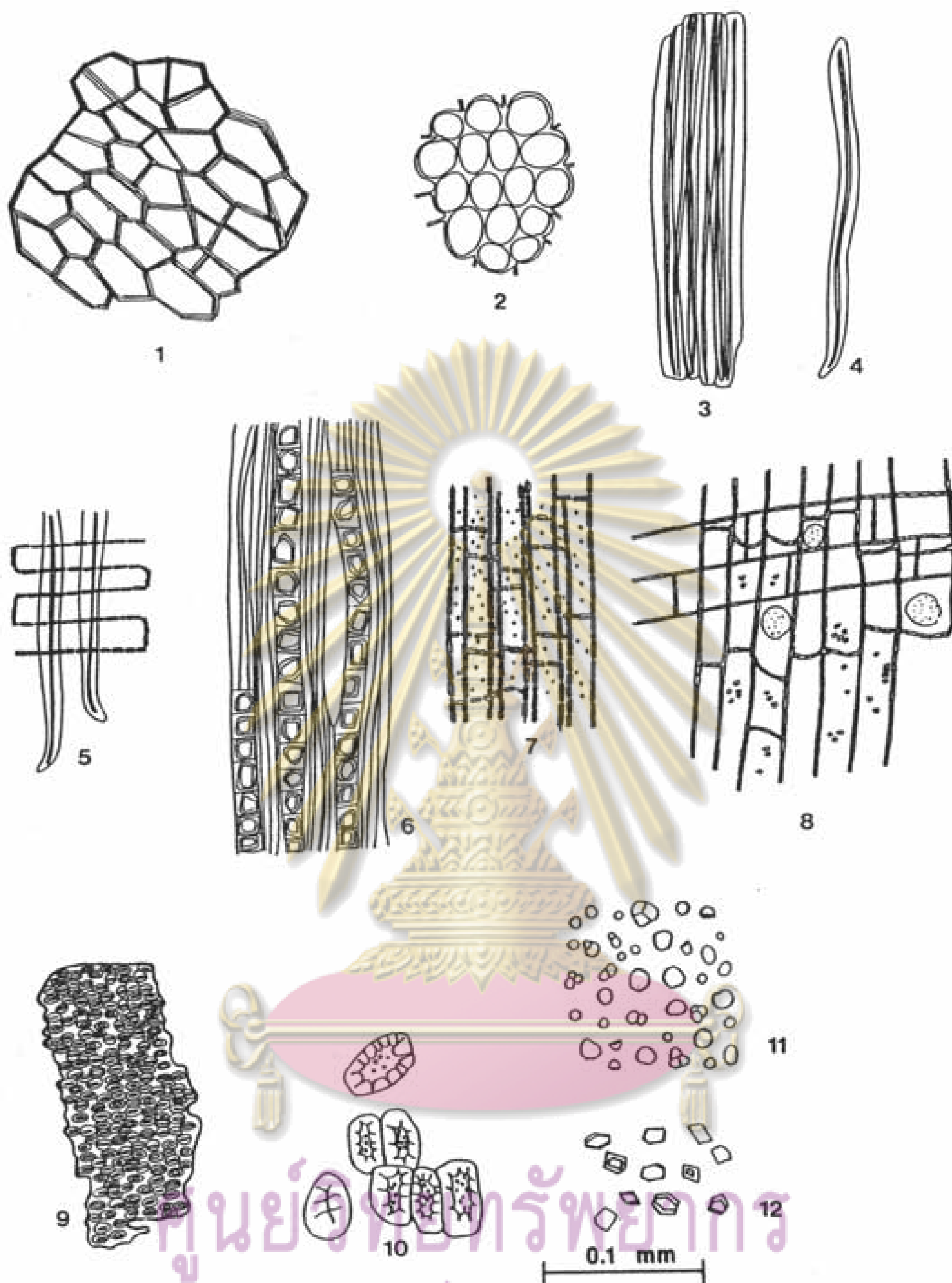
หอสมุดกลาง สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 14 Powdered *Derris reticulata* Craib stem

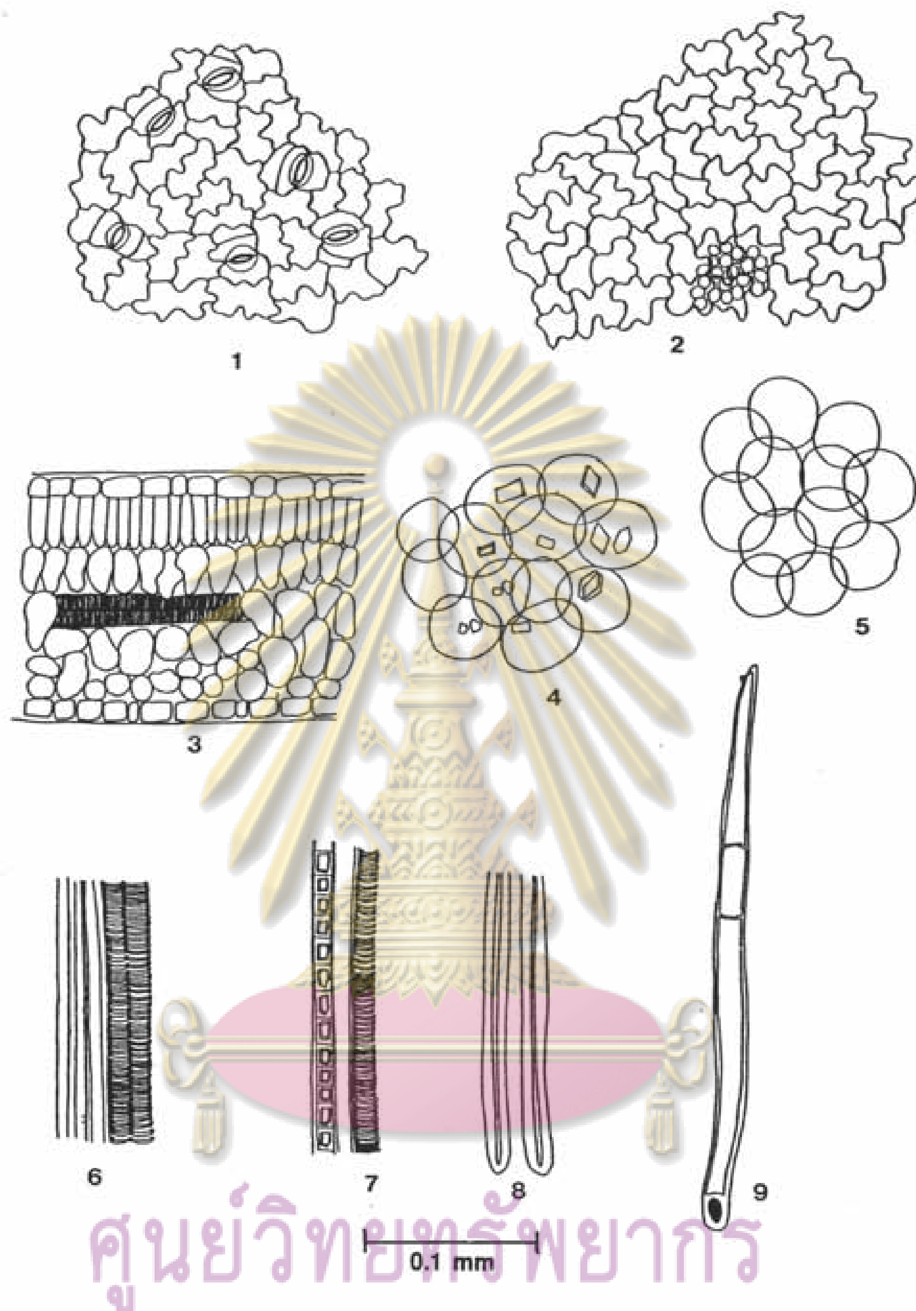
1. cork cells in sectional view
2. medullary ray (m.r.) in tangential longitudinal view
3. fibres and calcium oxalate prism sheath
4. fibres
5. xylem parenchyma
6. prisms of calcium oxalate
7. simple and compound starch granules
8. brownish substance
9. sclereids
10. fragment of bordered pitted vessels



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 15 Powdered *Derris scandens* Benth. stem

1. cork in surface view
2. parenchymatous cells
3. groups of fibres
4. fibre
5. part of medullary ray showing xylem parenehyma with underlying fibres
6. group of fibres with part of calcium oxalate prism sheath
7. fragments of xylem parenchyma with moderately thickened walls and numerous pits
8. part of medullary ray showing xylem parenchyma with underlying xylem parenchyma and containing brownish substances
9. fragment of bordered pitted vessels
10. sclereids
11. simple and compound starch granules
12. prisms of calcium oxalate



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 16 Powdered *Derris reticulata* Craib leaf

1. lower epidermis in surface view with paracytic stomata
2. upper epidermis in surface view and part of underlying palisade cells
3. part of the lamina in sectional view
4. the fragments of parenchymatous layer containing calcium oxalate prisms
5. the fragments of parenchymatous layers
6. fragment of fibres associated with small vessel from a veinlet
7. fragment of fibres and calcium oxalate prism sheath associated with small vessel from a veinlet
8. fragments of fibres
9. non-glandular multicellular trichome

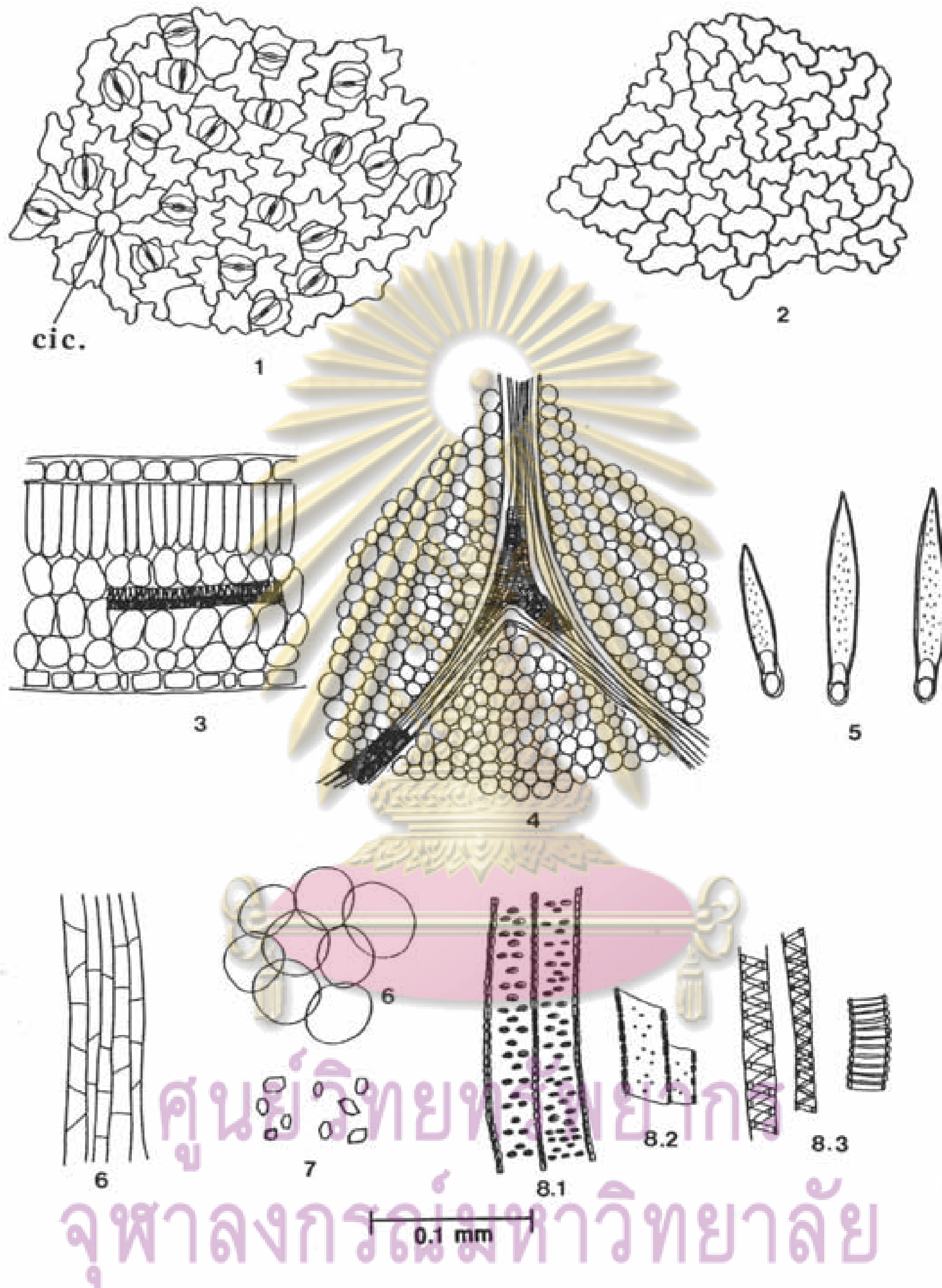


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Figure 17 Powdered *Derris scandens* Benth. leaf

1. lower epidermis in surface view with paracytic stomata and cicatrix (cic.)
2. upper epidermis in surface view
3. part of the lamina in sectional view
4. the fragment of mesophyll with fibro-vascular tissues
5. non-glandular bicellular trichomes
6. parenchyma
7. prisms of calcium oxalate
8. fragments of vessels ; bordered pitted vessels (8.1), pitted vessels (8.2), spiral vessels (8.3)

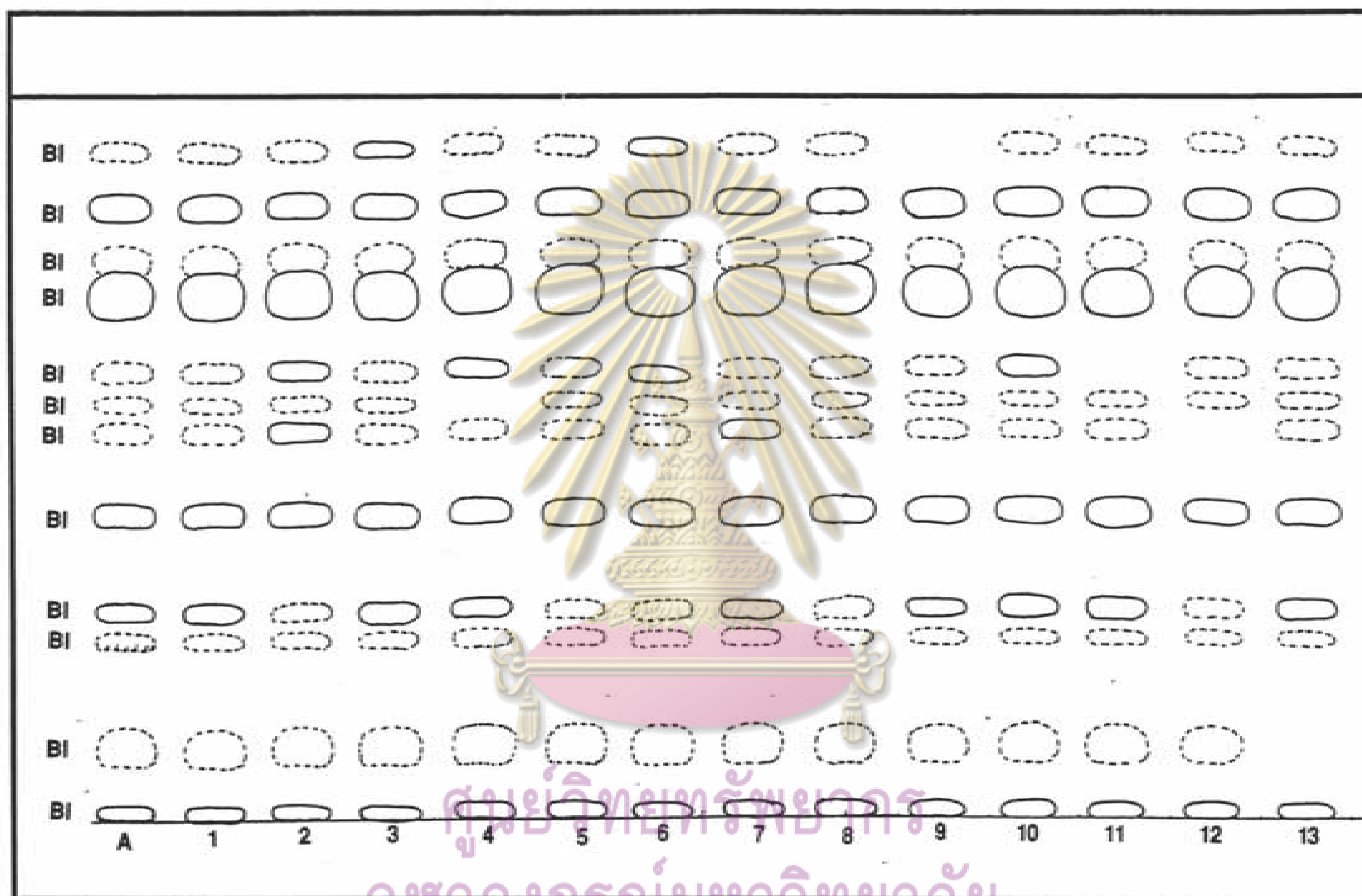

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Figure 18 Thin-layer chromatogram of Cha-aem Thai which were purchased from various local traditional drug distributors (1-13) and compared with authentic sample (*Derris reticulata* Craib, Cha-aem nuea) (A).
Developing solvents : Chloroform-ethyl acetate (6:4)
Detection : UV 254 nm

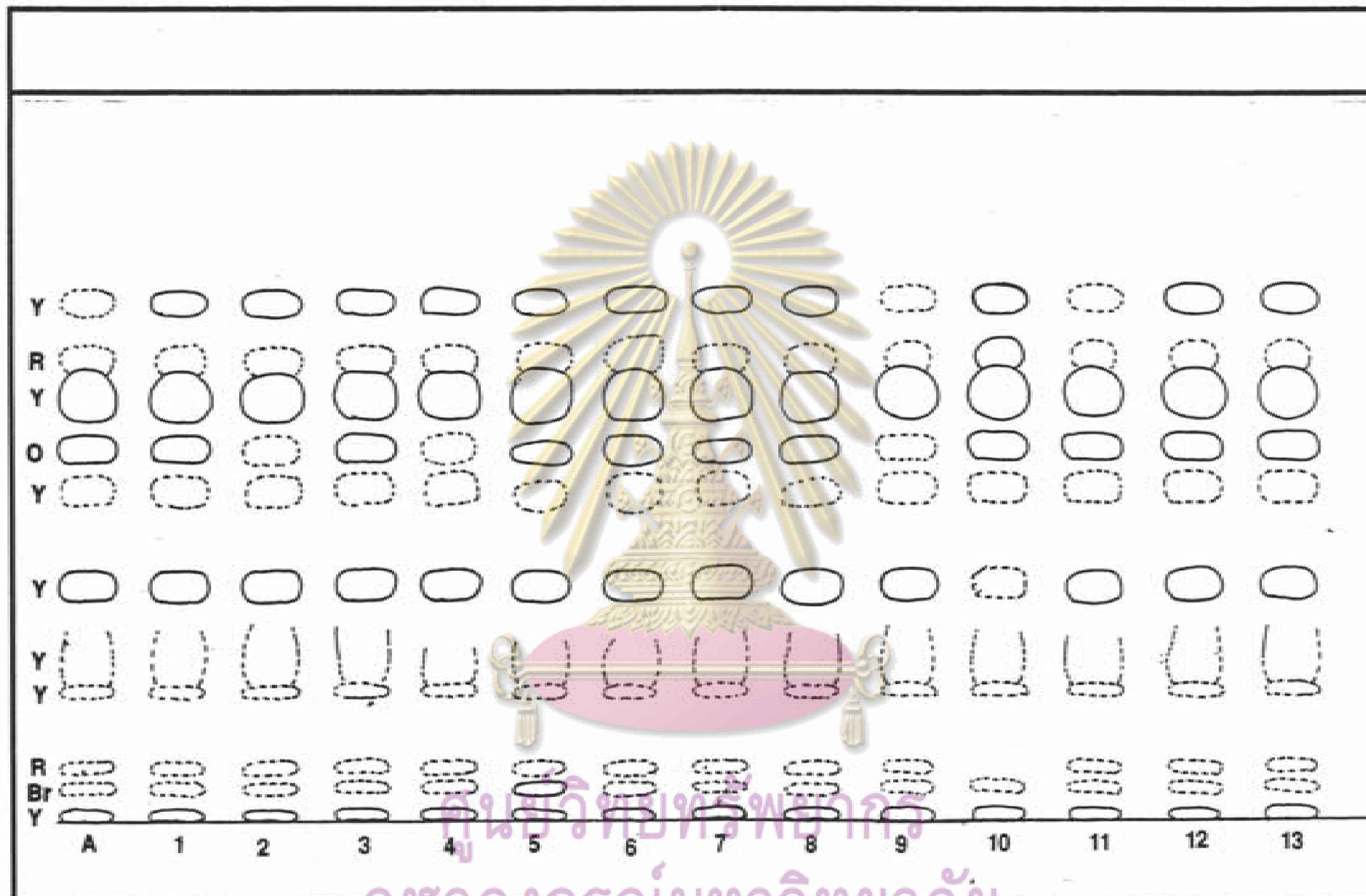

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Figure 19 Thin-layer chromatogram of Cha-aem Thai which were purchased from various local traditional drug distributors (1-13) and compared with authentic sample (*Derris reticulata* Craib, Cha-aem nuea) (A).
Developing solvents : Chloroform-ethyl acetate (6:4)
Detection : NP / PEG

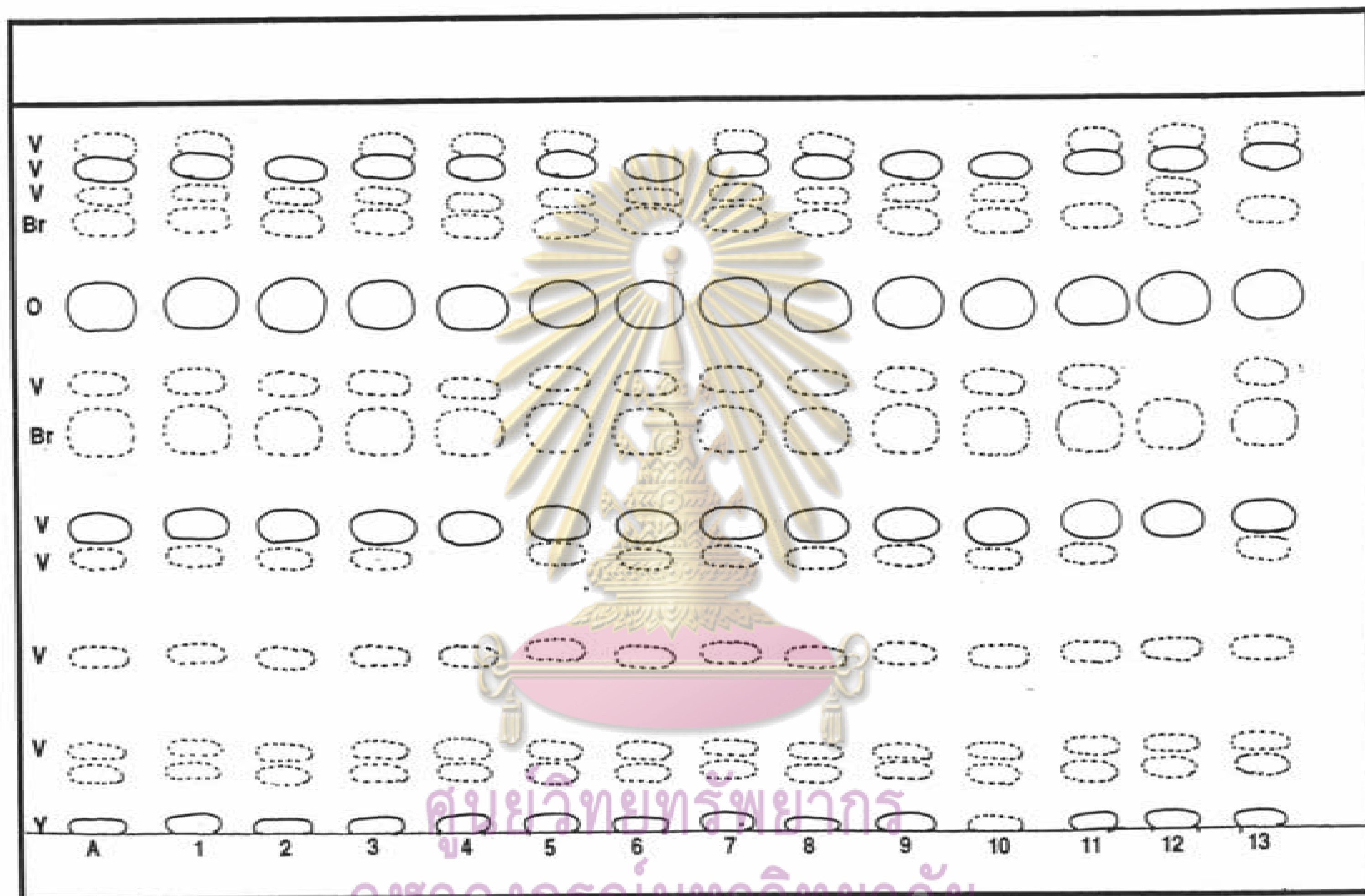

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Figure 20 Thin-layer chromatogram of Cha-aem Thai which were purchased from various local traditional drug distributors (1-13) and compared with authentic sample (*Derris reticulata* Craib, Cha-aem nuea) (A).
Developing solvents : Chloroform : ethyl acetace (6:4)
Detection : Anisaldehyde-sulphuric acid spraying reagent

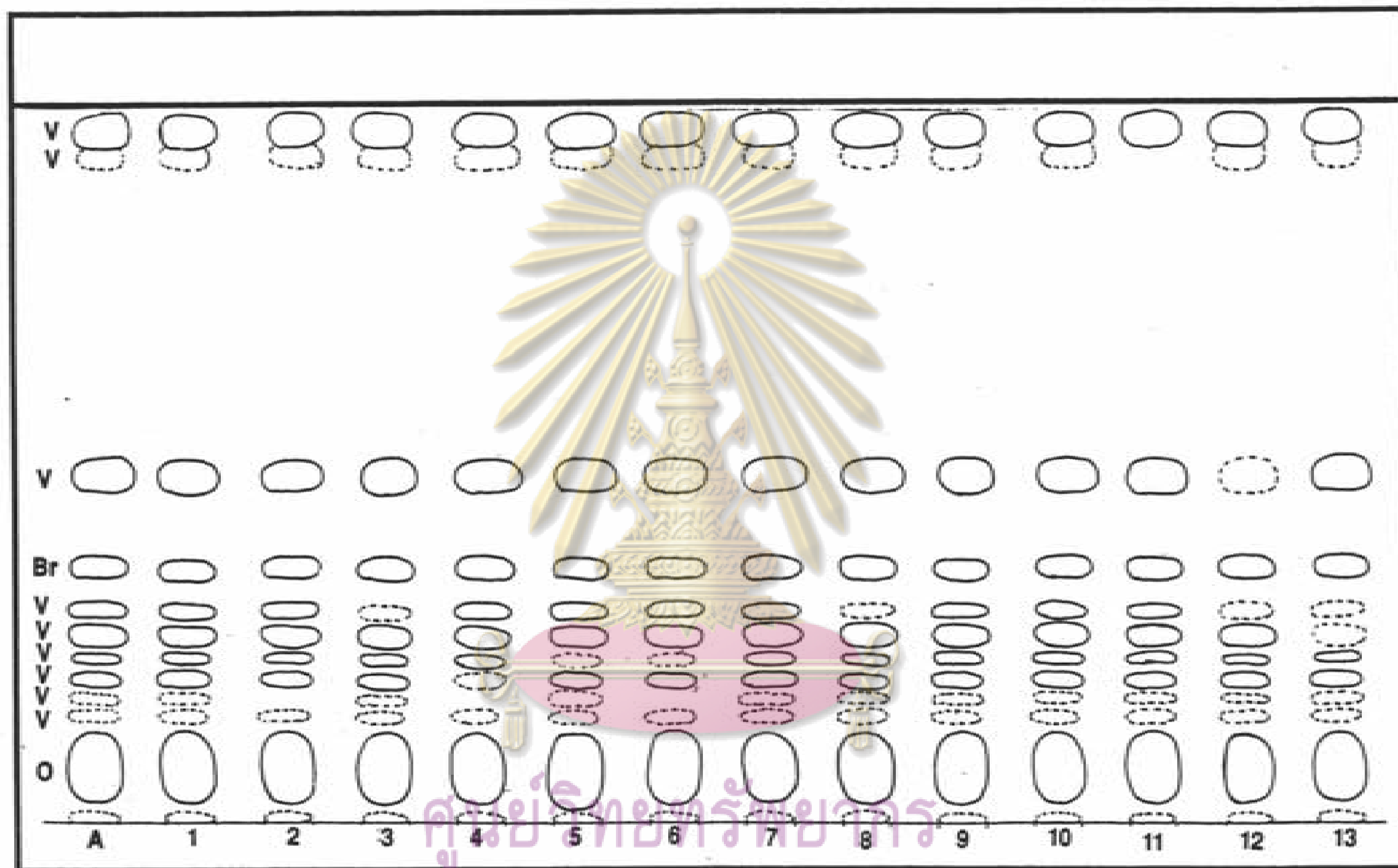

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Figure 21 Thin-layer chromatogram of Cha-aem Thai which were purchased from various local traditional drug distributors (1-13) and compared with authentic sample (*Derris reticulata* Craib, Cha-aem nuea) (A).
Developing solvents : Ethyl acetate-formic acid-glacial acetic acid-water (11:11:11:27)
Detection : 10% H_2SO_4

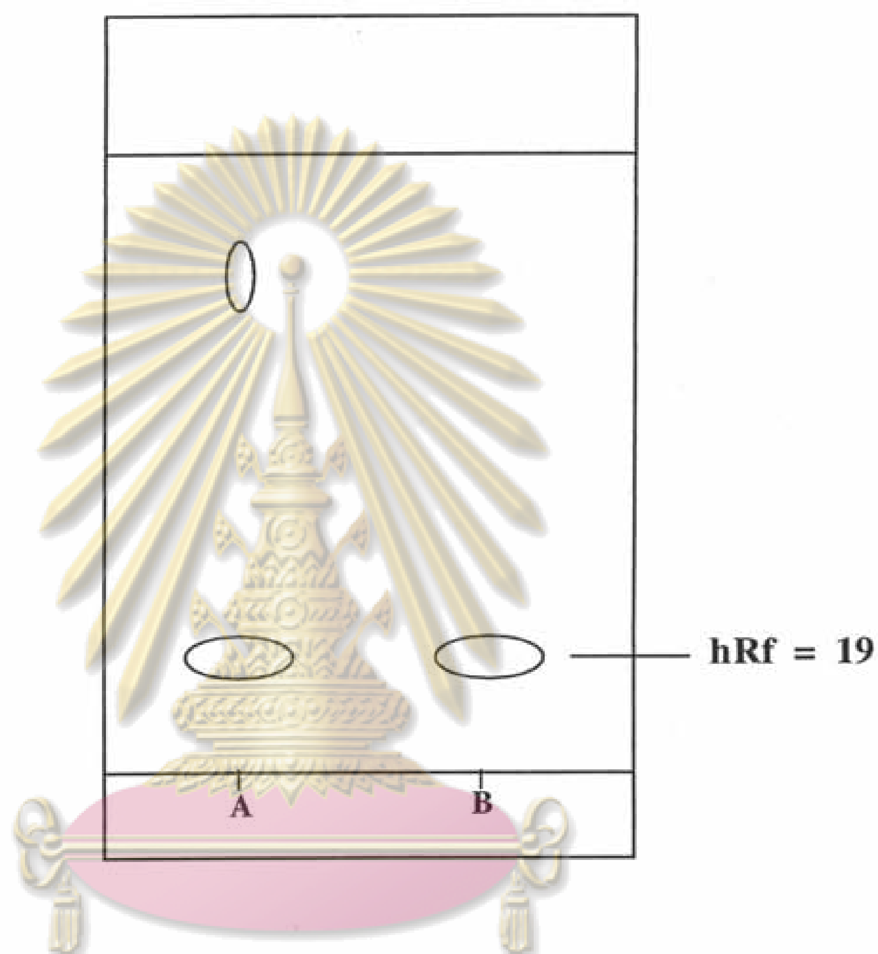

Figure 22 Thin-Layer chromatogram of aqueous portion (DR-W) of *Derris reticulata* Craib

Developing solvent : Aqueous phenol : Water + 0.002% Oxine (89:11)

Detection : Anisaldehyde : H_2SO_4

A = The aqueous portion (DR-W) of *D. reticulata* Craib

B = Sucrose

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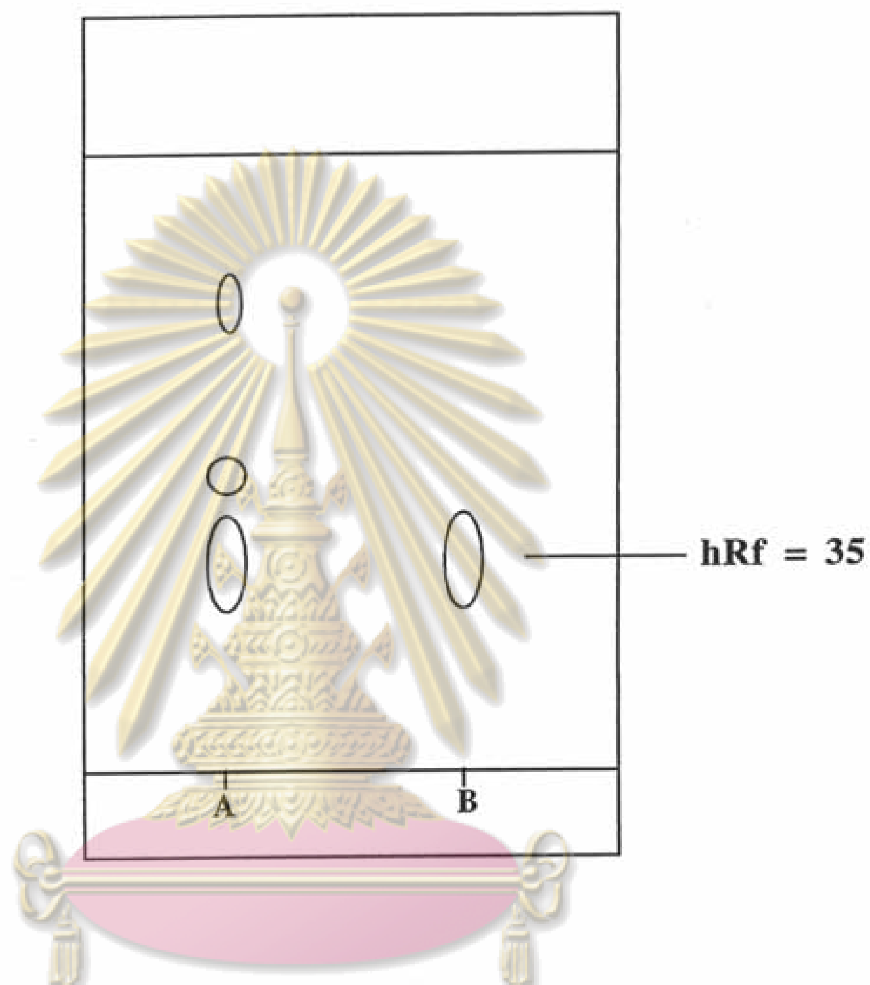

Figure 23 Thin-Layer chromatogram of aqueous portion (DR-W) of *Derris reticulata* Craib

Developing solvent : Formic acid : n-Butanol : tertiary-Butanol : Water (15:30:40:15)

Detection : 10% H_2SO_4 in Ethanol

A = The aqueous portion (DR-W) of *D. reticulata* Craib

B = Sucrose

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