CHAPTER IV

RESULTS

1. HPLC Analysis of Colchicine and Colchicoside in *G. superba* Seeds

1.1 HPLC Chromatogram of Ethanolic Extracts of *G. superba* Seeds

Under the established HPLC conditions, all ethanolic extracts of various sources of *G. superba* seeds showed very similar HPLC chromatograms. A typical one is shown in Fig.13. It can be seen that there are only three main peaks, which are well separated from one another. The retention times of these peaks were found to be 10.77, 18.91 and 23.37 min, respectively (Fig.13).

1.2 Peak Identification of HPLC Chromatogram

Identification of these peaks in HPLC chromatogram was first performed by comparing their retention times (Rt) with the retention times of various colchicine derivative standards , including colchicine , colchicoside , and 3-demethylcolchicine. It appeared that the peak of Rt 10.77 min was corresponded to

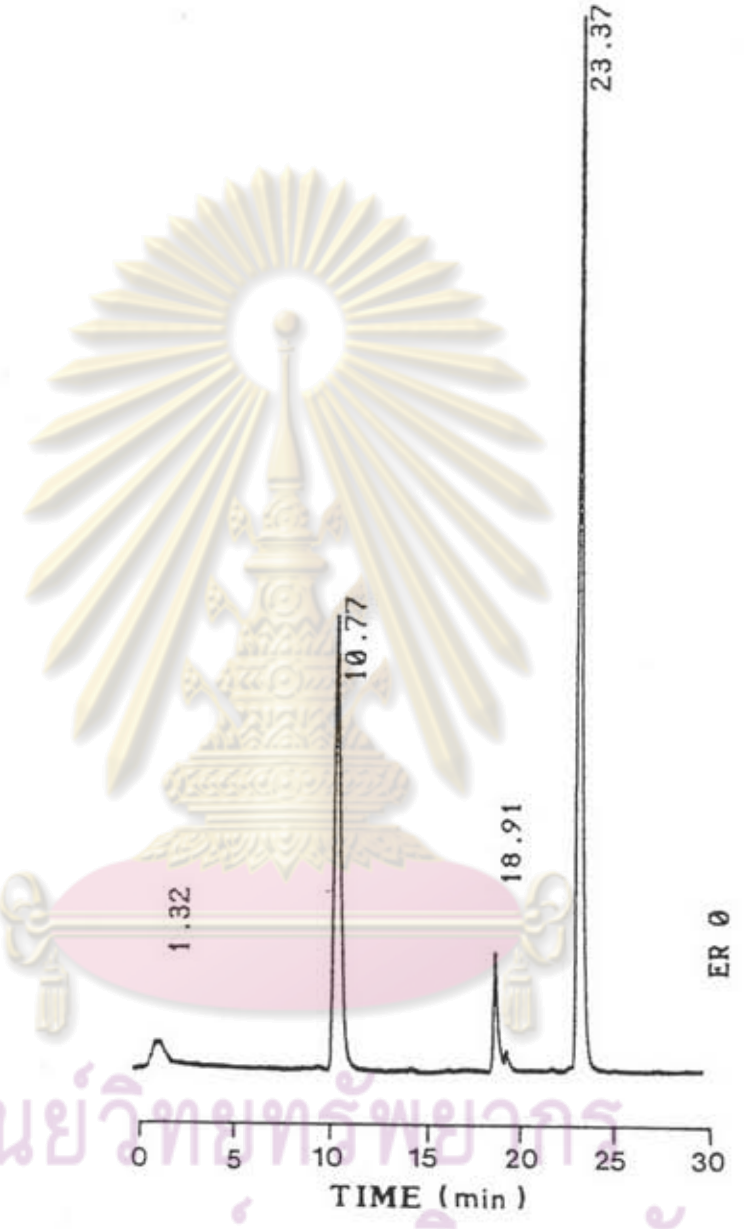


Fig.13 A typical HPLC chromatogram of ethanolic extract of *G. superba* seeds.

the peak of colchicoside, Rt 18.91 min corresponded to 3-demethylcolchicine and the major one with the Rt 23.37 min corresponded to colchicine (Fig.14). Their structures are also shown in Fig.15.

In addition to the HPLC method , TLC was also used to confirm the presence of the major three alkaloids in the seed ethanolic extracts. Two different developing solvent systems (both on silica gel plates) were employed. One solvent system was chloroform: methanol: 10 % acetic acid ; 85:15:1 (S_1) and the other was benzene : ethyl acetate : diethylamine : methanol; 5:4:1:2 (S_2). Both TLC systems showed essentially three spots on the silica gel plates (under 254 nm of UV light). While S_1 showed the Rf values of 0.13, 0.56 and 0.62, S_2 showed the Rf values of 0.12, 0.47 and 0.65 (developed 2 times) which were all corresponded respectively to the Rf values of authentic colchicoside , 3-demethylcolchicine and colchicine in the S_1 and S_2 systems (Fig.16).

Moreover, the final confirmation was also carried out by examining the characteristic UV-absorption spectra of these compounds in comparison with those of colchicoside, 3-demethylcolchicine and colchicine. This was performed by collecting fractions corresponding to their peaks (Fig.13) and each of which was subjected to UV-scanning. The resulted absorption spectrum was

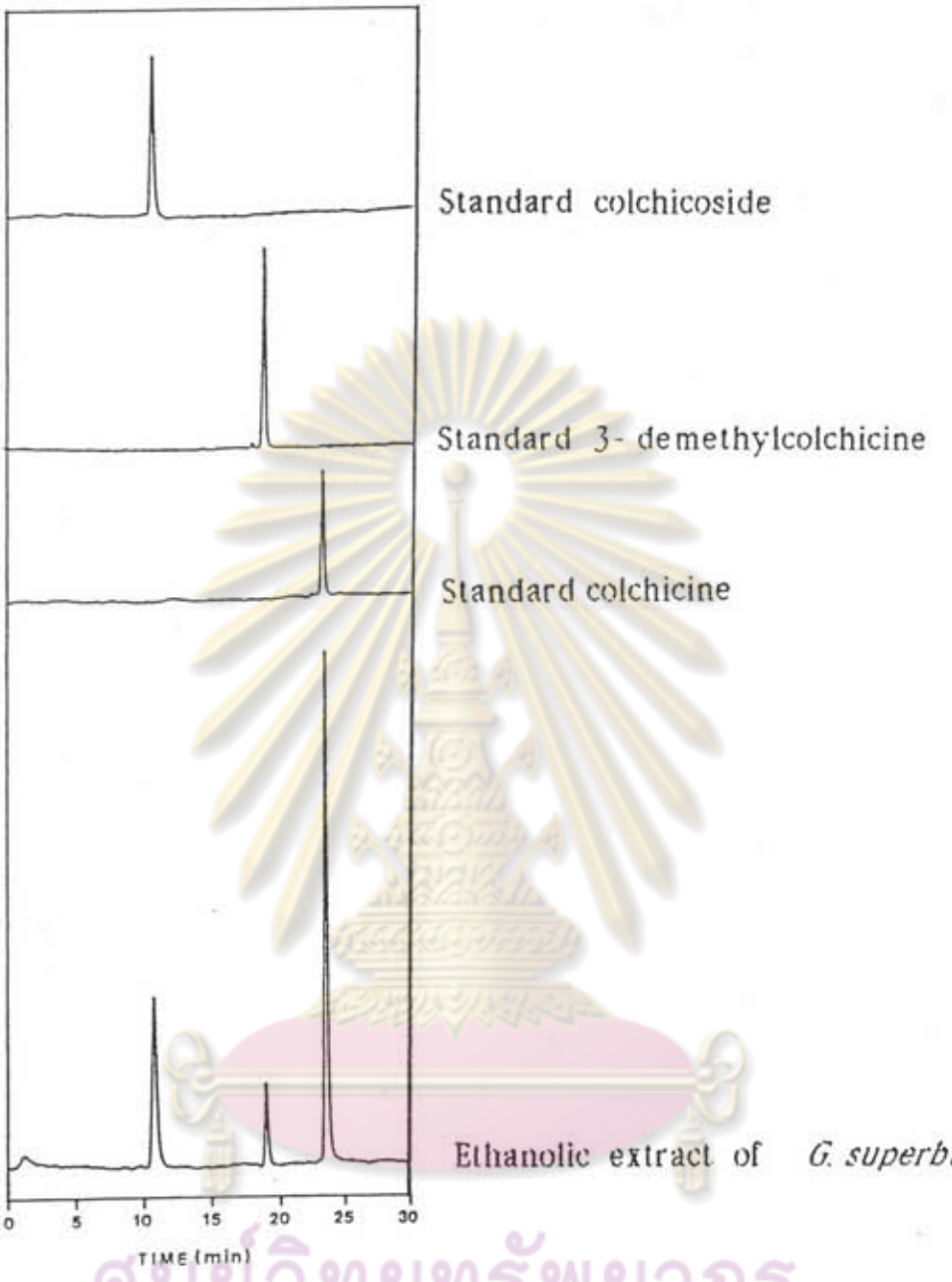
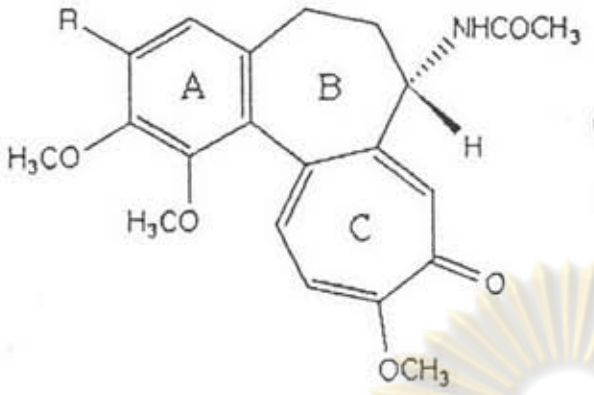


Fig.14 HPLC chromatograms of ethanolic extract of *G. superba* seeds compared with those of various colchicine derivative standards.



Colchicine R = OCH_3

Colchicoside R = $OC_6H_{11}O_5$

3 - Demethylcolchicine R = OH

Fig.15 The structures of colchicine, colchicoside and 3-demethylcolchicine.

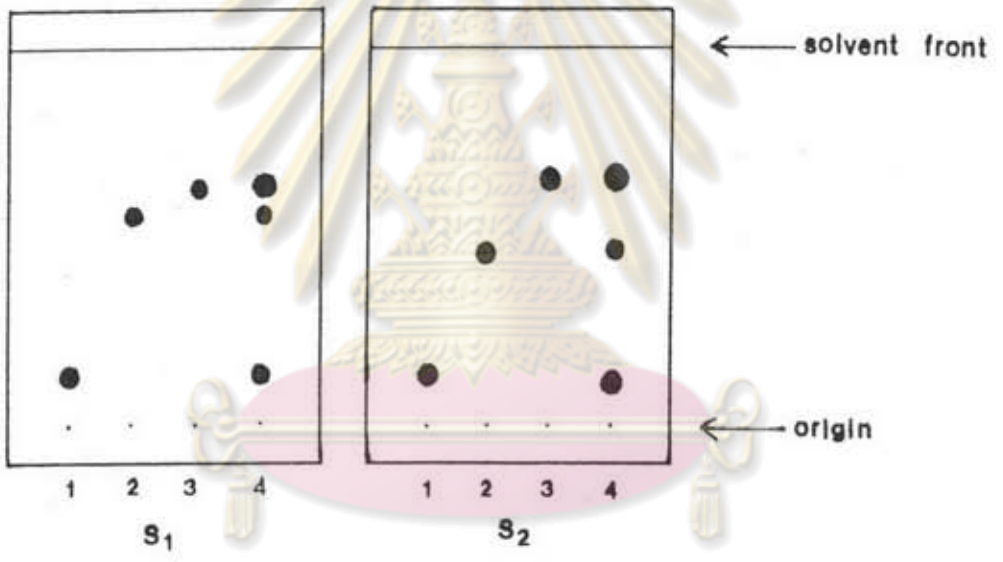


Fig.16 TLC-separation on silica gel plates of chemical constituents of ethanolic extracts of *G. superba* seeds under two different developing solvent systems :

(S_1) chloroform:methanol:10 % acetic acid, 85:15:1

(S_2) benzene:ethyl acetate:diethylamine:methanol, 5:4:1:2

(run two times)

standards : 1) colchicoside 2) 3-demethylcolchicine 3) colchicine

sample : 4) ethanolic extract of *G. superba* seeds.

then compared with the spectrum of each authentic compound. It can be seen in Fig.17 that the HPLC peaks assigned as colchicoside , 3-demethylcolchicine and colchicine had their spectra absolutely identical to those of their authentic compounds , and notably that they were all identical with two maximum absorption values at about 240 and 350 nm.

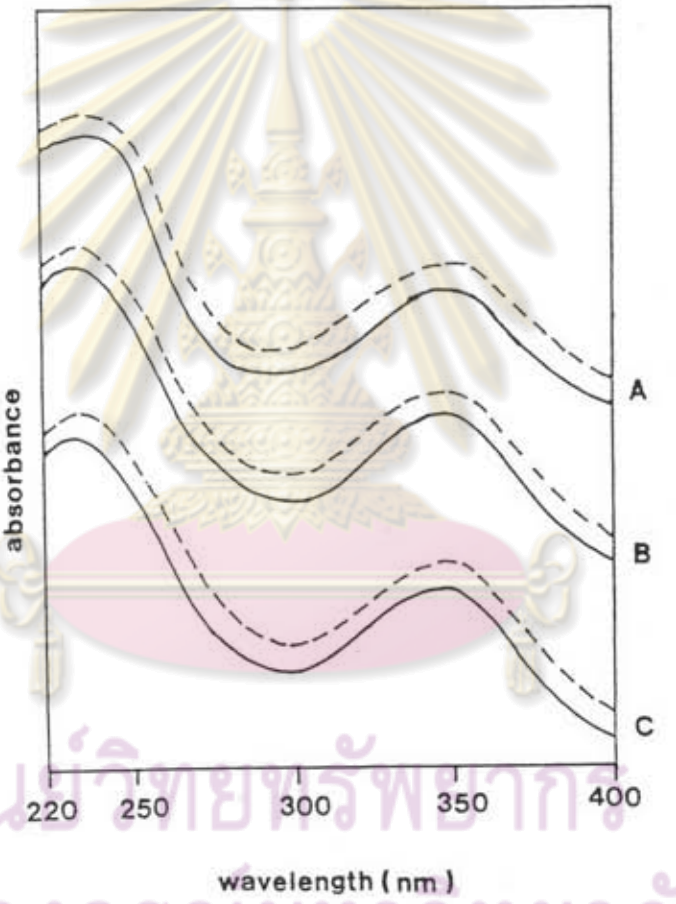


Fig.17 Absorption spectra of the HPLC peaks assigned as colchicoside , 3-demethylcolchicine and colchicine as compared with the spectra of their authentic compounds.

A) Colchicoside B) 3-Demethylcolchicine C) Colchicine

——— fraction from HPLC peak ; ----- authentic compound.

1.3 Calibration Curves of Standard Colchicine and Colchicoside

The standard curves of colchicine and colchicoside which were obtained by the HPLC method are shown in Fig.18. Each curve showed linearity of the peak area - concentration relationship between the concentration range of 5-100 µg/ml for colchicine(Fig.18A) and 5-50 µg/ml for colchicoside (Fig.18B). Results of the regression analysis and the correlation coefficients (r) were found to be 0.9998 for colchicine and 0.9969 for colchicoside. Regression equations of standards are listed below :

For colchicine: $y = 6251.057\ x - 1125.75$, $n^* = 8$, $r=0.9998$

For colchicoside: $y = 4597.127\ x - 1515.53$, $n^* = 6$, $r=0.9969$

* n = number of standard concentration levels.

1.4 Colchicine and Colchicoside Contents in *G.superba* Seeds

Since the HPLC chromatogram (Fig.13) suggested that colchicine and colchicoside were the two major constituents of *G. superba* seeds and that both alkaloids could be quantitated simultaneously , determination of their content in *G. superba* seeds of various sources was carried out. The purpose of this experiment was to study the variation of colchicine and colchicoside

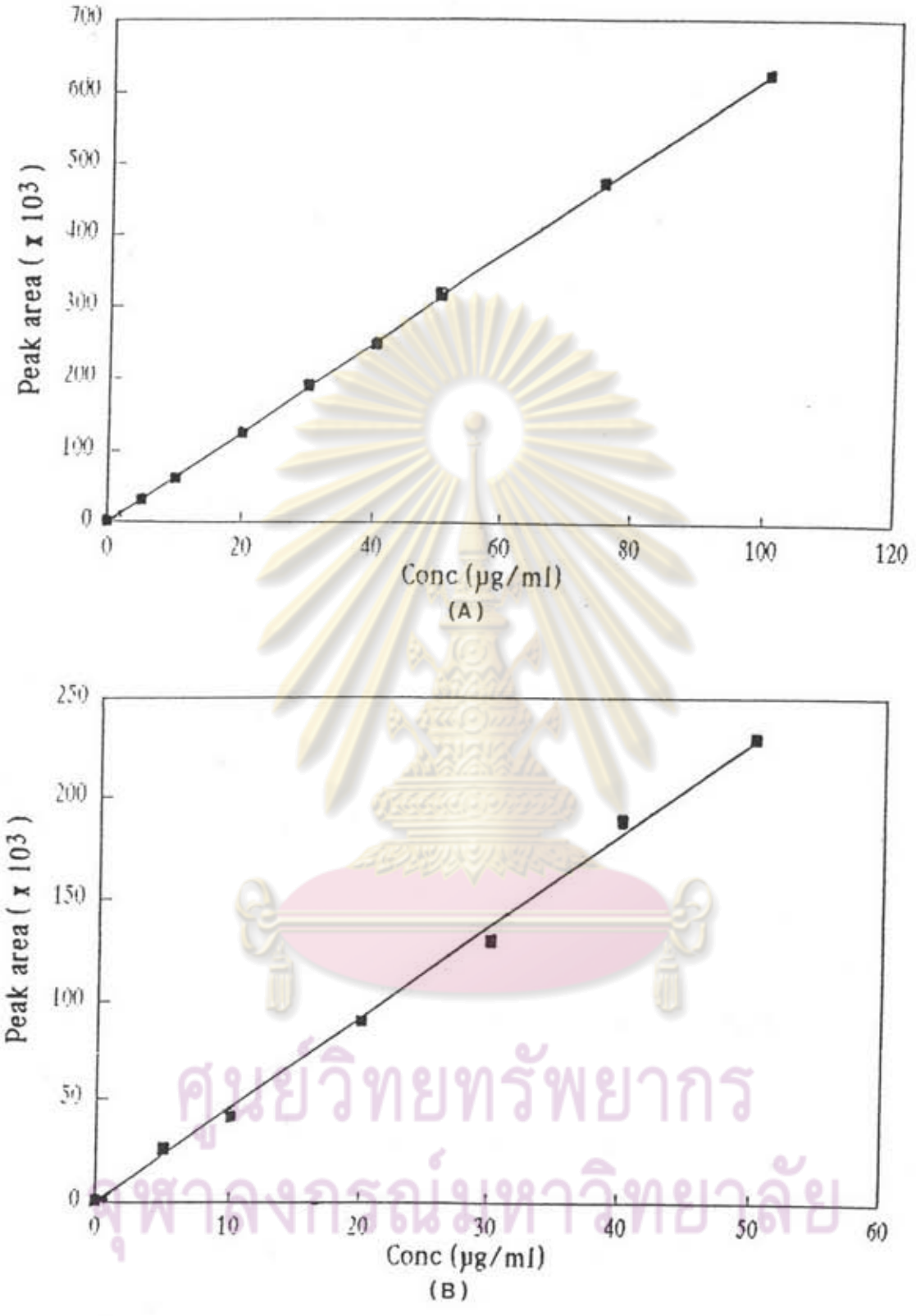


Fig.18 Calibration curves of standard colchicine and colchicoside by HPLC method.

A) Colchicine B) Colchicoside

contents in *G. superba* seeds which had been produced from different geographic conditions of Thailand.

Based on the peak areas of colchicine and colchicoside in the HPLC chromatograms of various samples, the seed content of both compounds was calculated using the established standard curves (Fig.18A and 18B). The results showed that *G. superba* seeds obtained from various parts of Thailand contained relatively high level of colchicine , ranging from 0.83-1.46 % (w/w) and colchicoside , 0.67-1.27 % (w/w). As shown in Table 8 and Fig.19, the seeds from Chumphon 91 (1.46 %) and from Chanthaburi 2 (1.43 %) showed highest content of colchicine. They also contained high level of colchicoside with 1.16 % and 1.05 % , respectively. The ratio of colchicine and colchicoside in both samples was found to be about 55:45. This ratio was also similar to all other seed samples (Fig.19) except the one from Prachin Buri which was the only sample that showed lower content of colchicine (1.04 %) than colchicoside (1.27 %), with the ratio of 45:55 (Fig.19),respectively.

From Chiang Mai, however , the *G. superba* seeds appeared to contain the lowest level of colchicine (0.83%) and colchicoside (0.67 %). It should be noted that the plants in Chiang Mai were originally cultivated using the seeds from India. This is different from the other

Table 8 Colchicine and colchicoside contents in the seeds of *G. superba* obtained from various parts of Thailand by HPLC method.

Source of [a] *G. superba* seeds	Colchicine [b] (% w/w)	Colchicoside [b] (% w/w)	Total colchicine and colchicoside [b] (% w/w)
Chanthaburi 1	1.305±0.045	1.084±0.043	2.389±0.044
Chanthaburi 2	1.426±0.028	1.049±0.022	2.477±0.025
Prachin Buri	1.041±0.033	1.268±0.048	2.309±0.040
Lop Buri	1.259±0.051	0.983±0.041	2.243±0.046
Chiang Mai	0.834±0.032	0.674±0.011	1.508±0.021
Chumphon 91	1.456±0.032	1.162±0.029	2.618±0.030
Chumphon 89	1.146±0.020	0.732±0.022	1.878±0.021
Chumphon 88	1.065±0.044	0.770±0.038	1.835±0.041
Songkhla	1.133±0.019	0.790±0.024	1.923±0.022

a : See Plant Materials in Chapter III

b : Mean ± standard deviation (n = 6)

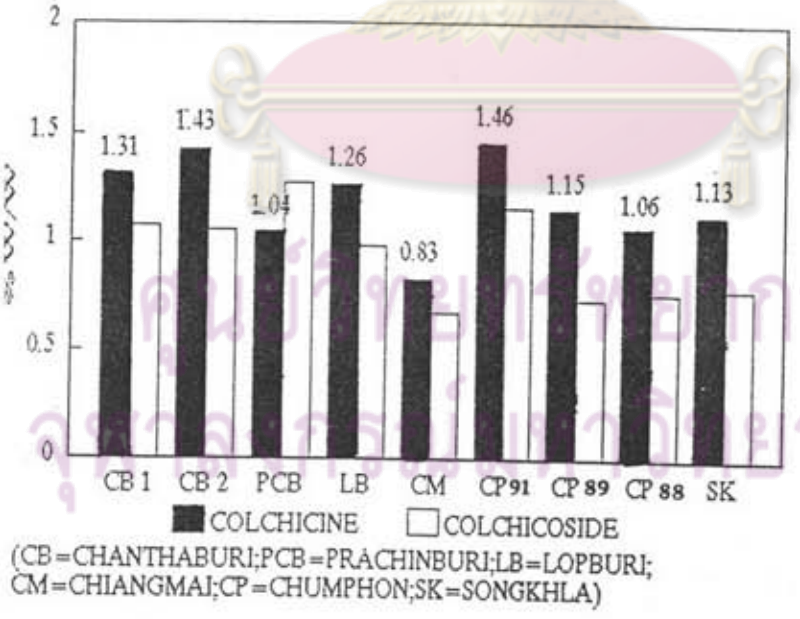


Fig.19 Bar graph of colchicine and colchicoside contents in the seeds of *G. superba* obtained from various parts of Thailand.

G. superba plants which were grown from the tubers of Thai cultivar.

In Chumphon's experimental field of Thai Commodities Company , it should be noted that the seed colchicine content increased continuously from the crops of 1988 (1.06 %) to 1991 (1.46 %) (Table 8 and Fig.19).

In term of colchicine and colchicoside summation (Table 8), both alkaloids showed the values over 2.2 % of dry weight for the seeds obtained from the eastern and central provinces. From the south, the content appeared to be slightly lower than 2 % except for the crop of Chumphon 1991 which went highly to 2.6 % . For Chiang Mai samples, the total colchicine and colchicoside content was found to be lowest (1.5 %).

1.5 Determination of Total Content of Colchicine and Its Derivatives in *G. superba* Seeds by UV-Spectrophotometric Method

As mentioned earlier, the HPLC chromatogram (Fig.13) indicated that the ethanolic extract of *G. superba* seeds was relatively clean. Only three components of colchicine , colchicoside and 3-demethyl-colchicine could be detected by the UV-detector set to the wavelength of 350 nm. As a result, it would be

possible to use the same wavelength to determine the total colchicine derivatives in the extract by UV-spectrophotometry. In doing this, a standard curve of colchicine was first constructed. It was found that the curve showed linearity in a wide range of colchicine concentration (Fig.20). The lowest colchicine concentration that could be detected was 0.39 µg/ml and the upper concentration limit was at least 50 µg/ml.

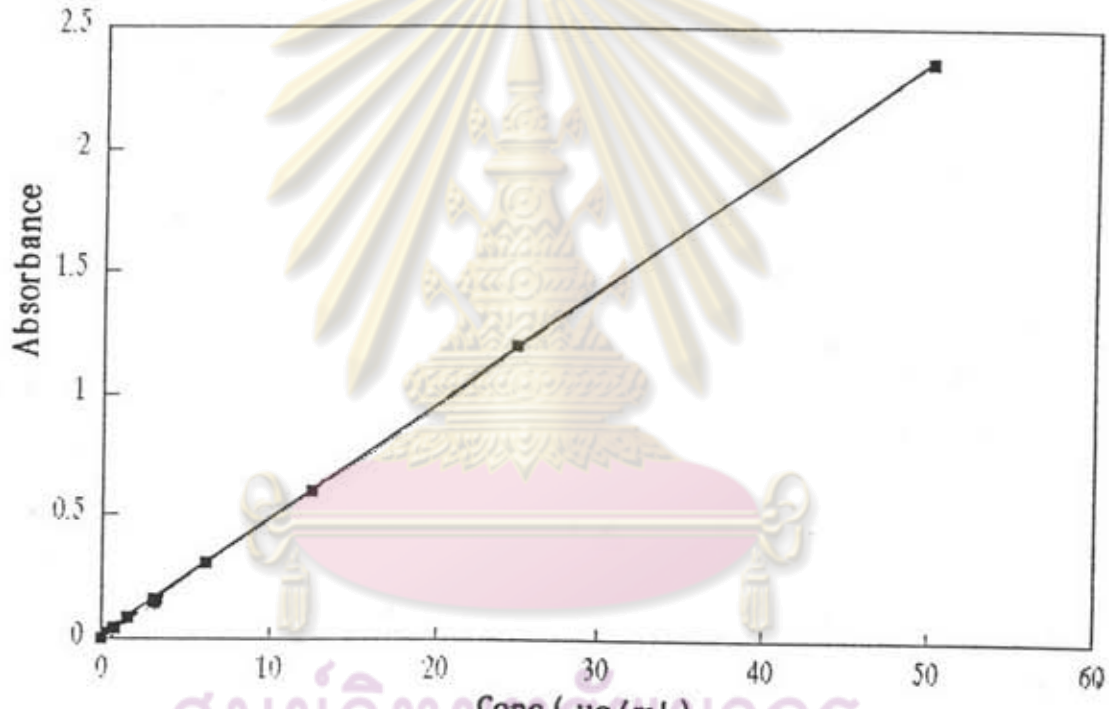


Fig.20 Calibration curve of standard colchicine by UV-spectrophotometric method.

With this calibration curve of colchicine, the resulted content of total colchicine derivatives in the ethanolic extracts of *G. superba* seeds was found to be closed to the values of total colchicine and colchicoside

in the same extracts as determined by the HPLC method (Table 9 and Fig.21).

As shown in Table 9, more than seven out of nine samples showed less than 10 % difference between the value of total colchicine derivatives determinded by UV-spectrophotometry and the value of total colchicine and colchicoside determined by HPLC. It should be noted that these seven samples namely Chanthaburi 1 , Prachin Buri , Chiang Mai , Chumphon 91 , Chumphon 89 , Chumphon 88, and Songkhla, had small peak of 3-demethyl-colchicine (Fig.22 A). The samples from Chanthaburi 2 and Lop Buri, on the other hand , showed relatively high amount of this compound (Fig.22 B). Therefore, in general, the spectrophotometric method could replace the HPLC method for the determination of total colchicine and colchicoside in *G. superba* seeds.

Table 9 Total colchicine and its derivatives in *G. superba* seeds determined by UV-spectrophotometry compared with the total value of colchicine and colchicoside by HPLC

Source of [a] *G. superba* seeds	Total colchicine derivatives (%w/w) by UV-spectrophotometry [b]	Colchicine and colchicoside (%w/w) by HPLC [c]	% Difference
Chanthaburi 1	2.485±0.063	2.389±0.044	3.86
Chanthaburi 2	2.776±0.052	2.477±0.025	10.77
Prachin Buri	2.364±0.012	2.309±0.040	2.33
Lop Buri	2.577±0.016	2.243±0.046	12.96
Chiang Mai	1.502±0.040	1.508±0.021	0.40
Chumphon 91	2.687±0.060	2.618±0.030	2.57
Chumphon 89	2.044±0.053	1.878±0.021	8.12
Chumphon 88	1.930±0.053	1.835±0.041	4.92
Songkhla	1.955±0.033	1.923±0.022	1.64

a : See Plant Materials in Chapter III

b : Mean ± standard deviation (n=3)

c : Mean ± standard deviation (n=6)

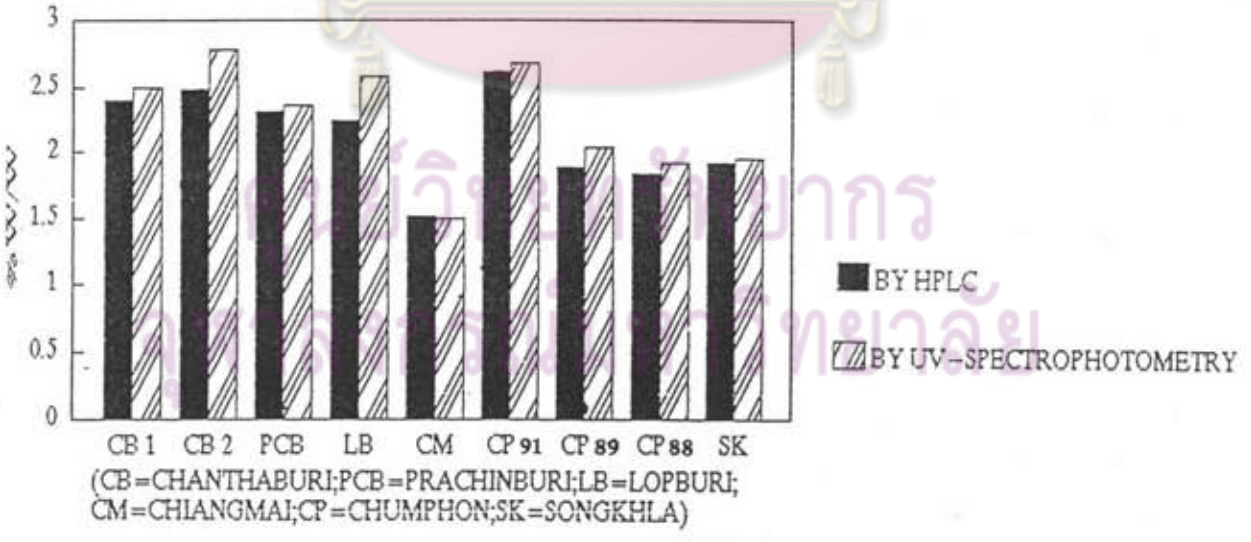


Fig.21 Bar graph of total colchicine and its derivatives in *G. superba* seeds determined by UV-spectrophotometry and those of total colchicine and colchicoside determined by HPLC.

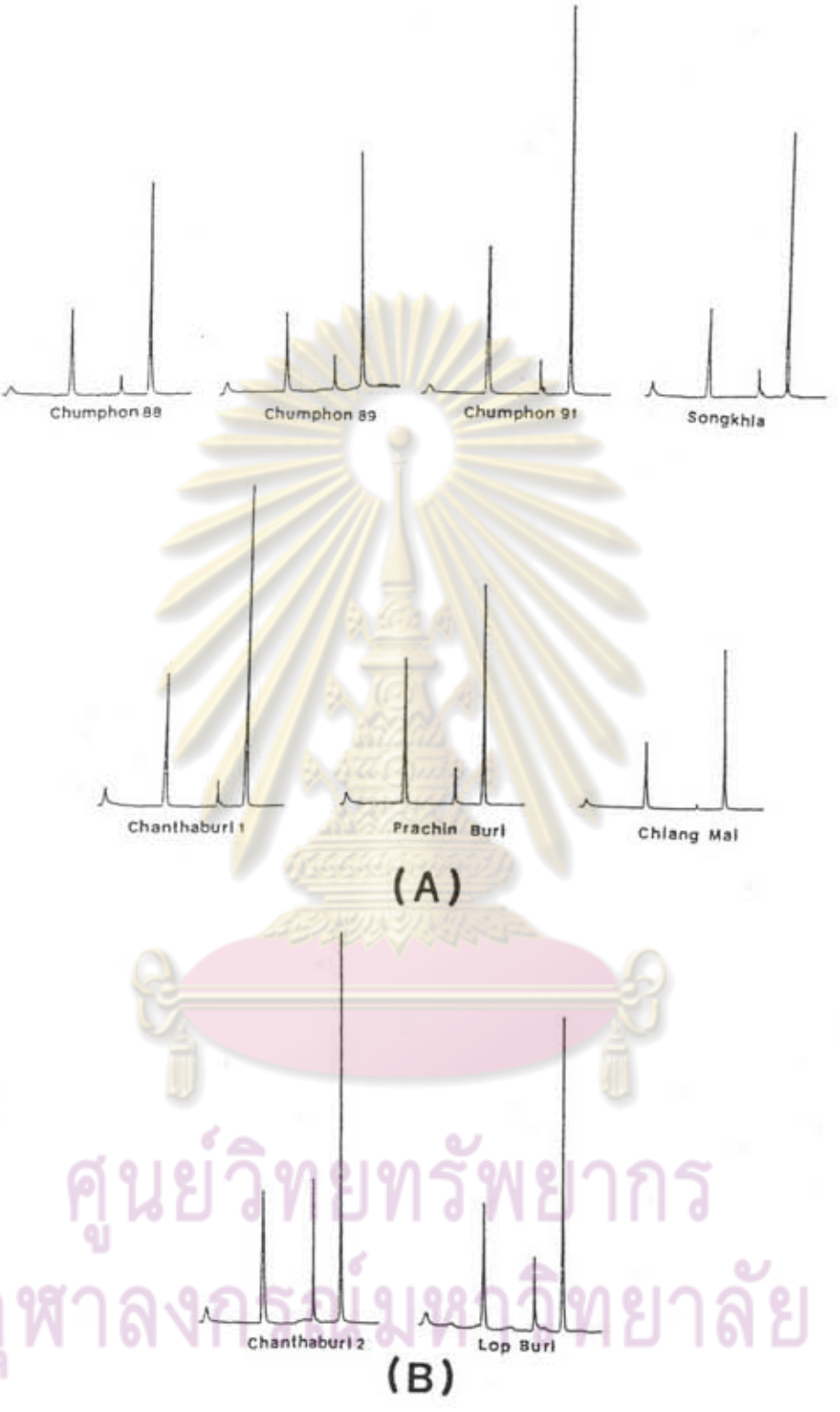


Fig.22 HPLC chromatograms of ethanolic extracts of *G.superba* seeds from various sources of Thailand.

A) small peak of 3-DMC seed samples

B) relatively high peak of 3-DMC seed samples

2. TLC-Densitometric Analysis of Colchicine in Various Plant Parts of *G. superba*

2.1 Optimization of TLC-Densitometric Conditions for Colchicine Determination

2.1.1 Extraction Conditions

Initially, optimum proportions of powdered plant materials from various parts of *G. superba* and volumes of extracting solvent (95 % ethanol) were investigated in order to obtain best extraction of colchicine. This was carried out by varying various sample sizes and volumes of the extracting solvent followed by extraction under reflux at 60-70 ^{o}c. After one hour, colchicine content in each ethanolic extract was then quantitated by TLC-densitometric method. The results, as summarized in Table 10 , showed that the optimum proportions for the powdered seeds and pericarps were 40mg per 10 ml 95 % ethanol, for powdered tubers and flowers: 200 mg/10 ml and for powdered leaves and stems : 1000 mg/10 ml. These material : solvent ratios were used throughout in this study.

Table 10 Optimum proportion of powdered samples from various plant parts of *G. superba* and volume of extracting solvent (95% ethanol)

Plant parts	Powdered samples (mg)	Volume of 95% ethanol (ml)
Seeds	40	10
Pericarps	40	10
Tubers	200	10
Flowers	200	10
Leaves	1000	10
Stems	1000	10

2.1.2 Development of TLC Solvent System

In order to search for a suitable developing solvent system for TLC separation of colchicine from other substances in various ethanolic extracts, a number of solvent systems were tried. Table 11 shows the list of the solvent systems used in the experiment and the resulted Rf values of colchicine in each system.

According to Table 11, almost all the developing solvent systems gave relatively high Rf values of colchicine (0.74-0.81) except the systems of chloroform: methanol : 10 % acetic acid ratio 85:15:1 and benzene: ethyl acetate : diethylamine : methanol, ratio 5:4:1:2

Table 11 Various solvent systems tried for TLC separation of colchicine in ethanolic extracts of various *G. superba* parts

Developing solvent systems used with Si gel 60 F_{254}	Ratio	Rf value * of colchicine
Chloroform : methanol : 10% acetic acid	85 : 15 : 1	0.62
Chloroform : acetone : diethylamine	7 : 2 : 1	0.74
	5 : 4 : 1	0.75
Benzene : ethyl acetate : diethylamine : methanol	5 : 4 : 1 : 2	0.53
	4 : 3 : 2 : 1	0.75
	4 : 4 : 1 : 2	0.76
	5 : 4 : 2 : 3	0.81

* Rf value was calculated from the migration distance of colchicine divided by the migration distance of the solvent.

which gave the Rf values of 0.62 and 0.53, respectively. The latter two systems were then used for separating colchicine from other components in the plant materials. The results showed that the solvent system of chloroform : methanol : 10 % acetic acid (85:15:1) gave better than the other system in the separation of colchicine in every plant part used in the experiment. No interference from other components was observed either under 254 or 365 UV light under this TLC conditions (Fig.23). Furthermore, the UV-spectrum of the spot of putative colchicine from various plant parts were indistinguishable from the

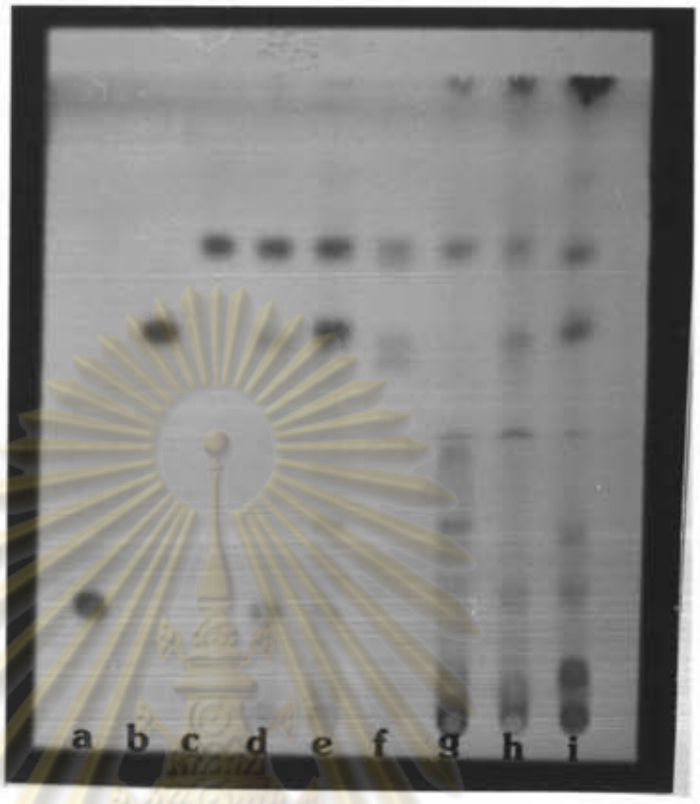


Fig.23 TLC patterns of ethanolic extracts obtained from various plant parts of *G. superba* under 254 UV light.
standards : a) colchicoside b) 3-DMC c) colchicine
samples : d) seeds e) pericarps f) tubers g) flowers
h) stems i) leaves
solvent system : chloroform : methanol : 10 % acetic acid
(85:15:1)

spectrum of authentic colchicine (Fig.24). These results indicated that colchicine was completely separated from other compounds. Therefore , the solvent system of chloroform: methanol: 10 % acetic acid (85:15:1) was used throughout in this study.

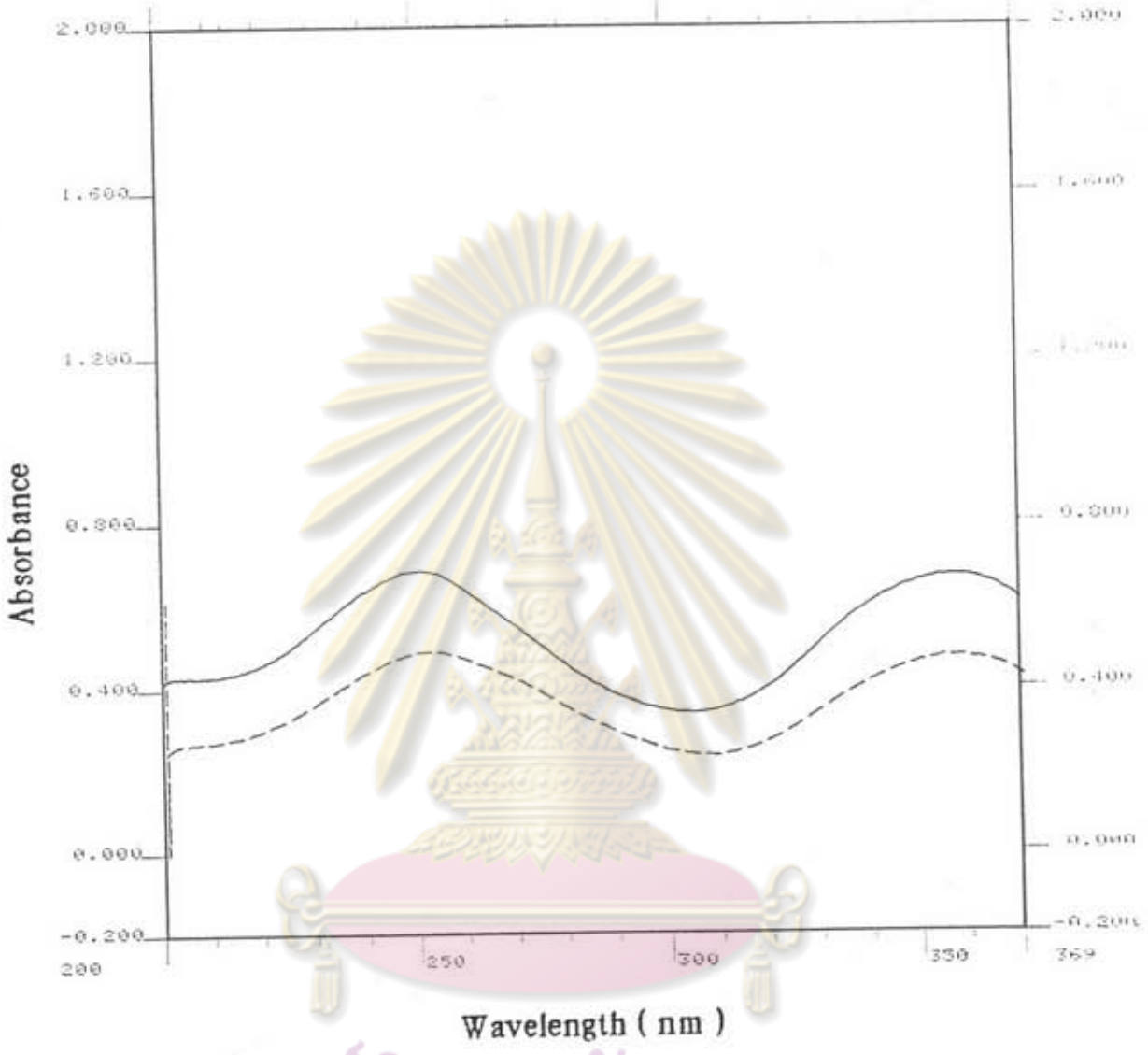


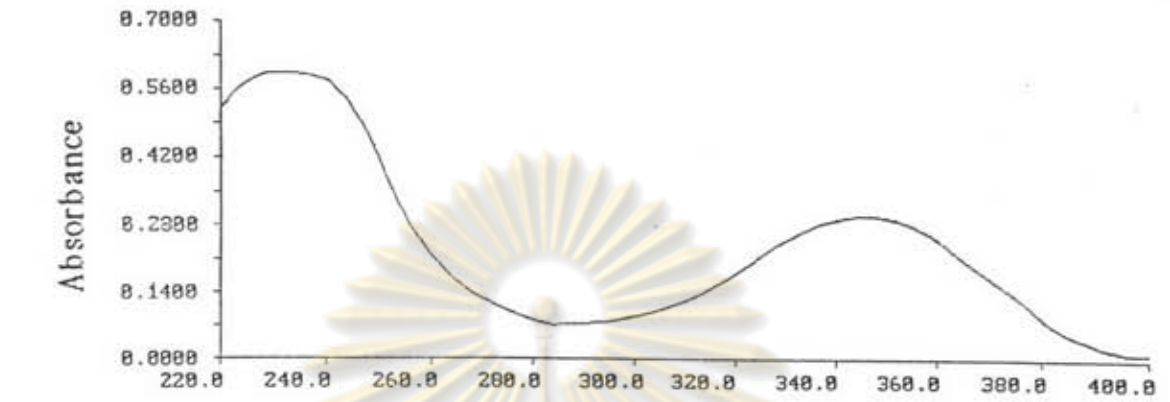
Fig.24 UV-absorption spectra of authentic colchicine (-----) and the compound of similar Rf values obtained from various parts of *G. superba* (———).

2.1.3 Determination of Optimum Wavelength for TLC-Densitometric Analysis

The optimum wavelength used for TLC-densitometric analysis of colchicine was determined by the absorption spectrum colchicine. The spectrum was obtained by using two method : UV-spectrophotometric and TLC-densitometric. With UV-spectrophotometric method, standard colchicine solution 6.25 µg/ml 95 % EtOH was used to obtain the absorption spectrum of colchicine (Fig.25A). The colchicine solution was scan in a 1 cm cell in the ultraviolet range from 220 to 400 nm using 95 % ethanol as blank solution. The spectrum showed two values of maximum absorption, 235 and 350 nm, which were closed to the values reported earlier (243 and 350 nm, BP, 1988 and 243 and 350.5 nm, Merck Index, 1989).

With TLC-densitometric method, the absorption spectrum of colchicine was obtained directly from the colchicine spot on a silica plate which was developed using the solvent system established as described earlier. the resulted absorption spectrum obtained is shown in Fig.25B. It can be seen that there are two wavelength values of maximum absorption : 250 and 353 nm. To avoid possible interference of other compounds at the short UV wavelength (250 nm),the wavelength of 350 nm was chosen for the TLC-densitometric determination of colchicine

throughout in this study.



(A)

(B)

Fig.25 UV-absorption spectra of colchicine

A) by UV-spectrophotometry B) by TLC-densitometry

2.2 TLC-Densitometric Chromatograms of the Crude Ethanolic Extracts of Various *G. superba* Parts

Using the established optimum conditions of extraction, solvent system and TLC scanning, TLC-densitometric chromatograms of crude ethanolic extracts of various parts of *G. superba* were obtained. The plant parts used for the study included seeds, pericarps, flowers, tubers, leaves and stems. As shown in Fig.26, all parts of *G. superba* contained colchicine which was well separated from other components under the established conditions. The peak near colchicine (less Rf value) was believed to be 3-demethylcolchicine (3-DMC) since it co-chromatographed with the standard 3-DMC. Similar to colchicine, 3-DMC appeared to be present in every part of *G. superba* plant. Another standard, colchicoside, was also found to be co-chromatographed with a peak in the seeds, no other parts were detected. The present of colchicine, colchicoside and 3-DMC in the seeds was, therefore, consistent with the previous finding (section 1.2) using HPLC method.

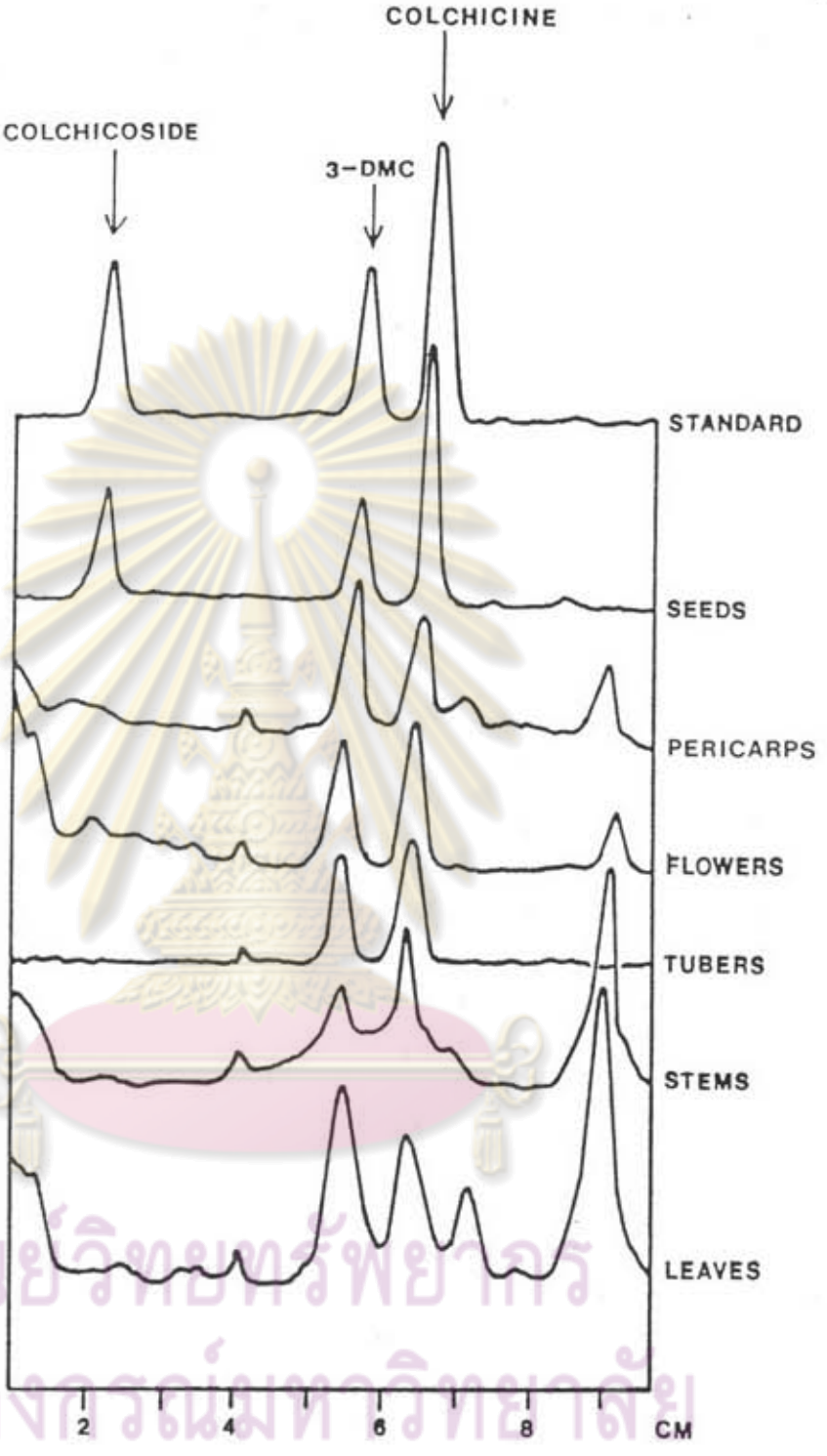


Fig.26 TLC-densitometric chromatograms of the crude ethanolic extracts of various plant parts of *G. superba*

2.3 Method Validation

2.3.1 Calibration Curve

The calibration curve of standard colchicine which was obtained by plotting between the peak areas against colchicine concentration is shown in Table 12 and Fig.27. The calibration curve was linear in the range between 5 to 60 µg/ml for a 5 µl application volume of each colchicine concentration equivalent to 0.025 to 0.30 µg colchicine per each spot. The correlation coefficient (r) was found to be 0.9991 and regression equation was $y = 215103.5 x + 280.9683$ (n=7),

where y = peak area of colchicine

x = colchicine concentration (µg/5µl)

n = the number of standard colchicine concentration levels

r = correlation coefficient

2.3.2 Accuracy

Six known concentrations of colchicine were prepared and then were subjected to TLC-densitometric analysis. Subsequently , each calculated colchicine content was compared with its actual concentration. The results obtained are shown in Table 13. The mean percent coefficient of variation was 1.88 at all concentrations

which seemed to be well acceptable.

Table 12 Relationship between colchicine concentrations and their peak areas as determined by TLC-densitometric method.

Colchicine concentration		Peak area [a]
µg / ml	µg / 5 µl	(x 1000)
5	0.025	5.09±0.244
10	0.05	11.75±0.261
20	0.10	22.93±0.184
30	0.15	31.49±0.944
40	0.20	43.23±0.589
50	0.25	54.04±0.462
60	0.30	64.95±0.325
r^b = 0.9991		

a : Mean ± standard deviation (n = 3)

b : r = Correlation coefficient (n = 7)

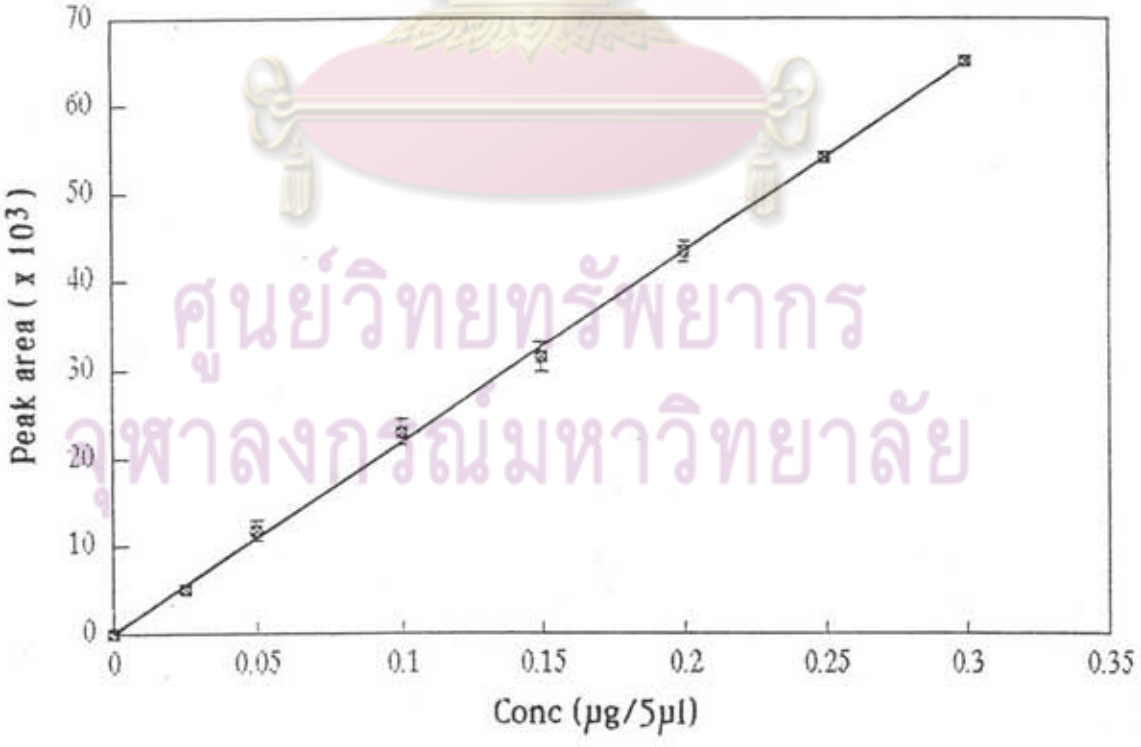


Fig.27 Calibration curve of standard colchicine obtained by TLC-densitometry.

Table 13 Calculated concentrations of colchicine compared with their known concentrations by TLC-densitometric method.

Known conc. (µg /ml)	Calculated conc. [a] (µg / ml)	% CV [b]
10.20	10.16±0.17	0.39
20.40	19.91±0.38	2.40
25.50	25.23±0.34	1.06
40.80	39.56±0.70	3.04
51.00	49.25±0.74	3.43
61.20	60.61±0.59	0.96

a : Mean ± standard deviation (n = 3)

b : % coefficient of variation

Furthermore, the accuracy of the TLC-densitometry was also confirmed by HPLC method using the same conditions described in the previous experiment (see(5) in Chapter III). Ethanolic extracts (5 samples) of *G. superba* seeds were determined for colchicine content by both TLC-densitometric and HPLC methods. The colchicine contents obtained by both methods were then compared with each other. The results showed that the colchicine content determined by both methods were very closed from one another with the mean percent coefficient of variation of 1.68 at all samples (Table 14). This suggested that the TLC-densitometric method was reliable.

Table 14 Colchicine content in *G. superba* seeds determined by TLC-densitometric method compared with HPLC method.

Sample no.	Colchicine (% w/w)		% CV [c]
	HPLC [a]	TLC-densitometry [b]	
1	1.456±0.0316	1.480±0.0037	1.62
2	1.305±0.0452	1.340±0.0117	2.61
3	1.428±0.0284	1.437±0.0181	0.63
4	1.259±0.0510	1.282±0.0052	1.79
5	0.834±0.0314	0.849±0.0122	1.77

a : Mean ± standard deviation (n=6)

b : Mean ± standard deviation (n=3)

c : % coefficient of variation

2.3.3 Precision

In order to evaluate the precision of TLC-densitometric method, ten replicate samples (n=10) of a crude ethanolic extract of *G. superba* seeds were analyzed according to TLC-densitometric procedure. The results obtained are presented in Table 15. The percent coefficient of variation was 3.15 which was still acceptable.

Table 15 Precision analysis of colchicine content in *G. superba* seeds by TLC-densitometric method.

Run no.	Colchicine (% w/w)
1	1.164
2	1.194
3	1.170
4	1.161
5	1.215
6	1.254
7	1.144
8	1.158
9	1.143
10	1.139
Mean (X)	1.174
Standard deviation (SD)	0.037
% Coefficient of variation (% CV)	3.15

2.4 Colchicine Content in Various Parts of *G. superba* Plant

2.4.1 Distribution of Colchicine in the Whole Plant

The complete separation of colchicine from other components in the crude extracts of various *G. superba* parts (Fig.23) allowed colchicine be determined by densitometric method. Based on the integrated areas under the peaks of colchicine appeared in the densitometric chromatograms (Fig.26), the quantitative distribution of colchicine in the whole *G. superba* plant could be examined. This was carried out by separating the whole mature plant into seeds,pericarps, flowers, tubers, leaves and stems. The leaves and stems, because of the height of the plant, were further divided into small portions (nine portions) from the bottom to the top.

It was found that the colchicine content was distributed in the mature *G. superba* plant according to Fig.28. The seeds apparently contained the highest amount of colchicine. Its content was found to be up to 1.35 % of dry weight as compared to 0.86 % for the pericarps , 0.40 % for the flowers and 0.26 % for the tubers. The leaves and the stems showed the lowest

Fig.28 Distribution of colchicine in the mature *G.superba* plant. The values were obtained from three plants (For the pericarps, seeds, flowers, and tubers , n=3 ; For the leaves and stems, n=1). The percent standard deviation is between 0.06 and 4.21 % at all sample portions.

amount, approximately 0.06 and 0.05% (w/w), respectively. However, it appeared that the leaves and the stems near by the tuber and shoot contained higher colchicine content than the middle portion of both parts (Fig.29).

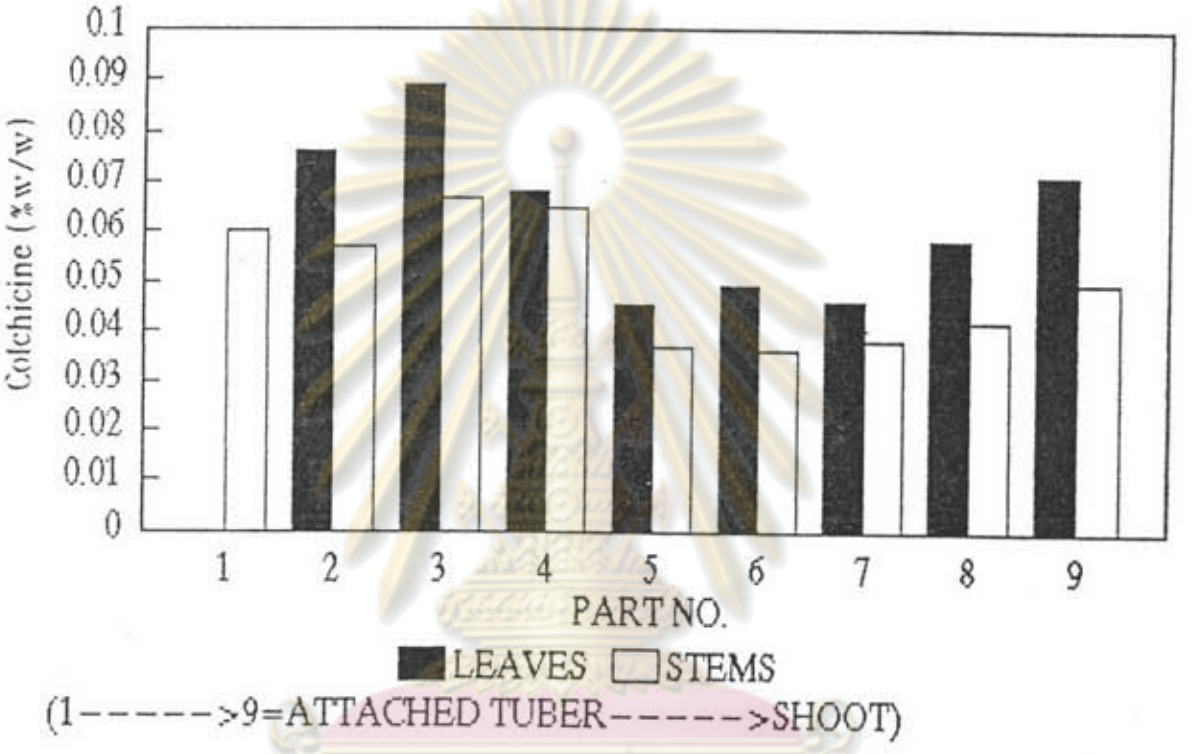


Fig.29 Bar graph of colchicine content in leaves and stems obtained from various portions of the mature *G. superba* plant. The values were obtained from three plants (n=1).

2.4.2 Colchicine Content in Premature and Mature Plants

G. superba plants with two months before maturation were collected and subjected to colchicine determination in order to compare with the mature plants

with respect to their colchicine distribution. The fresh premature plants were separated into the parts of seeds, pericarps, floweres, tubers, leaves and stems and, after drying, were determined for their colchicine content.

Fig.30 shows the resulted colchicine content in each parts of the premature *G. superba* as compared with the results from mature plant. It can be seen that while the colchicine content in the seeds, flowers, tubers, leaves and stems were not significantly different between the two plants, the content in the young pericarps appeared to be two times higher than that in the mature pericarps. Since both types of plants were collected from the same place (Amphur Khlung, Chanthaburi), the difference in the colchicine content should have come from the internal factor of the plant. Therefore, changes in colchicine content during the maturation of capsules were investigated in more detial.

2.4.3 Changes in Colchicine Content in *G.superba* Pericarps and Seeds During Maturation

In this experiment, the collected capsules were divided into six groups based on their maturity and capsule sizes. These groups included young capsules with less than 3 cm long , 3-4 cm , 4-5 cm , or more than 5 cm long and ripe capsules with non exploded and exploded.

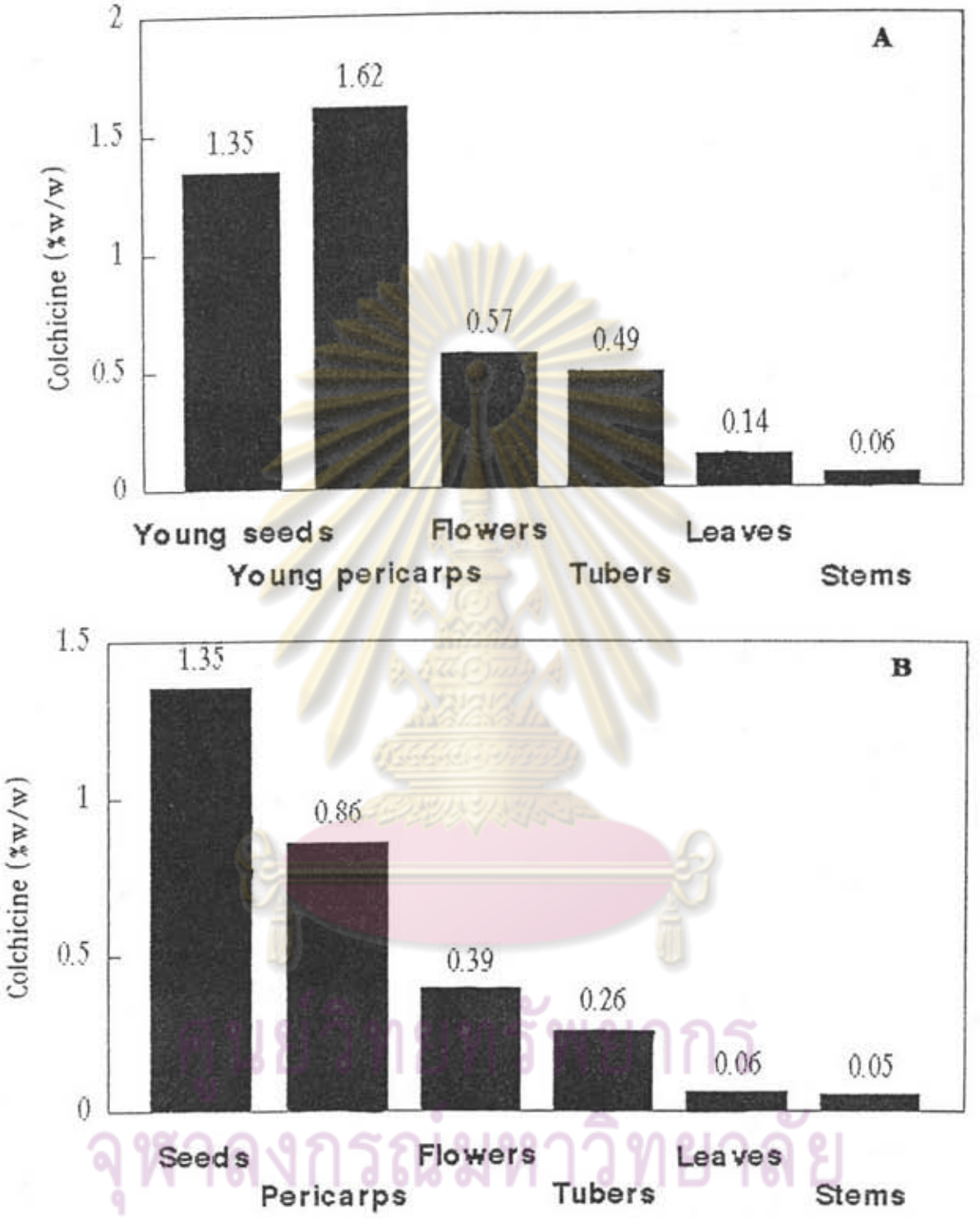


Fig.30 Comparison of colchicine content in various parts of *G. superba* between the premature (A) and mature (B) plants. The plants were collected from Amphur Khlung, Chanthaburi in August 1992 (premature) and October 1992 (mature plant).

In each group, the seeds were separated from the capsules and colchicine content in both the seeds and the remaining capsules (pericarps) were determined by TLC-densitometric analysis. As shown in Table 16 and Fig.31 , the young capsules with different sizes seemed to have no significant different content of colchicine in their seeds on the basis of percent dry weight (1.06-1.17%), although with the seeds of very young capsule (less than 3 cm) which contained high colchicine content (1.33 %). It should be noted that the size of the seeds obtained from the 3-cm capsules were very small and light in weight whereas the seeds from the bigger capsules were bigger and heavier. Therefore, although the colchicine content on the basis of percent dry weight was relatively similar, the total colchicine content per total weight of the seeds must be considerably different.

The results of the colchicine content in the pericarps are shown in Tabel 16 and Fig.32. It can be seen that the colchicine content in the pericarps obtained from various capsules seemed to be different in their quantities and apparently showed the tendency of a decrease in colchicine content during the process of maturity.

Table 16 Colchicine content in *G. superba* seeds and pericarps obtained from various capsules different in their maturity and capsule sizes.

Maturity and capsule sizes	Colchicine (% w/w)	
	Seeds *	Pericarps *
young capsules, < 3 cm long	1.33±0.015	1.68±0.053
young capsules, 3-4 cm long	1.17±0.056	1.46±0.015
young capsules, 4-5 cm long	1.17±0.035	1.41±0.030
young capsules, > 5 cm long	1.06±0.045	1.17±0.002
ripe capsules, not exploded	1.06±0.006	0.92±0.048
ripe capsules, exploded	1.35±0.004	0.32±0.010

* Mean ± standard deviation (n = 3)

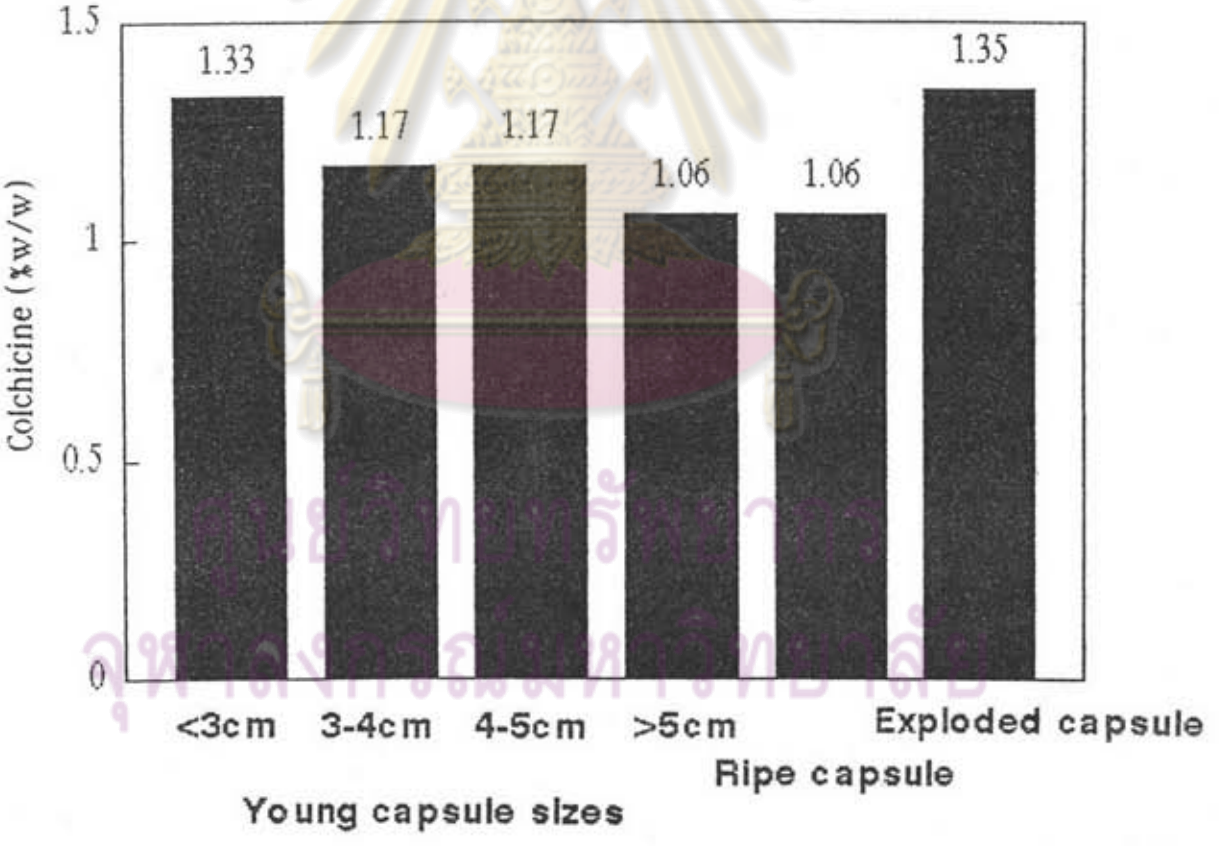


Fig.31 Bar graph of colchicine content in *G.superba* seeds obtained from various capsules different in their maturity and capsule sizes.

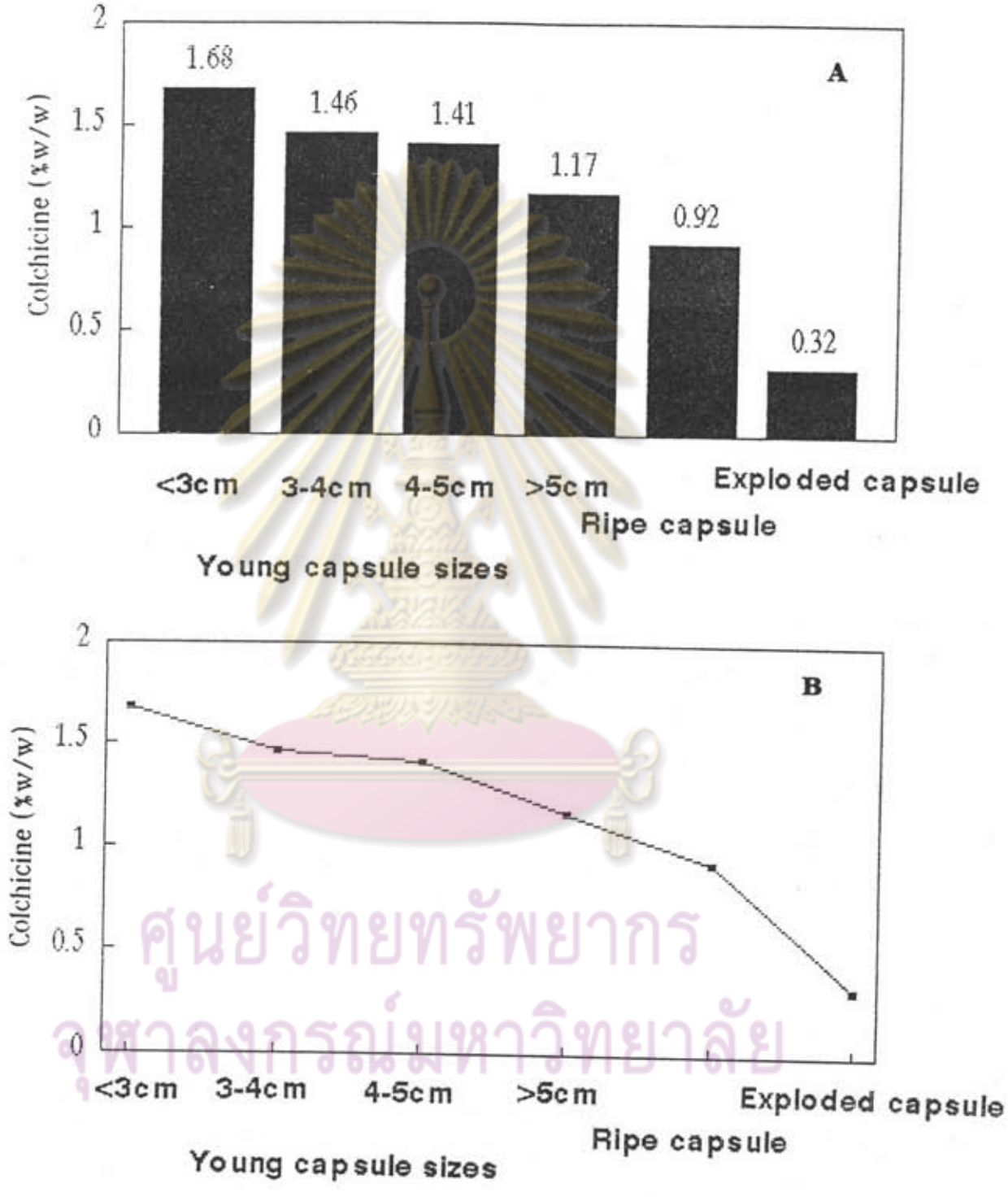


Fig.32 Colchicine content in *G. superba* pericarps obtained from various capsules different in their maturity and capsule sizes. A) Bar graph B) Line graph