CHAPTER 3

Electrocoagulation of Natural Dyes

In this section, coloring matter from nine natural sources: black glutinous rice, seed lac, turmeric, jackfruit wood, roselle, beet root, gardenia, betel nut and dark bean were extracted by aqueous alcoholic solutions. Coloring matter was removed under similar electrocoagulation conditions as reported in Chapter 2.

3.1 Electrocagulation of natural pigment from black glutinous rice

3.1.1 Introduction

Rice is a major cereal crop in the developing world and an important staple food source for over half of the world's population. Although widely consumed as white rice, there are many special cultivars of rice that contain color pigments, such as black rice and red rice. Southeastern Asia (20-28° latitude) is the major production area for this agricultural crop, and inhabitants of this area have a long history of black rice consumption [26].

Black rice (*Oryza sativa* L. Indica), having dark purple colored grains, is a major rice crop in South Asia and Mainland China. It is broadly known as enriched rice with medicinal effects. Anthocyanin pigments, cyanidin 3-glucoside and peonidin 3-glucoside, were isolated from this plant [27]. Black glutinous rice is a unique feature of many of the traditional glutinous rice. It has been used for making diverse products including food, drink and cosmetics. According to the popular of healthy food, black

glutinous rice is considered as one of the high potential materials for making such products. [28]

In the following study, the isolation of coloring matter from black glutinous rice was firstly extract by an aqueous-alcoholic solvent system and then subjected to electrocoagulation treatment.

3.1.2 Experiment

3.1.2.1 Plant material

Dry grain of black glutinous rice was obtained from Chiang Mai's local market.

Picture 3.1 Black glutinous rice sample (powdered).

3.1.2.2 Instrumental and apparatus

(1) UV/VIS spectrophotometer, model Genesys 10 spectrophotometer, Thermo scientific, USA.

(2) DC power supplier, model GPR-1810 HD and GPS-3030D; Good Will Instrument Co.Ltd., Taiwan.

(3) Vacuum rotary evaporator, model rotavapor-R; Buchi Vacuum pump, model PC-1; VacUUbrand, Imed, USA.

(4) Soxhlet apparatus

(5) pH meter, model pH; Precisa, Merck, Germany.

(6) TLC plates (aluminium), silica gel 60 F254; Merck, Germany.

(7) Aluminium plates (dimension 15 x 3.5-4 x 0.05cm)

3.1.2.3 Chemicals

(1) Ethanol (absolute) C_2H_5OH; E. Merck, Darmstadt, Germany.

(2) Methanol CH_3OH; Analytical Reagent Grade; Fisher Scientific UK Limited, UK.

(3) *n*-Butanol $C_4H_{10}O$ Analytical Reagent Grade; Fisher Scientific UK Limited, UK.

(4) Hydrochloric Acid Hydrochloric acid solution HCl (37%); Carlo Erba Reagent Co., Ronando, MI, Italy.

(5) Sodium chloride NaCl (99.9%, AR grade) Ajax Chemical Co., Sydney, Australia.

3.1.2.4 Procedure

(a) Extraction of coloring matter from black glutinous rice

Dried grain of black glutinous rice were blended and then divided into 3 sets. The sample set including 6 samples, each sample contained approximately 10 g of powdered black glutinous rice. The solvent systems and extraction conditions used for the first set were aqueous solutions as shown in table 3.1. Solvent systems for other 2 sets are 95% and 85% aqueous ethanol respectively.

The solution was then filtered, evaporated and dried under vacuum to afford the crude extract.

Table 3.1 Solvent systems and extraction conditions used.

Sample Set	Sample number	Solvent system	Extraction condition*
I	1	Acidic solution; with 1% hydrochloric acid	Stir 24 hours at room temperature
	2	100% water	Stir 24 hours at room temperature
	3	Alkali solution; with 1% sodium hydroxide	Stir 24 hours at room temperature
	4	Acidic solution; with 1% hydrochloric acid	Reflux, 30 min
	5	100% water	Reflux, 30 min
	6	Alkali solution; with 1% sodium hydroxide	Reflux, 30 min
II	7	95% aqueous ethanol with 1% hydrochloric acid	Stir 24 hr at room temperature
	8	95% aqueous ethanol	Stir 24 hr at room temperature
	9	95% aqueous ethanol; with 1% sodium hydroxide	Stir 24 hr at room temperature
	10	95% aqueous ethanol; with 1% hydrochloric acid	Reflux, 30 min
	11	95% aqueous ethanol	Reflux, 30 min
	12	95% aqueous ethanol; with 1% sodium hydroxide	Reflux, 30 min

Sample Set	Sample number	Solvent system	Extraction condition*
III	13	80% aqueous ethanol; with 1% hydrochloric acid solution	Stir 24 hr at room temperature
	14	80% aqueous ethanol	Stir 24 hr at room temperature
	15	80% aqueous ethanol; with 1% sodium hydroxide	Stir 24 hr at room temperature
	16	80% aqueous ethanol; with 1% hydrochloric acid solution	Reflux, 30 min
	17	80% aqueous ethanol	Reflux, 30 min
	18	80% aqueous ethanol; with 1% sodium hydroxide	Reflux, 30 min

Note: 50 mL of solvent measured per 1 g of sample.

(b) Chromosome dyeing test

The crude extract (0.02) g was dissolved in methanol then the volume adjusted and centrifuged. The supernatant was taken to measure the absorbance to determine the maximum absorption wavelength (λ_{max}). A small amount of the crude extracts were tested for chromosome dyeing.

(c) Electrocoagulation of crude extract from black glutinous rice

(c-1) Experiment set I; electrocoagulation of aqueous crude extract.

To the 10 g of dried, powdered black glutinous rice sample, 500 mL distillate water was added then stirred overnight at room temperature. After that, the solution was filtered then collected. Sodium chloride (1.0 g) was added to the obtained solution which was adjusted to 500 mL by distillated water.

Two aluminum plates (dimensions 30 x 4.5 x 0.05 cm) were used as electrodes. These were spaced 3 cm apart and dipped 7 cm deep into the magnetically-stirred aqueous solution in a 600-mL beaker. Direct current (1.2 A, 16.7 V) from the DC power supplier was then passed through the solution for 8 minutes.

The black coagulum was obtained by filtration and then kept overnight to dry.. The coagulum was dissolved in 10% hydrochloric acid solution and extracted by *n*-butanol. The red solution in both aqueous and organic layer were evaporated and dried under vacuum, after that the deep purple solid residue was collected. The sample was analyzed by TLC (developed by *n*-butanol : CH_3OH (glacial) : H_2O = 40:10:20 v/v)[29], determined for λ_{max} and tested of chromosome dyeing activity.

(c-2) Experiment set II; electrocoagulation of of 95% ethanolic crude extract with 1% hydrochloric acid.

The process was repeated with the crude extract of black glutinous rice (sample no. 7). One gram of the sticky crude compound was re-dissolved in 85% aqueous ethanol and made up to a volume of 200 mL and 0.4 g of sodium chloride was added. The solution was placed in a 250 mL glass container and electrocoagulated with a pair of 15 x 3.5 x 0.05 cm aluminum electrodes, space 3 cm apart and dipped 6.5 cm into a magnetically-stirred solution. After 1 hour of electrolysis with direct current (0.3 A, 20.7 V), the mixture was filtered and the filtrate was evaporated to dryness. The precipitate obtained from the electrocoagulation was dissolved in 10% hydrochloric acid solution and the resulting acidic solution was extracted with *n*-butanol. The alcoholic solution was evaporated and the solid residue was collected.

The samples were studied for maximum adsorption wavelength determination and chromosome dyeing activity.

(c-3) Experiment set III; Recovered compound from the filtrate of EC process.

From the previous study (c-1 and c-2) EC technique was applied to extract coloring matter from black glutinous rice and mentioned on the recovered compound from coagulation of EC process. In this experiment, another condition (80% ethanolic solution with TFA and 0.5 A current) was used and the recovered compound from the filtrate was collected.

Ethanol (80%, 250 mL) with trifluoroacetic acid (7.5 mL; 3% v/v) was added to 25 g of the dry grain of black glutinous rice and stirred overnight at room temperature. After filtration the filtrate was made up to a volume of 250 mL with the same solvent and placed in a 400 mL beaker then 0.5 g of sodium chloride was added. A pair of 15 x 4 x 0.05 cm aluminum electrodes space 3 cm apart and dipped 5.5 cm into a magnetically-stirred solution. After 2 hour of electrolysis with direct current (0.5 A, 6.0-16.0 V), the mixture was filtered and the filtrate was evaporated.

The residue was dissolved in absolute ethanol and the remaining undissolved inorganic matter was then filtered off from the solution, which was evaporated to dryness in vacuum. The obtained red solid compound was then tested for chromosome dyeing activity.

3.1.3 Results and discussion

(a) Extraction of coloring matter from black glutinous rice

Black glutinous rice sample (as shown in picture 3.1) was extracted in various conditions. The results are as shown in table 3.2

Table 3.2 Percentage yield and color of crude extract from black glutinous rice.

sample	solvent	Weight of sample (g)	Extraction condition		Weight of crude extract (g)	% yield	Color
			condition	pH of solvent			
1	water	10.00	T_{room} 24 hr	1.10	0.64	6.40	dark red
2		10.00	T_{room} 24 hr	7.20	0.37	3.70	purple
3		10.03	T_{room} 24 hr	11.6	6.84	68.4	dark brown
4		10.06	Reflux 0.5 hr	1.10	9.00	89.5	dark red
5		10.03	Reflux 0.5 hr	7.50	2.91	29.1	purple
6		10.04	Reflux 0.5 hr	11.6	9.77	97.3	dark brown
7	95% ethanol	10.01	T_{room} 24 hr	1.10	0.54	5.39	red
8		10.11	T_{room} 24 hr	7.40	0.33	3.26	purple
9		10.02	T_{room} 24 hr	8.80	2.45	24.5	yellow
10		10.01	Reflux 0.5 hr	1.10	0.90	8.99	red
11		10.03	Reflux 0.5 hr	7.40	1.00	9.97	purple
12		10.03	Reflux 0.5 hr	8.40	5.53	55.1	yellow
13	85% ethanol	10.01	T_{room} 24 hr	0.70	0.62	6.19	red
14		10.00	T_{room} 24 hr	8.40	0.84	8.40	purple
15		10.05	T_{room} 24 hr	10.5	6.05	60.2	yellow
16		10.04	Reflux 0.5 hr	0.70	1.21	12.1	red
17		10.02	Reflux 0.5 hr	8.40	0.99	9.88	purple
18		10.03	Reflux 0.5 hr	10.5	7.04	70.2	brown

It was seen that, the alkali extract, by reflux method of all sample sets, produced the highest percentage yield of crude extract. The color shades of solutions are different depending on pH range: bright red color appeared in acidic condition, while purple and brown color was obtained in neutral and alkali condition respectively (Picture 3.2).

Picture 3.2 Color of black glutinous rice sample in 95% aqueous ethanol solution. (From left 95% aqueous ethanol, acidic condition and alkali condition).

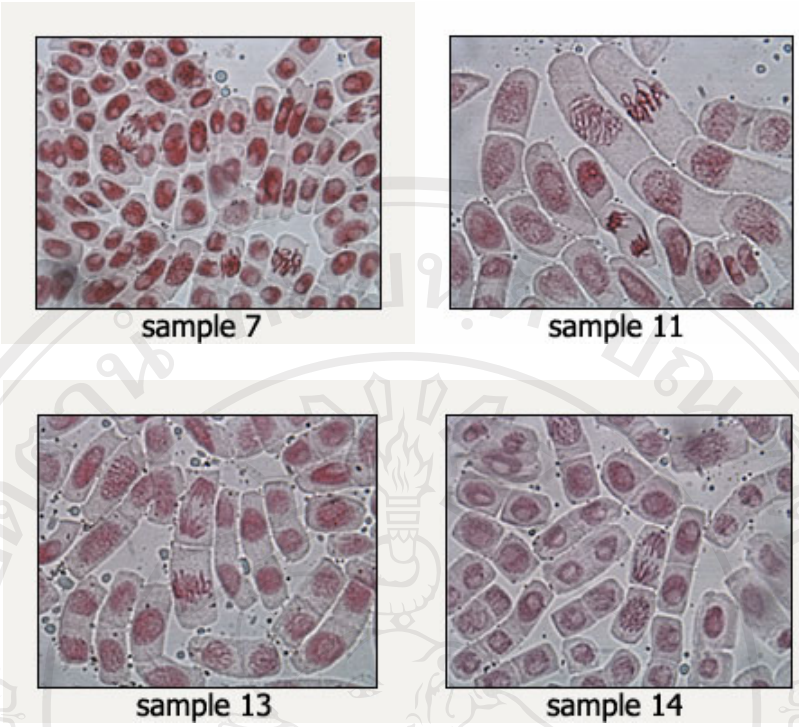
(b) Chromosome dyeing test

The natural color extracts from black glutinous rice were tested for chromosome dyeing, the results are as shown in table 3.3.

Table 3.3 Chromosome dyeing test and maximum absorption wavelength of the crude extracts from black glutinous rice.

Sample	Solvent	Weight of sample (g)	Extraction condition		λ_{max} (nm)	Chromosome dyeing test
			Condition	pH of solvent		
1	water	10.00	Troom 24 hr	1.1	535	*
2		10.00	Troom 24 hr	7.2	535	**
3		10.03	Troom 24 hr	11.6	-	-
4		10.06	Reflux 0.5 hr	1.1	535	*
5		10.03	Reflux 0.5 hr	7.5	535	*
6		10.04	Reflux 0.5 hr	11.6	-	-
7	95% ethanol	10.01	Troom 24 hr	1.1	535	***
8		10.11	Troom 24 hr	7.4	535	*
9		10.02	Troom 24 hr	8.8	-	-
10		10.01	Reflux 0.5 hr	1.1	535	*
11		10.03	Reflux 0.5 hr	7.4	535	***
12		10.03	Reflux 0.5 hr	8.4	-	-
13	85% ethanol	10.01	Troom 24 hr	0.7	535	*
14		10.00	Troom 24 hr	8.4	535	*
15		10.05	Troom 24 hr	10.5	-	-
16		10.04	Reflux 0.5 hr	0.7	535	*
17		10.02	Reflux 0.5 hr	8.4	535	*
18		10.03	Reflux 0.5 hr	10.5	-	-

***; best, **; good, * fair for chromosome dyeing and - ; negative test



Picture 3.3 Chromosome dyeing of some crude extracts from black glutinous rice.

As illustrated in picture 3.3, it was seen that, chromosome dyeing with crude extract no. 7 and no. 11 showed a clear and bright color. In addition, the methanolic solution of these samples showed the maximum wavelength absorption at 535 nm. Due to the simple aqueous extraction of sample no. 2 and the best chromosome dyeing of sample no.7, these samples were subjected to electrolysis in experiment c-1 and c-2 as follows.

(c) Electrocoagulation of crude extract from black glutinous rice

Picture 3.4 Black glutinous rice solution before (left) and after (right) electrocoagulation process.

(c-1) Experiment set I; electrocoagulation of aqueous crude extract.

In this experiment section, the precipitate from electrocoagulation was dissolved in 10% hydrochloric acid solution and extracted with *n*-butanol. The alcoholic solution was evaporated and the dark purple solid residue was obtained with 2 percentage yield. Furthermore, the aqueous layer was evaporated and the red solid residue was collected with the 4 percentage yield.

The dark purple solid sample taken from *n*-butanol layer was dissolved in methanol and separated on silica gel plate and compared with the crude extract. The results showed that after electrocoagulation the constituents of the recovered compounds were a somewhat changed.

Picture 3.5 solid compound obtained from the precipitate part of electrolysis of black glutinous rice, dark purple solid compound from *n*-butanol layer (left) and pink solid compound from acid layer (right).

Picture 3.6 Recovered compounds from EC precipitate of black glutinous rice. Right; 95% ethanolic solution of recovered compound from alcoholic solution. Left; 95% ethanolic solution of recovered compound from acid solution.

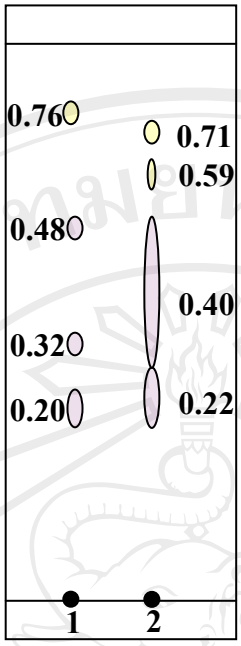


Figure 3.1 TLC chromatogram of crude extract of black glutinous rice (spot 1) and compound obtained from *n*-butanol layer of the precipitate from electrocoagulation process (spot 2).

Two samples which were obtained from this experiment section showed the maximum absorption wavelength at 575 (alcoholic layer) and 570 nm (acid layer). However, both of them showed the negative results for chromosome dyeing test.

(c-2) Experiment set II; electrocoagulation of crude extract of 95% ethanol with 1% hydrochloric acid)

In this experiment section, precipitate obtained from electrocoagulation was dissolved in 10% hydrochloric acid solution and extracted by *n*-butanol. The alcoholic solution was evaporated and the dark purple solid residue was obtained in a high yield (0.74 g or 74% yield from the starting sticky crude compound). However, the chromosome dyeing test showed negative result.

(c-3) Experiment set III; Recovered compound from the filtrate of EC process.

After 2 hours of electrolysis, the mixture was filtered and the filtrate was evaporated. The dark purple solid residue was dissolved in absolute ethanol and the remaining undissolved inorganic matter was then filtered off from the solution. After evaporated, the solid residue was collected, treated with a 3% trifluoroacetic acid solution, filtered, and washed free of acid with water. The 6.1 g (24% yield) of red dry solid residue was collected and showed the negative result of chromosome dyeing test.

3.1.4 Conclusions

In this study, when the EC method was applied, the coloring matter from black glutinous rice was coagulated in both aqueous and alcoholic solution. The coloring matter from this plant can be electrolysed and recovered in a dry-solid form. However, chromosome dyeing activity of the recovered compounds was lost.

.

3.2 Electrocagulation of seed lac.

3.2.1 Introduction

Lac[30] is a natural resin of outstanding properties and exceptional versatility. The only known commercial resin of animal origin, it is the hardened secretion of a tiny insect, *Laccifer lacca* (Kerr), popularly known as the lac insect. Lac insect is specific in its parasitism and thrives only on certain trees and bushes which are consequently called lac hosts. Young larvae lac insects attach themselves to the host tree by piercing the bark with their tubular mouths through which they suck the sap of the tree for their nourishment and start secreting lac, presumably, as a protective coating. Under this coating, the larvae continue to grow, and go on secreting more lac from inside.

Lac crop is collected by cutting down the lac bearing twigs of the hosts either week before larval emergence. Lac encrustations are separated from the twigs by either breaking off by hand or scraping with a knife or sickle. Lac, thus gathered, is known as sticklac and it is in this form that cultivators bring it to the market.

Sticklac contains, in addition to lac resin and its associated spirit soluble dye eythrolaccin and wax, certain water soluble components which include a dye (laccaic acid), sugars, albuminous matter, etc. In order to purify sticklad, the first step is to crush it coarsely and clean it by sieving out the dust and sand on a fine sieve and then to wash the residue with water. During this processing, not only the soluble constituents are removed but also such incidental impurities as dead insects, barks, twigs, wood chips and other materials that float in the water. The last traces of water soluble matter and most of these extraneous materials are removed by rubbing the lac grains under water against the rough side of the washing tub with or without addition of mild alkaline materials, washing soda etc. During washing, lac also separates. Seedlac, consists chiefly of the larger lac grains, sinks to the bottom of the tub.

Seedlac, which is only a semi-refined product, still contains some amount of impurities including sand, wood-chips etc. It is therefore further refined in one of two ways, viz. (1) by a process of hot filtration or (2) by solvent extraction i.e., by dissolving it in alcohol, filtering free from insoluble impurities and distilling off the solvent to recover the dissolved lac. Refine lac from the process are the familiar shellac of commerce.

The compositions of sticklac, seedlac and shellac as show in table 3.4

Table 3.4 Composition of sticklac, seedlac and shellac

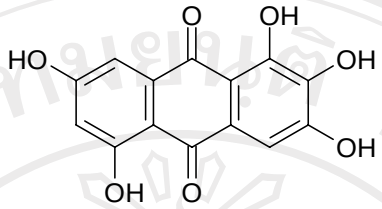
Constituent	**In sticklac**	**In seedlac**	**In shellac**
	Per cent	**Per cent**	**Per cent**
Resin	68.0	88.5	90.0
Dye	10.0	2.5	0.5
Wax	6.0	4.5	4.0
Gluten	5.5	2.0	2.8
Foreign bodies	6.5	-	-
Impurities	4.0	2.5	1.8

Ref: R. Bhattacharya, B.S. Gidvani, *London Shellac Res. Bur., Tech.,* 1938, 13

Apart from lac resin, there are a number of minor constituents in sticklac. They are the water-soluble dye known as lac dye, the water-insoluble but spirit-soluble dye erythrolaccin, the wax (lac wax), an odoriferous principle and some albuminous matter, sugar etc.

The colour of lac, even after washing free from water-soluble dye (laccaic acid) still remains light yellow to dark reddish brown depending upon the host and the crop. This is due to another colouring matter which is insoluble in water but soluble in almost all

solvents of lac. The water insoluble dye call erythrolaccin was isolated from seed lac by different procedure. Erythrolaccin is 1,2,5,7-tetrahydroxy-3-methyl anthraquinone of the following structure.[30]



In the following study, decolorization of lac and isolation of erythrolaccin from seedlac by electrocoagulation was attempted.

3.2.2 Experiment

3.2.2.1 Material

Seedlac sample was obtained from Thai Shellac (1980) Co.Ltd. Chiang Mai.

3.2.2.2 Instrument and apparatus

As indicated in 3.1.1.2

3.2.2.3 Chemicals

(1) *n*-Butanol $C_4H_{10}O$ (Analytical Reagent Grade); Fisher Scientific UK Ltd., UK.

(2) Hydrochloric acid solution HCl (37%); Carlo Erba Co., Ronando, MI, Italy.

(3) Diethyl ether $C_4H_{10}O$ (AR grade); Carlo Erba Reagent Co., Ronando, MI, Italy.

(4) Chloroform $CHCl_3$ (AR grade); Lab-Scan asia Co.Ltd., Thailand.

(5) Sodium carbonate Na_2CO_3; E. Merck, Darmstadt, Germany.

(6) Acetic acid CH_3COOH; E. Merck, Darmstadt, Germany.

(7) Ethanol C_2H_5OH (absolute); E. Merck, Darmstadt, Germany.

(8) Activated charcoal

3.2.2.4 Procedure

(a) Decolorization of lac and isolation of erythrolaccin

(a-1) Sample preparation

Seedlac sample was extracted with 95% ethanol by stirring overnight at room temperature. After filtration, the solution was diluted to 85% ethanolic solution by distilled water. The 4.6% 2.0% and 1.0% w/v solutions were prepared and used in the electrocoagulation experiments.

(a-2) Electrocoagulation procedure

Two aluminum plates (dimensions 15 x 4 x 0.05 cm) were used as electrodes. These were spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred aqueous solution (250 mL) of 4.6% w/v seedlac in a 400 mL beaker. Sodium chloride (0.5 g) was added as supporting electrolyte. Direct current (0.3 A, 22-27 V) from the d.c. power supplier was then passed through the solution. Every 15 minutes during a 2 hour period of electrolysis, a 4 mL sample of the solution was withdrawn, centrifuged and taken for an absorbance measurement at an appropriate wavelength.

(a-3) Recovery procedure

A 250 mL of seedlac solution was placed in a 400-mL beaker. Two aluminium plates (15.0 X 4.0 x 0.05 cm.) were used as electrodes. These were dipped 3 cm apart and 5.5 cm deep into the magnetically-stirred solution (4.6%, 2.0% and 1.0% w/v). Sodium chloride 0.5 g was added as a supporting electrolyte. Direct current (0.3 A, 22-27 V) from a d.c. power supplier was then passed through the solution via the two electrodes for 2 hour (then 1, 0.5 hour for other set of 1.0% w/v solution). The resulting mixture was filtered through a buchner funnel. The filtrate was evaporated and the brown-resinous

residue was dissolved in absolute ethanol. Sodium chloride was then filtered off from the solution and after evaporating, the brown resinous lac was collected.

The precipitate was dissolved in 10% hydrochloric acid solution. The obtained acidic solution was extracted with *n*-butanol. Alcoholic solution was evaporated, the solid compound was collected as a recovery extract, which was taken for determination of IR spectrum.

(b) Isolation of erythrolaccin from seedlac by classical method [5a]

Seedlac (100 g) was allowed to stand in contact with 1:1 ether-chloroform mixture (200 mL) at room temperature for 24 hours. The solvent was then distilled off from the extract and the residue treated with 10% sodium carbonate. The violet solution, after filtering free from the wax was acidified with 10% acetic acid. The sticky brown crude dye that separated was mixed with shredded filter paper and extracted with boiling ether-chloroform mixture (40 mL). The extract was filtered then concentrated and determined for IR pattern.

(c) Decolourization of seedlac by activated charcoal

of Seedlac crude extract (1.5 g) was dissolved in absolute ethanol. Activated charcoal (0.15 g) was added and the solution was heated until boiling for 10 minutes. Activated charcoal was filtered off the solution which was then evaporated and the dark brown-resinous lac was collected.

3.2.3 Results and discussion

(a) Decolorization of lac and isolation of erythrolaccin

Electrocoagulation procedure

In this experiment section, the seedlac solution (85% ethanolic) was taken for UV-Vis absorption measurement. The appropriate wavelength of maximum absorption in visible-range at 450 nm was chose for the investigation of lac's amount during EC process. The plot of absorbance and electrolysis time for 4.6% w/v seedlac solution was shown in Figure 3.2 and fractions taken during 2 hours of electrolysis was shown in Picture 3.7

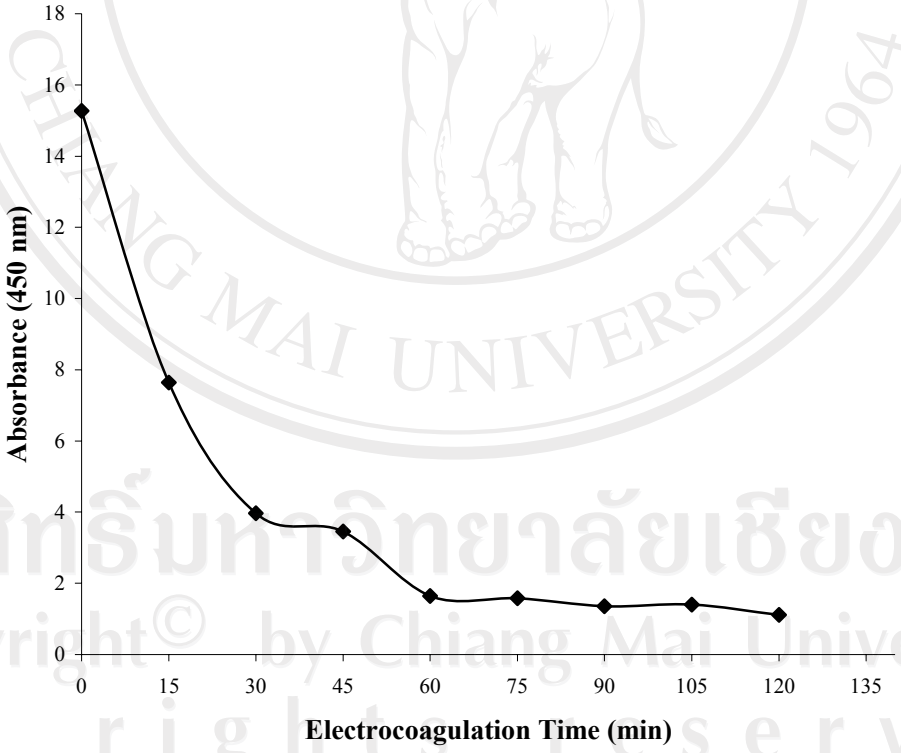
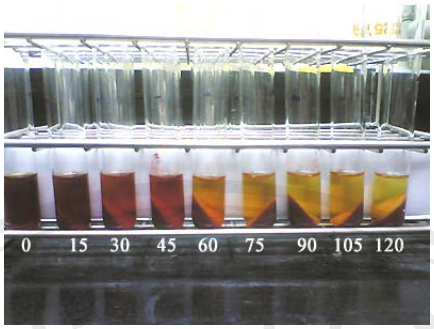


Figure 3.2 Plot of absorbance and electrolysis time for seedlac solution 4.6% w/v in 85% ethanol.

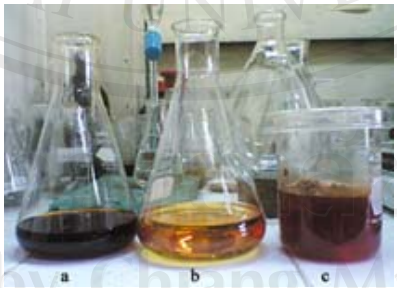


Picture 3.7 Seedlac sample solution (4.6% w/v in85% ethanol) taken every 15 minutes during 2-hour electrolysis.

As shown in Figure 3.2 it has been seen that the constituents in sample solution were coagulated nearly complete in 60 minutes of electrolysis time.

Recovery procedure

Picture 3.8 shows seedlac solution before and after electrocoagulation. As illustrated in the picture seedlac solution after EC process shows the bright-clear yellow color due to the removal of some coloring matter.



Picture 3.8 Seedlac solution (4.6% w/v in 85% ethanol).
From left; (a) solution of crude seedlac, (b) filtrate after 2 hours electrolysis and (c) precipitate dissolved in 10% HCl solution.

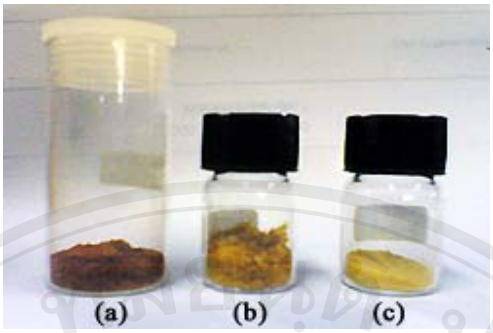
Table 3.5 shows percentage recovery of compounds from filtrate and precipitate after electrolysis at the various sample concentration. and electrolysis time. Recovered compounds are shown in Picture 3.9

Table 3.5 Weight and percentage yield of compounds obtained from filtrate and precipitate after EC process of seedlac sample solutions.

Seedlac sample solution (%w/v in 85% ethanol)	**EC time (hour)**	**Grams of compounds recovered form filtrate (%yield)**	**Grams of compounds recovered form precipitate (%yield)**
4.6	2	1.89 (16.6%) [(a)]	1.16 (10.1%)
2.0	2	1.01 (20.2%) [(b)]	1.22 (24.4%)
1.0	2	0.39 (15.6%) [(c)]	1.55 (62%)
1.0	1	0.62 (24.8%)	0.37 (14.8%)
1.0	0.5	0.68 (27.2%)	*0.28 (11.2%)

*treated with 10% HCl and determined for IR spectrum.

(a), (b) and (c) are illustrated in Picture 3.9.



Picture 3.9 Lac from filtrate of electrolysis**.**

(a) Form seedlac solution 4.6% w/v in 85% ethanol (2 hours electrolysis)

(b) From seedlac solution 2.0 % w/v in 85% ethanol (2 hours electrolysis)

(c) From seedlac solution 1.0% w/v in 85% ethanol (2 hours electrolysis)

IR spectra of crude seedlac, recovered lac from filtrate and recovered compounds from coagulum after EC process of seedlac solution were studied and the spectra are shown in Figures 3.3, 3.4 and 3.5 respectively.

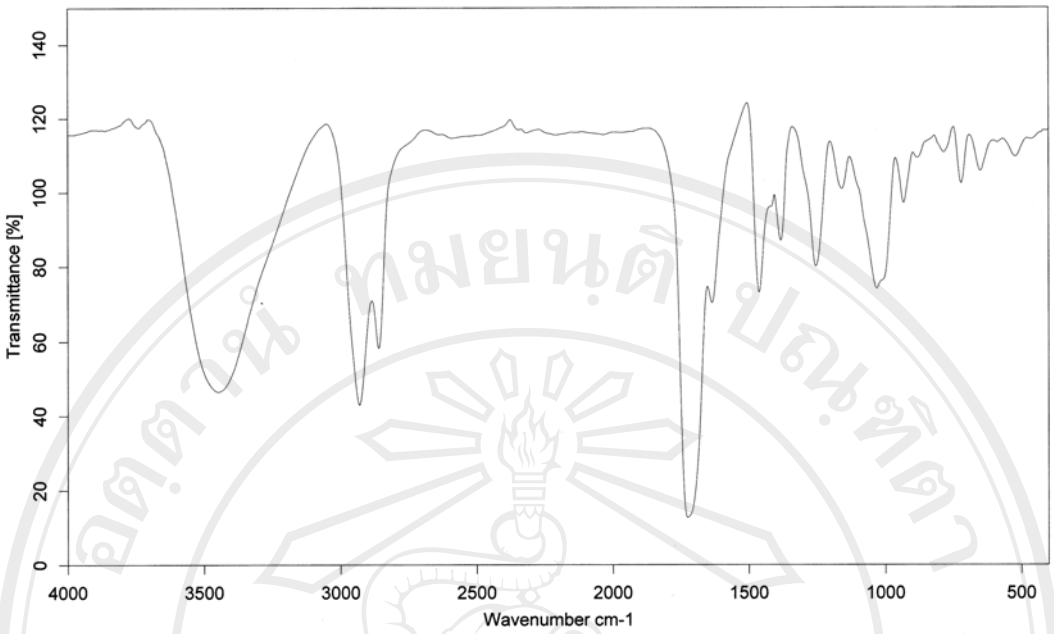


Figure 3.3 IR spectrum of crude seedlac**.**

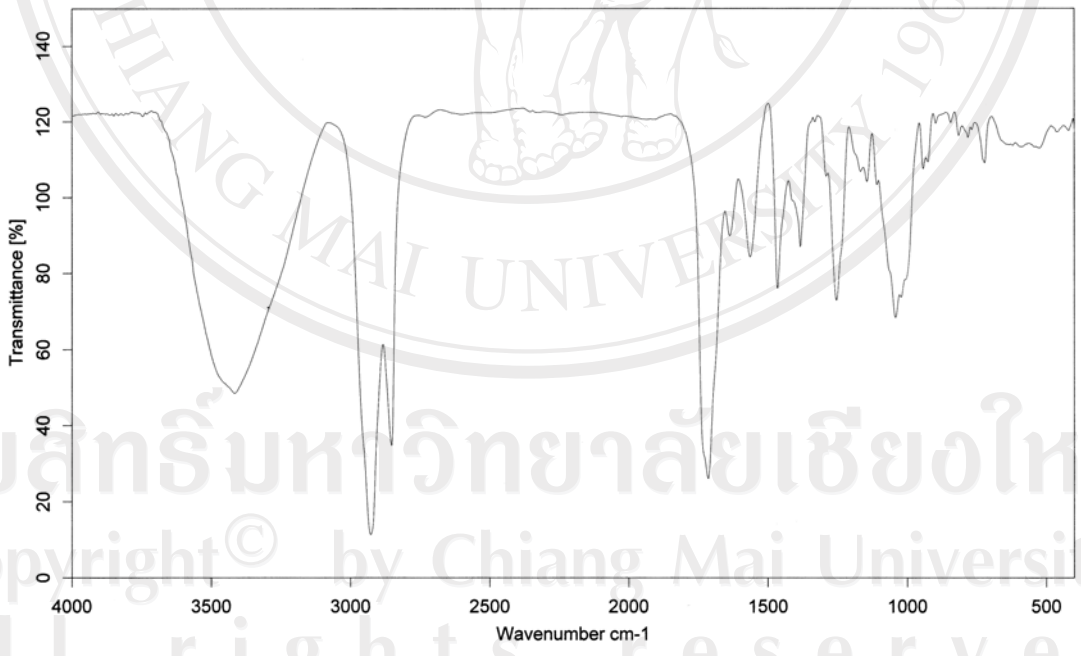


Figure 3.4 IR spectrum of lac recovered from filtrate of seedlac solution (1% w/v in 85% ethanol), 1 hour electrolysis.

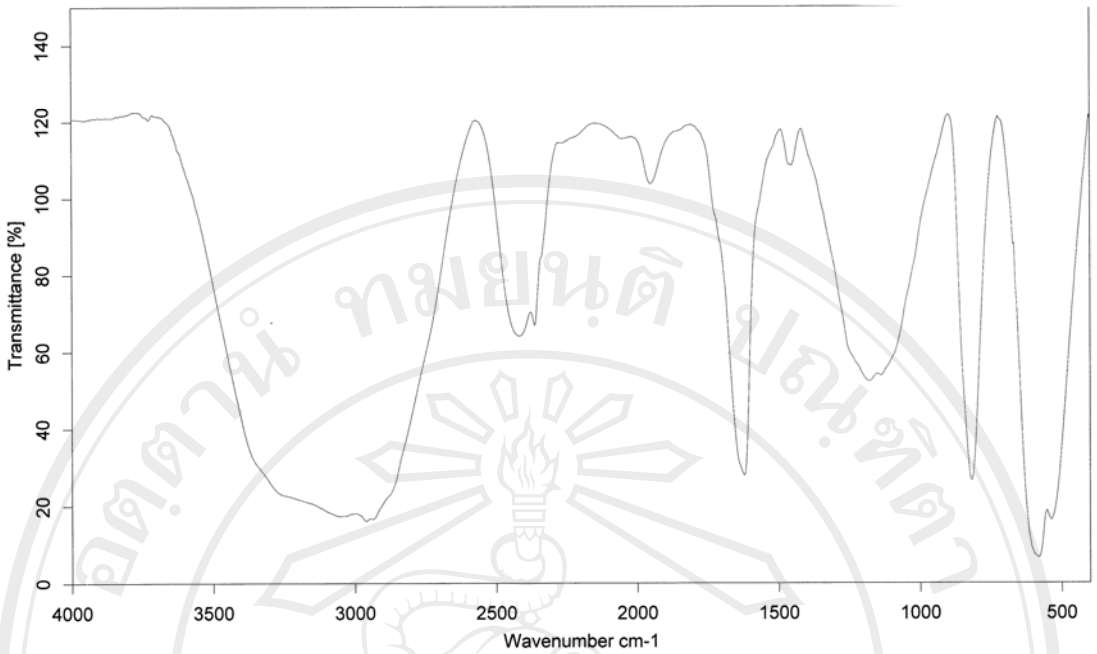


Figure 3.5 IR spectrum of dark-purple compound obtained from coagulum of electrocoagulation of seedlac.

Isolation of erythrolaccin from seedlac by classical method

IR spectrum of erythrolaccin obtained from seedlac by classical method shows Figure 3.6.

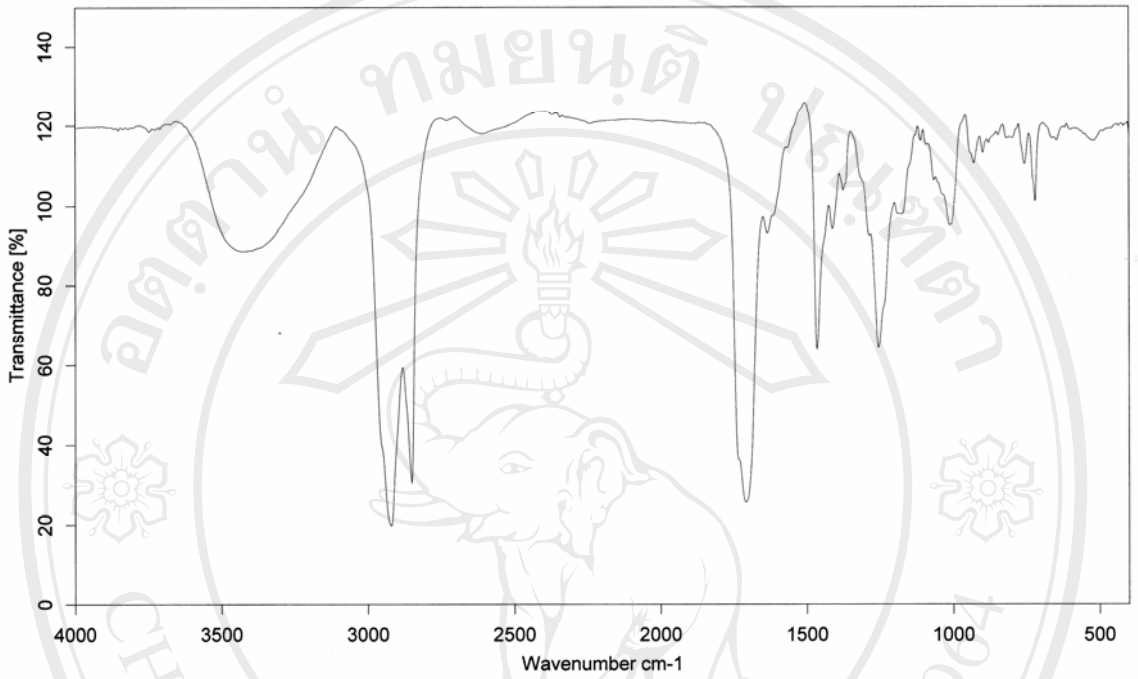
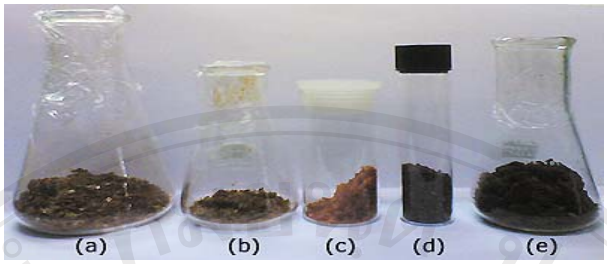


Figure 3.6 IR spectrum of compound isolated form seed lac by classical method of erythrolaccin isolating.

IR (KBr): υ_{max} cm^{-1} 3400 (br, OH stretching), 1675 (v.s, quinone), 1450 (s, C=C skelton for condense system), 810-750 (s, meta disubstituted), 780-760 (s, 1,2,3-trisubstituted)

Decolourization of seedlac by activated charcoal

Picture 3.10 shows crude seedlac and recovered compounds from filtrate and coagulate after electrolysis, compared with lac decolorized by activated charcoal. It can be seen that electrocoagulation is more efficient than carbon treatment in decolorizing seedlac.



Picture 3.10 Seedlac samples
(a) crude seedlac, (b) lac decolorized by activated charcoal, (c) lac decolorized by electrolysis for 2 hours (4.6% w/v in 85% ethanol), (d) recovered compounds from precipitate of electrolysis, (e) precipitate after removal of color.

In an attempt to isolate erythrolaccin from seedlac, an aqueous alcoholic solution of this substance was treated. The recovered colouring matter obtained, however, was not identical to that which was isolated from seedlac by an established procedure for erythrolaccin isolation,[30a] thus suggesting that the recovered pigment, though potentially useful in itself, might be an artifact. This might occur due to the fact that the coagulation time required in this case was rather long (1-2 hours), which according to our experience, can be somewhat detrimental in general to the coagulated compounds. Also, it has been mentioned that in a prolonged electrolyzing operation of this type, certain compounds present in the solutions may undergo reactions (e.g. oxidation or reduction) before coagulating.[32,33]

As to the reason why erythrolaccin was only slowly electrocoagulated when it possesses adjacent phenolic groups, this is not clear, although it was mentioned some time ago that erythrolaccin does not all occur in a free state in lac or seedlac but is

partially bound with the resinous substance.[30b] On the other hand, however, it was noted that the electrolysed seedlac was very pale in colour. Normally, this material is decolorized using various forms of carbon but the results are usually far from satisfactory. We have found that electrocoagulation is far more efficient than carbon treatment in decolorizing seedlac by coagulating out the yellow erythrolaccin. However, in our hand the yield of decolorized seedlac was still low, due to the fact that a good part of the resinous substances themselves tended to co-coagulate with the coloring matter.

3.2.4 Conclusions

It has been determined that electrocoagulation is an efficient method for removing coloring matter from seedlac.

3.3 Electrocoagulation of curcumin from turmeric.

3.3.1 Introduction

The dried ground rhizome of the perennial herb *Curcuma longa* Linn., called turmeric in English, haldi in Hindi and ukon in Japanese, has been used in asian medicine since the second millennium BC. *Curcuma spp*. contains turmerin (a water-soluble peptide), essential oils (with composition such as turmerones, atlantones and zingiberene) and curcuminoids including curcumin (diferuloylmethane; [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] (Figure 3.7). Curcuminoids can be defined as phenolic compounds derived from the roots of *Curcuma spp*. (Zingiberaceae)[34].

Curcumin is a low molecular weight polyphenol, non-nutritive, non-toxic chemical in turmeric that has been used for treatment of wounds, jaundice, and rheumatoid arthritis. Curcumin, a symmetrical diphenolic dien, exhibits numerous biological activities including anti-cancer, anti-inflammatory and anti-angiogenesis activities.[35]

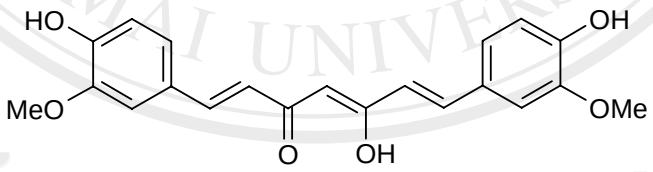


Figure 3.7 Curcumin structure

In this study, electrocoagulation of authentic curcumin was operated in a systematic manner then applied to the isolation of curcumin from turmeric. The final recovery extract was compared with the reference compound.

3.3.2 Experiment

3.3.2.1 Plant material

Dried powder turmeric purchased from local market in Chiang Mai.

3.3.2.2 Instrumental and apparatus

As indicated in 3.1.1.2

3.3.2.3 Chemicals

(9) Curcumin (1,7-Bis(4-hydroxy-3-methoxyphenyl)1,6-heptadiene-3,5-dione; (4-[OH]-3-$(CH_3O)C_6H_3CH=CHCO]_2CH_2$; for synthesis); Merck, Darmstadt, Germany.

(10) *n*-Butanol $C_4H_{10}O$ (AR grade); Fisher Scientific UK Ltd., UK.

(11) Hydrochloric acid solution HCl (37%); Carlo Erba Reagent Co., Ronando, MI, Italy.

(12) Ethanol C_2H_5OH (absolute); E. Merck Darmstadt, Germany.

(13) Benzene C_6H_6 ; Carlo Erba Reagent Co., Ronando, MI, Italy.

(14) Methanol CH_3OH; AR grade; Fisher Scientific UK Ltd., UK.

(15) Sodium chloride NaCl (99.9%, AR grade) Ajax Chemical Co., Sydney, Australia.

3.3.2.4 Procedure

(a) Model studies of electrocoagulation of curcumin

(a-1) Sample preparation

A 0.01% (w/v), 500 mL sample solution of authentic curcumin was prepared in 85% aqueous ethanolic solution. Sodium chloride (0.5 g or 0.2% w/v) was added as supporting electrolyte. The solution was divided into 3 parts: the first 50 mL was scanned for maximum absorption wavelength (λ_{max}), the next 250 mL was studied in EC process and the last 200 mL for recovering experiment.

(a-2) Electrocoagulation procedure

Two aluminum plates (dimensions 15 x 4 cm) were used as electrodes. These were spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred aqueous solution (250 mL) of standard curcumin in a 400-mL beaker. Direct current (0.3 A, 28.2-30.1 V) from the DC power supplier was then passed through the solution. Every 15 min during a 2 hour period of electrolysis, a 4 mL sample of the solution was withdrawn, centrifuged and taken for an absorbance measurement at an appropriate wavelength.

The measured absorbance was then converted into the residual weight percentage of the compound by a calibration curve obtained from a plot of the absorbance versus the concentration for each compound.

(a-3) Recovery of curcumin

A 250 mL, 0.048% w/v standard solution of curcumin was prepared in 85% aqueous ethanol and placed in 400-mL beaker. Two aluminium plates (15.0 X 4.0 x 0.05 cm.) were used as electrodes. These were dipped 3 cm apart and 5.5 cm deep into the magnetically-stirred solution then sodium chloride 0.5 g was added as a supporting electrolyte. Direct current (0.3 A, 30.1 V) was then passed through the solution *via* the two electrodes for 30 min.

The resulting mixture was filtered**,** the filtrate was evaporated and the solid residue was dissolved in absolute ethanol. Sodium chloride was then filtered off from the solution and after evaporating, the residue was collected. The precipitate was dissolved in 10% hydrochloric acid solution. The obtained acidic solution was extracted with 1-butanol. The alcoholic solution was evaporated and the solid compound was collected as a recovered curcumin, which was taken for determination of melting point, TLC and IR.

(b) Electrocoagulation of curcumin from turmeric

Extraction of curcumin from turmeric

Dried powder turmeric (78.22 g) was extracted with hexane for 72 hr in a Soxhlet apparatus to remove lipids, after that 85% aqueous ethanol was replaced for hexane and re-extraction was done for another 72 hr. The combined brown solution obtained was evaporated and dried under vacuum and the sticky brown residue was collected.

A 0.2% w/v, 250 mL sample of the crude extract was prepared in 85% ethanolic solution then 0.5 g of sodium chloride was added. The solution was placed in 400-mL beaker, two aluminium plates (15.0 X 4.0 x 0.05 cm.) were used as electrodes. These were dipped 3 cm apart and 5 cm deep into the magnetically-stirred solution. Direct current (0.3 A, 29.8 V) was then passed through the solution *via* the two electrodes for 30

min. The mixture after electrocoagulation process was filtered and extracted with the same process as EC of standard curcumin. The compounds obtained from both parts were taken for TLC and IR compared with authentic sample.

3.3.3 Results and discussion

Model study of electrocoagulation of curcumin

A standard solution of curcumin in 85% ethanoic solution showed a visible absorption wavelength at 430 nm. A calibration curve was prepared as shown in Figure 3.8. The residual weight percentages of the compound after calibrating with standard calibration curve are shown in Table 3.6. Figure 3.9 shows the plot of residual weight percentage versus electrolysis time and Picture 3.11 shows fractions taken during 2 hr of electrolysis.

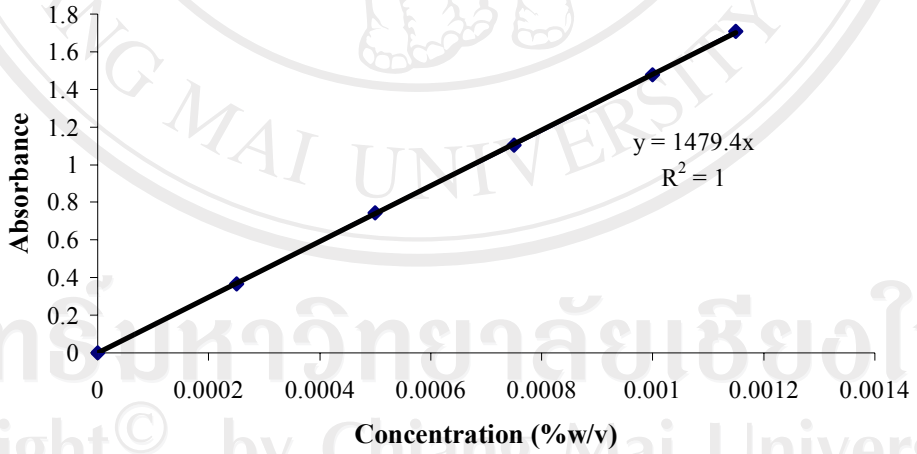


Figure 3.8 Calibration curve of standard curcumin.

Table 3.6 Residual weight percentage of curcumin during elctrocoagulation process.

	Electrolysis Time (min)								
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Residual Weight Percentage (* $x10^{-3}$)	0.01	2.39*	1.33*	0.76*	0.57*	0.48*	0.40*	0.37*	0.44*

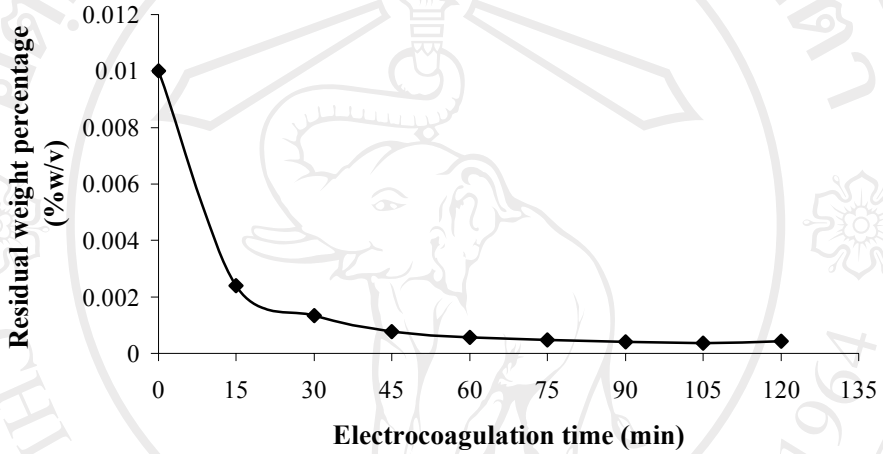
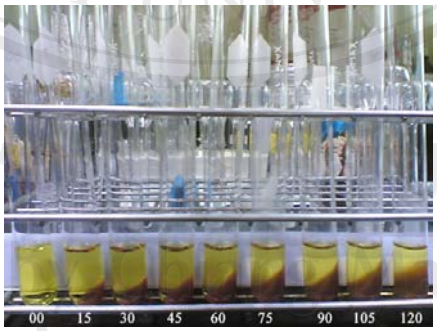


Figure 3.9 Electrocoagulation curve of standard curcumin.



Picture 3.11 EC fraction of standard curcumin.

The results show that curcumin in sample solution can be largely coagulated within 30 min, however, the coagulation is not complete. Furthermore, as shown in Picture 3.11, the bright yellow color of curcumin still appeared in the filtrate until the end of electrolysis.

After EC process 64% yield of recovered curcumin was obtained from precipitate part and 24% yield from filtrate part. The melting point of recovered curcumin from precipitate was 168^{o}C (while standard curcumin's is 172 ^{o}C). Recovered compounds were analyzed by TLC (silica gel; benzene: MeOH = 20:3 v/v), the results showed that all recovered and authentic curcurmin are identical (R_f 0.08, 0.24 and 0.28). IR spectra of standard and recovered curcumin are shown in Figure 3.10, 3.11 and 3.12 respectively.

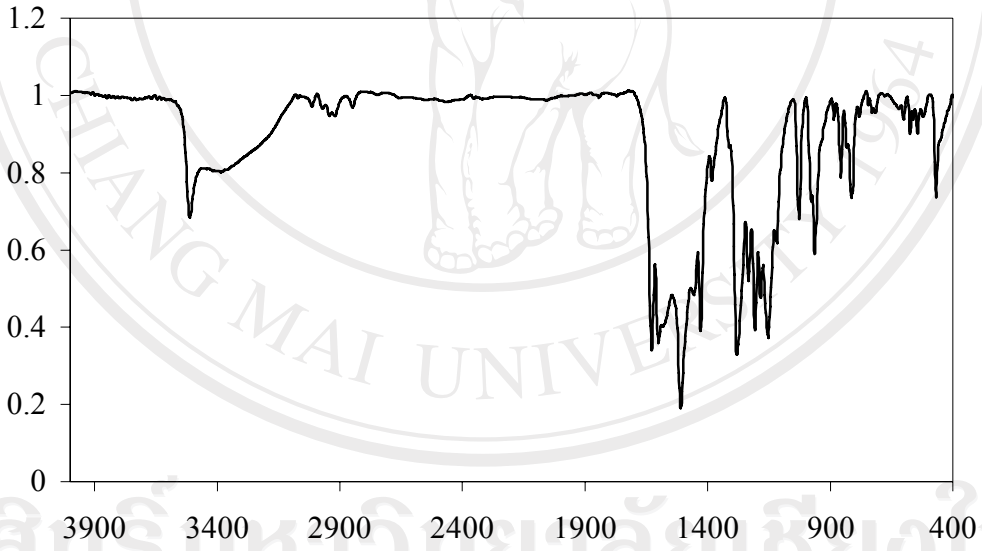


Figure 3.10 IR spectrum of standard curcumin.

IR (KBr): υ_{max} cm^{-1} 3400 (br, OH stretching), 1650, 1600 (m, C=C streching), 1645,-1620 (m→ s, C=C (conjugate with carbonyl group) streching), 1650 (v.s, C=O enol form)

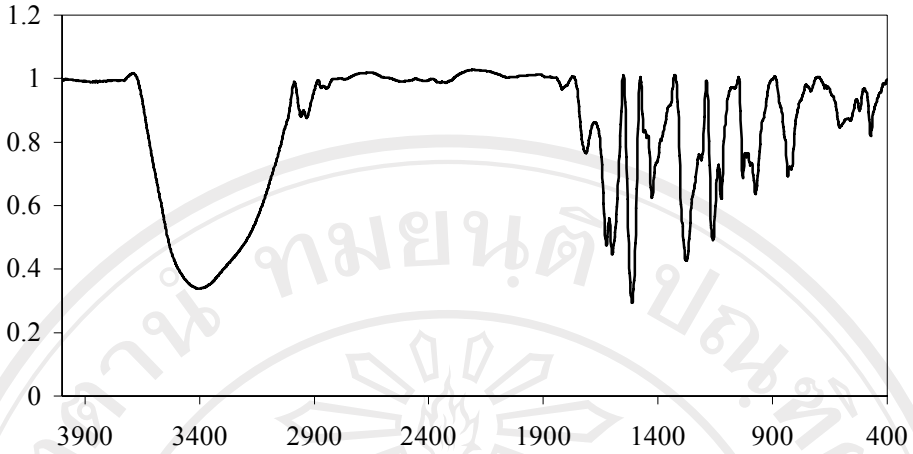


Figure 3.11 IR spectrum of recovered curcumin obtained from precipitate part after EC process.

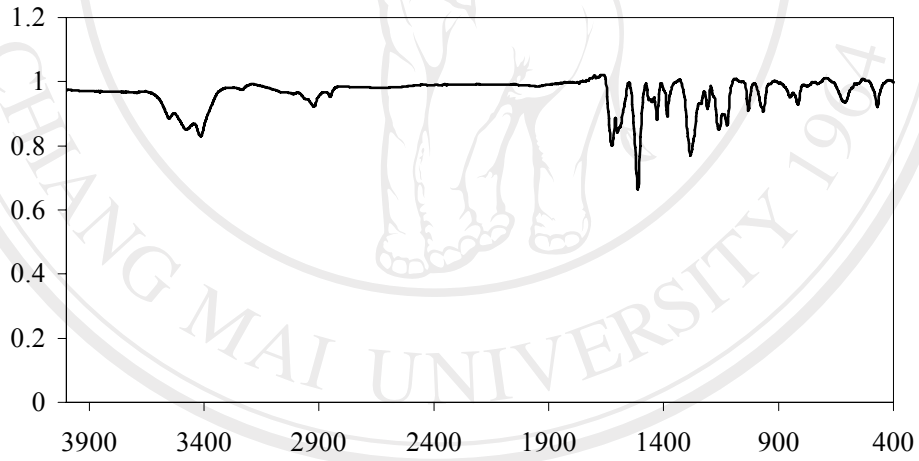


Figure 3.12 IR spectrum of recovered curcumin from filtrate part after EC process.

In experimental section b, turmeric sample was extracted by alcoholic solution and 9.12 g (11.6% yield) of crude extract was obtained. When re-dissolved in aqueous 85% ethanolic solution, the 0.2% w/v crude extract solution was then placed for 30 min of EC process and 0.21 g (42% yield) of compound was obtained from the filtrate part while 0.28 g (56% yield) of compound was obtained from the coagulated fraction.

The two recovered compounds and authentic curcumin were developed on silica gel plate (developed with benzene: methanol = 20:3 v/v). The results showed that all compounds are identical (R_f 0.10, 0.17 and 0.30).

However, IR spectrum pattern of both EC samples was different from the authentic one, as shown in Figure 3.13

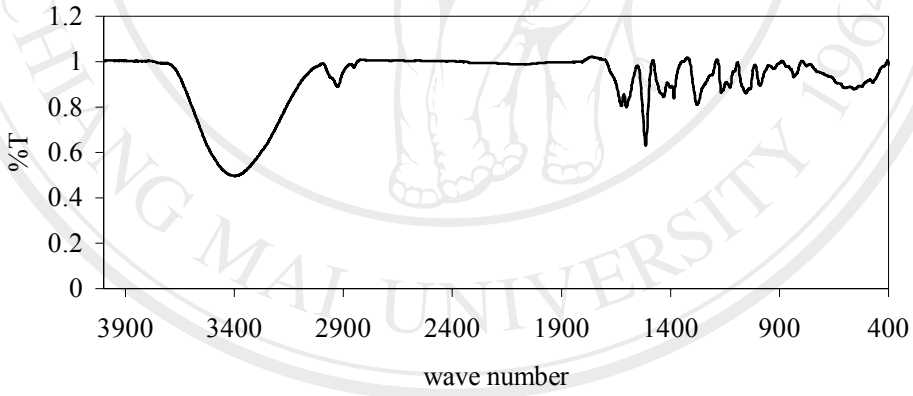


Figure 3.13 IR spectrum of recovered compound from EC filtrate of turmeric.

From the previous studies (chapter 2), polyphenolic compounds such as quinones and flavonoids with two (or more) adjacent hydroxyl group, or at least hydroxyl group flanking carbonyl carbon could be coagulated well. When the model study electrocoaglation of curcumin was investigated, curcumin which has two separate hydroxyl group in their molecule could not coagulated completely. The amount of

recovered curcumin from coagulated was two times more than those from filtrate. This coagulation seems to occur via a physical adsorption of aluminium hydroxide gel.

3.3.4 Conclusions

Among various sample preparations of curcumin from turmeric sample, the results were nearly the same as authentic sample. Curcumin was found both in filtrate and coagulate. However, IR pattern of recovered filtrate compound was not identical to the authentic one.

3.4 Isolation of morin from jackfruit hard wood by electrocoagulation

3.4.1 Introduction

The jackfruit, *Artocarpus heterophyllus* Lam., of the family Moraceae, is also called jak-fruit, jak, jaca, and in Malaysia and the Philippines, *nangka*; in Thailand, *khanun*; in Cambodia, *khnor*; in Laos, *mak mi* or *may mi*; in Vietnam, *mit*. The tree is 30 to 70 ft (9-21 m) tall, with evergreen, alternate, glossy, somewhat leathery leaves up to 9 inch (22.5 cm) long, oval on mature wood, sometimes oblong or deeply lobed on young shoots. All parts contain sticky, white latex. Short, stout flowering twigs emerge from the trunk and large branches, or even from the soil-covered base of very old trees.

Jackwood is an important timber, it changes with age from orange or yellow to brown or dark-red; is termite proof, fairly resistant to fungal and bacterial decay, seasons without difficulty, resembles mahogany and is superior to teak for furniture, construction, turnery, masts, oars, implements, brush backs and music instruments. Besides the yellow colorant, *morin*, the wood contains the colorless *cyanomaclurin* and a new yellow coloring matter, *artocarpin*, was reported by workers in Bombay in 1955. Six other flavonoids have been isolated at the National Chemical Laboratory, Poona.[36]

In Thailand jackfruit hard wood is a natural colorant which has been used as a dyeing material. In 2004, Kongsiriraung [37], studied a cotton dyeing from safflower and jackfruit core. Extraction condition by ratio of jackfruit core to water = 1:20 at 100 ˚C, 1 hour was used. The solution was in yellow shade. The moderate pH value was in the range of 7.20 – 7.90. The mordant was used in two ways, pre-mordanting and after – mordanting. Colorfastness to washing was tested. The results when compared with gray scale showed that the cotton dyed by jackfruit core and potassium dichromate as an after – mordant had a good level of fixation.

As indicated in the previous studies, morin could be coagulated by electrocoagulation method (chapter 2). In this experiment section, electrocoagulation was applied to extract morin from jackfruit core sample. The final recovery extract was compared with the reference compound.

3.4.2 Experiment

3.4.2.1 Plant material

Jackfruit wood was obtained from local area of Chiang Mai, Thailand.

3.4.2.2 Instrument and apparatus

As indicated in 3.1.1.2

3.4.2.3 Chemicals

(1) Morin (3,5,7,2',4'-pentahydroxyflavone) $C_{15}H_{10}O_7$; May&Baker Ltd. Dagenham, England.

(2) *n*-Butanol $C_4H_{10}O$ (AR grade); Fisher Scientific UK Ltd., UK.

(3) Hydrochloric acid solution HCl (37%); Carlo Erba Reagent Co., Ronando, MI, Italy.

(4) Ethanol C_2H_5OH (Absolute); E. Merck Darmstadt, Germany.

(5) Sodium chloride NaCl (99.9%, AR grade); Ajax Chemical Co., Sydney, Australia.

3.4.2.4 Procedure

Electrocoagulation and recovery of morin from jackfruit wood

A 35 g sample of dried small pieces of jackfruit wood was extracted with absolute ethanol (600 mL) using Soxhlet apparatus for 12 hr (or until the colorless solution was obtained). Finally the 485 mL of extractant was collected. Distillated water was added to an extraction solution and made up to a final volume of 250 mL. This solution was crude extract in 85% ethanolic solution.

The solution was placed in 400-mL beaker, two aluminium plates (15.0 x 4.0 x 0.05 cm.) were used as electrodes. These were dipped 3 cm apart and 5 cm deep into the magnetically-stirred solution. Direct current (0.3 A) was then passed through the solution *via* the two electrodes for 30 min. The mixture was filtered, the precipitate was collected while the same condition of EC process was repeated for another 30 min with the filtrate.

The combined coagulum was dissolved in 50 mL of 10% hydrochloric acid solution. The obtained acidic solution was extracted with 30 mL of *n*-butanol. Alcoholic solution was evaporated, the solid compound was collected and then washed with a small amount of 10% hydrochloric acid solution. After filtration the solid compound was obtained.

The filtrate after EC process was then evaporated until dry then dissolved in absolute ethanol. Sodium chloride was then filtered off from the solution and after evaporating, the solid residue was obtained. The compounds obtained from both parts were taken for TLC and IR compared with jackfruit wood's crude extract and the authentic morin.

A 250 mL 75% aqueous solution of crude extract was prepared and placed in 400 mL beaker. The same EC process as above was repeated. The 0.4 A of direct current was selected during 30 min of the process. Extraction steps were the same as the experimentation above and obtained compounds were taken for TLC and IR compared with jackfruit wood crude extract and the authentic morin.

3.4.3 Results and discussion

After 30 min (2 times) of EC process, 0.01 g (0.064% yield) of solid compound was obtained from the precipitate, while 0.57 g (3.65% yield) of solid compound was obtained from the filtrate. TLC chromatogram (silica gel; developed with ethyl acetate: methanol: water = 100:17:13 v/v) showed that the filtrate after EC process appeared at the same position as the crude extract and authentic morin (R_f = 0.74) but different in color, while the components contained in precipitate could not be separated by this system. TLC chromatogram and IR spectra of samples are shown in Figures 3.14, 3.15 and 3.16 respectively.

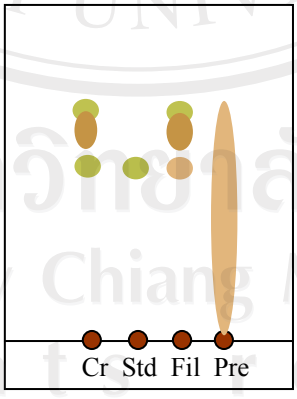


Figure 3.14 TLC chromatogram of (from left to right) jackfruit wood crude extract, authentic morin, EC filtrate and precipitate of the crude extract.

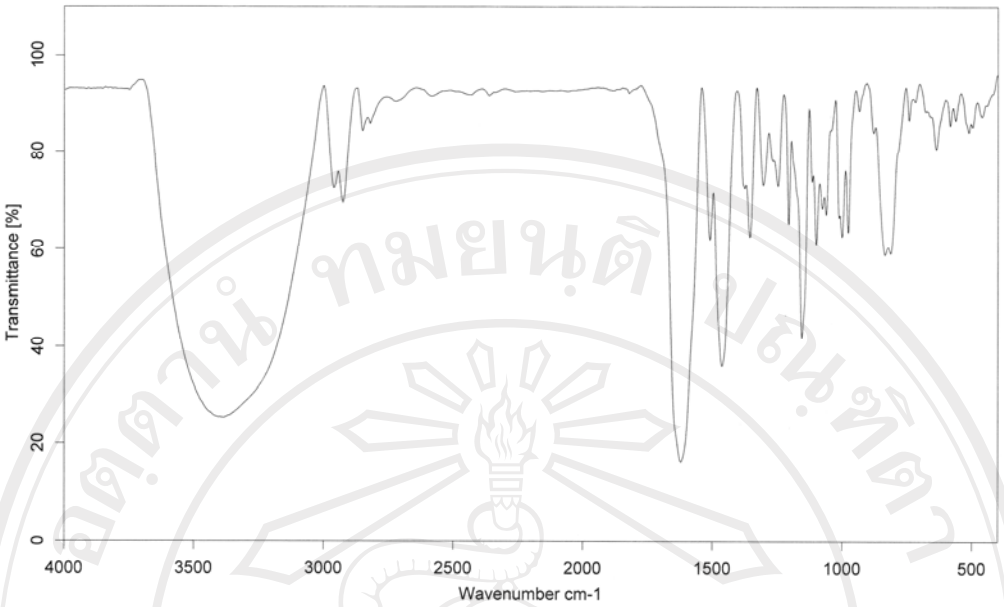


Figure 3.15 IR spectrum of recovered compound from EC filtrate of jackfruit wood (85% ethanol).

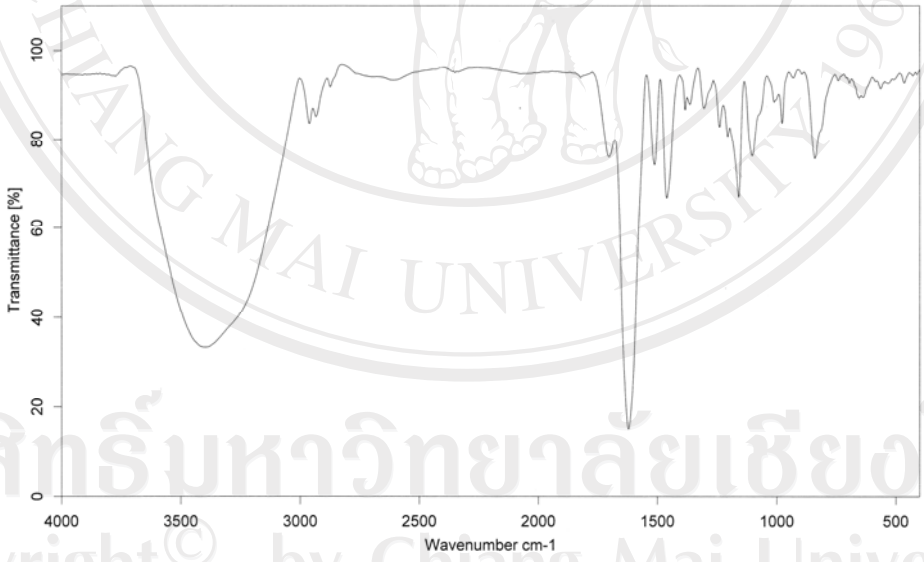


Figure 3.16 IR spectrum of recovered compound from EC precipitate of jackfruit wood (85% ethanol).

When higher current (0.4 A) was employed in EC process 0.21 g (1.3% yield) of compound was obtained from the precipitate, while 0.32 g (2.1% yield) of compound was obtained from the filtrate. However, when all compounds were analyzed by TLC, the results were the same as previous experiment.

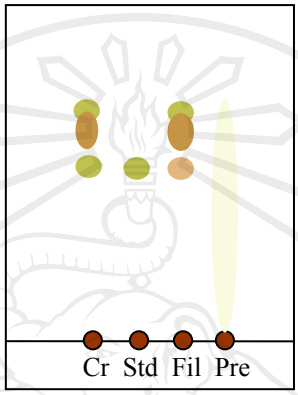


Figure 3.17 TLC chromatogram of (from left to right) jackfruit wood crude extract, authentic morin, EC filtrate and precipitate of the crude extract.(0.4 A direct current)

IR spectra of recovered compounds are shown in Figures 3.18-3.19 in comparison with standard morin in Figure 3.20.

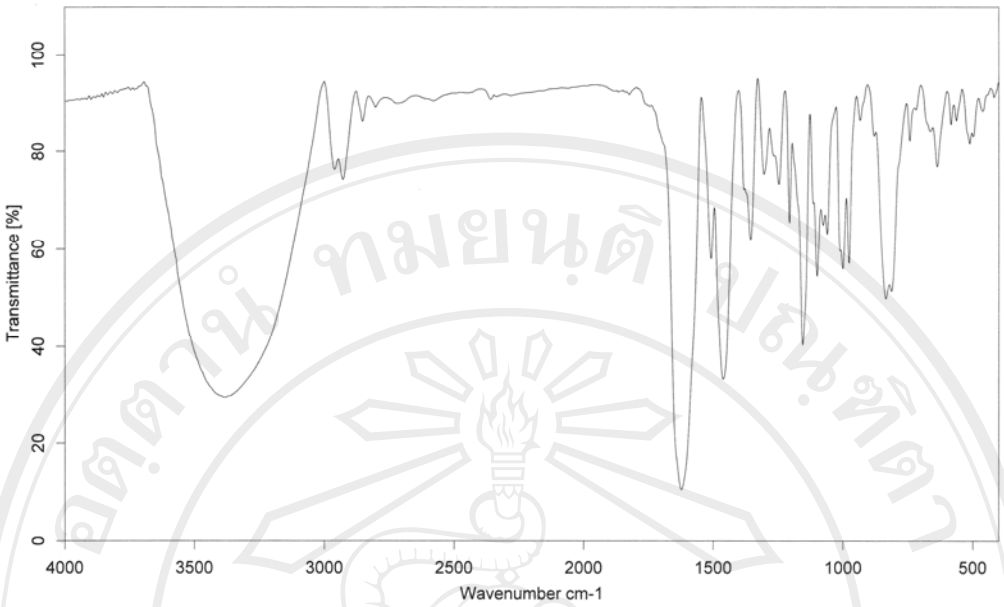


Figure 3.18 IR spectrum of recovered compound from EC filtrate of jack fruit wood (75% ethanol).

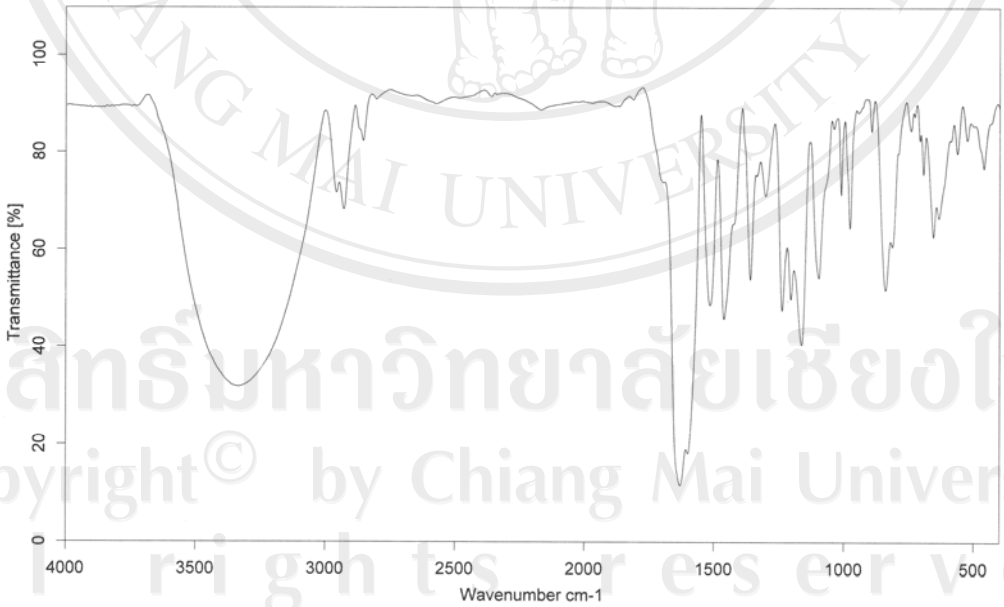


Figure 3.19 IR spectrum of recovered compound from EC precipitate of jackfruit wood (75% ethanol).

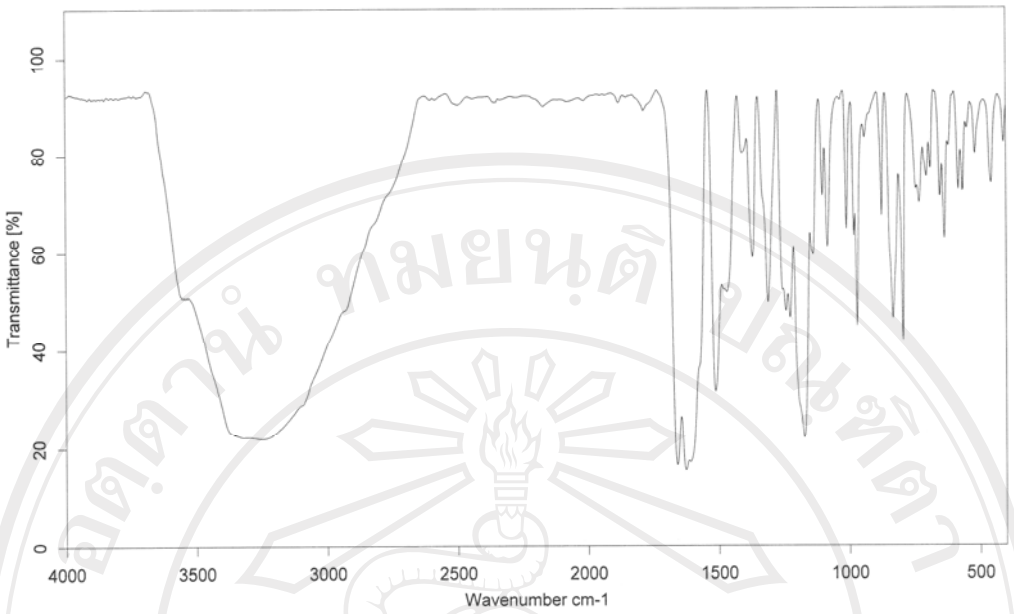


Figure 3.20 IR spectrum of standard morin.

IR (KBr): υ_{max} cm^{-1} 3300 (br, OH stretching), 1700 (s, C=O streching), 1160 (s, C-O stretching), 810-750 (v.s, metadisubstituted), 725-680 (m → s, metadisubsituted)

3.4.4 Conclusions

When electrocoagulation was applied to the isolation of morin from jackfruit wood, the results show that electrolysis recovered compound is not identical to the uncoagulated reference.

3.5 Electrocoagulation of Roselle

3.5.1 Introduction

Anthocyanins, the biggest group of water soluble natural pigments in plants, are responsible for the attractive colors of flowers, fruits and vegetables, contributing largely to the aesthetic quality of plant-derived products. These polyphenolic substances are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts. There is widespread interest in anthocyanin application and their degradation reactions in many fields of science. For example, the most important industrial application of plant anthocyanin research is in the food industry, and especially for wine commercialization. Another major interest of the food industry is in their use as natural colorants to replace synthetic red dyes. Recently, the biological activities of anthocyanin materials, such as their antioxidant activities, ability to protect from atherosclerosis and anti-carcinogenic activity have been investigated. These material are shown to have some beneficial effects in the treatment of diseases.

Anthocyanin exhibit greater stability under acidic conditions, but under normal processing and storage conditions readily convert to colorless derivatives and subsequently to insoluble brown pigments. The chemical nature of an anthocyanin, its concentration, the presence of pigment mixtures, the pH, the presence of compounds known as copigments and sometimes the presence of certain metal ions seem to be the major factors influencing the color of anthocyanins.[38]

Roselle (*Hibiscus sabdariffa* L.), an annual shrub, is commonly used to make jellies, jams and beverages. The brilliant red color and unique flavor make it a valuable food product. The anthocyanin pigments that create the color are responsible for the wide range of coloring in many foods.[39]

Hua, Y. (2005)[40] studied on extraction and purification of roselle calyx main red pigment. In this study, the roselle calyx red pigment was extracted and purified by paper chromatography. The results showed that the roselle calyx pigment was a kind of anthocyanin. The pigment would appear in purplish red, light red and light orange color spots when the pigment was analyzed by paper chromatography. The extraction yield of the purplish red pigment was 0.704 %, accounting for 46.93% of the total pigment content.

In this experiment, electrocoagulation was applied to isolating coloring matter from roselle in aqueous and aqueous alcoholic solution.

3.5.2 Experiment

3.5.2.1 Plant material

Dried-blended roselle sample was purchased from Rajawadee garden, Chiang Mai, Thailand.

3.5.2.2 Instrument and apparatus

As indicated in 3.1.1.2

3.5.2.3 Chemicals

(1) *n*-Butanol $C_4H_{10}O$ (AR grade); Fisher Scientific UK Ltd., UK.

(2) Hydrochloric acid solution HCl (37%); Carlo Erba Reagent Co., Ronando, MI, Italy.

(3) Ethanol C_2H_5OH (absolute); E. Merck Darmstadt, Germany.

(4) Sodium chloride NaCl (99.9%, AR grade); Ajax Chemical Co., Sydney, Australia.

3.5.2.4 Procedure

(a) Extraction of roselle sample

Dried-blended roselle sample (27 g) was extracted by 300 mL of hot distilled water (60 ^{o}C). After 30 min the mixture was filtered and roselle sample was re-extracted until colourless solution was obtained. When the extraction completed the red solution was collected and adjusted volume to 600 mL by distilled water.

(b) Electrocoagulation of roselle

Sodium chloride (0.5 g) was added to 250 mL of extractant, a small quantity of this solution was taken for UV-Vis absorbance (190-800 nm) measurement.

Direct current (0.5 A, 7.6 – 8.4 V) was then passed through the solution via the two aluminium plates electrodes (15 x 4 x 0.05 cm spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred solution) for 2 hr. Every 15 min during a period of electrolysis, a 4 mL sample of the solution was withdrawn, centrifuged and taken for an absorbance measurement at an appropriate wavelength (520 and 530 nm), then measured absorbance was plotted with electrocoagulation time.

The resulting mixture was filtered through a Buchner funnel. The filtrate was evaporated and dried in vacuum and the green solid compound was obtained. A small quantity of compound was dissolved in distilled water then measured for the UV-Vis absorbance and taken for TLC test (silica gel; *n*-butanol : glacial acetic acid: water = 40 : 10 : 20).

The same experiment was repeated with 2.5 A direct current and 1.5 hr of electrolysis. The precipitate was collected and extraction steps were the same as previous experimentation and obtained compounds were taken for TLC compared with the crude extract of roselle.

3.5.3 Results and discussion

After the electrolysis was operated, fractions of roselle solution were measured for absorbance at 520 and 530 nm. The first wavelength was proposed by Tsaia, P.J. *et.al*,[39] the latter obtained from the experiment. The absorbance of solution at definite time is shown in Table 3.7. The plot of absorbance and electrolysis time of roselle extracted solution and the fractions taken during a 2 hr period of electrolysis are shown in Figure 3.21 and Picture 3.12 respectively.

Table 3.7 Absorbance of roselle solution during elctrocoagulation process (0.5 A).

	Electrolysis Time (min)								
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Absorbance (520 nm)	2.46	2.20	2.06	1.95	2.26	2.15	2.16	2.08	2.06
Absorbance (530 nm)	2.47	2.08	2.00	1.91	2.44	2.39	2.33	2.30	2.27

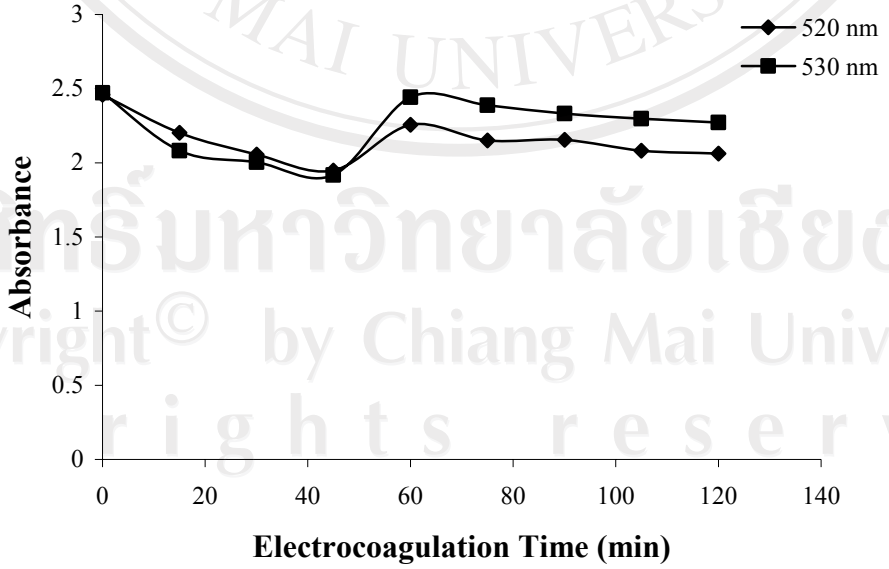
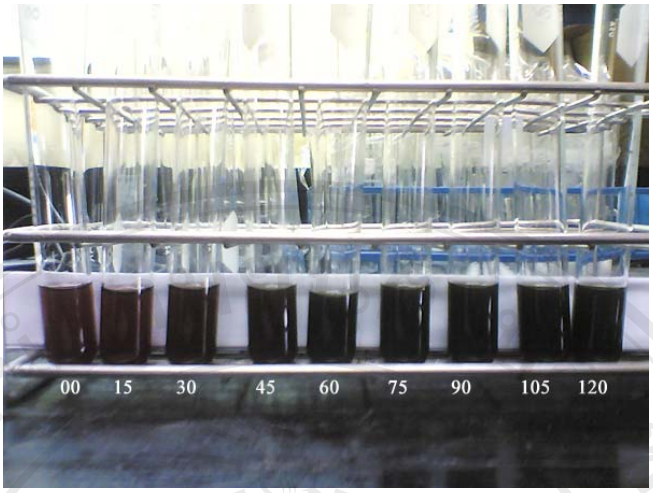


Figure 3.21 Electrocoagulation curve of roselle solution (aqueous, 0.5 A).



Picture 3.12 EC fractions of roselle (aqueous, 0.5 A)

A green solid compound (5.69 g, 50.6 % yield) was obtained from the filtrate. The aqueous solution of this compound showed the absorbance at 205 (λ_{max}), 585 and 770 nm. The small amount of coagulum was obtained but could not be weighted.

When higher current was selected (2.5 A) for electrocoagulation process, the result shows that the pale yellow solution was obtained after electrocoagulation. The absorbance of filtrate during electrolysis is shown in Table 3.8. The plots of absorbance and electrolysis time and fractions taken during 1.5 hr electrolysis are shown in Figure 3.22 and Picture 3.13, respectively.

Table 3.8 Absorbance of roselle solution during elctrocoagulation process (2.5 A).

	Electrolysis Time (min)						
	0	**15**	**30**	**45**	**60**	**75**	**90**
Absorbance (520 nm)	2.29	2.09	1.56	1.11	1.03	0.66	0.05
Absorbance (530 nm)	2.30	2.13	1.51	1.14	1.17	1.14	0.04

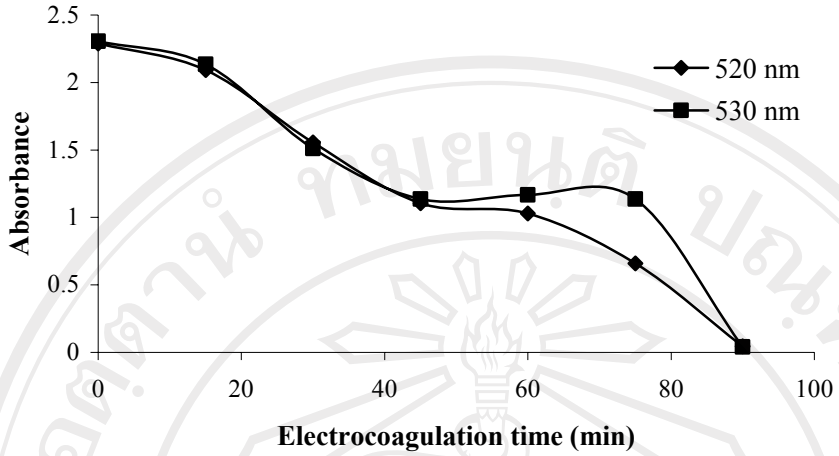
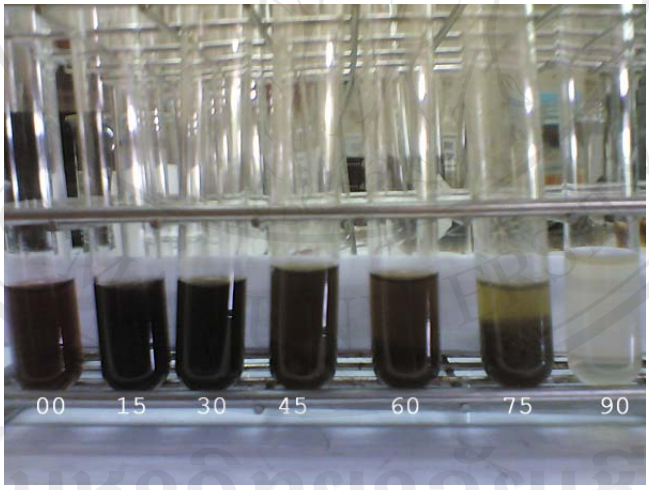


Figure 3.22 Electrocoagulation curve of roselle (aqueous, 2.5 A, 1.5 hr)



Picture 3.13 Electrocoagulation fractions of Roselle (aqueous, 2.5 A, 1.5 hr)

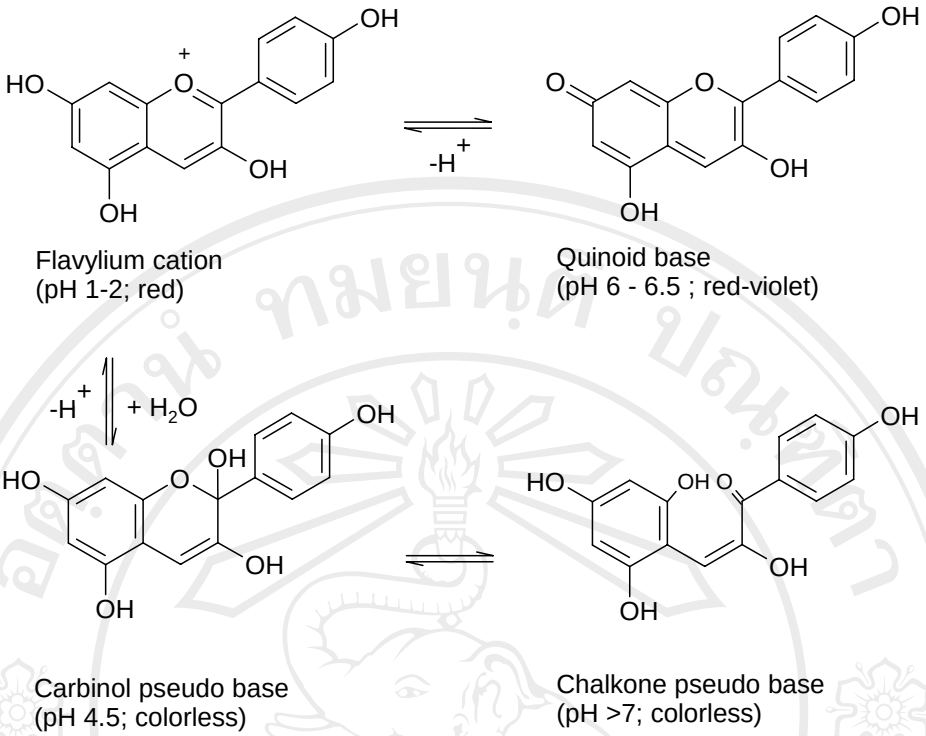
A solid compound (1.12 g, 9.7% yields) was obtained from the dark-green precipitate after EC process. This compound showed the spot in the same area as crude extract when analyzed by TLC (R_f 0.30-0.42 (green) and 0.50-0.64 (yellow)).

Precipitate and filtrate were obtained from EC process of roselle extract as shown in Picture 3.14.

Picture 3.14 Precipitate (left) and filtrate (right) of roselle solution after electrolysis.

From the studies above, rosell aqueous solution could be coagulated completely by a high current (2.5 A) electrocoagulation. However, the color changing of solution was interesting. The color of a solution strongly changed from red to green and finally colorless. This behavior refers to the changing in structure of anthocyanin content.

Anthocyanins give colors from blue through various shades to red. The content of anthocyanin is often low but can amount to up to a third of the dry weight. The anthocyanins are glycosides, the actual chromophores of which are their aglycones, the anthocyanidins. A common feature of all known anthocyanidins is the skeleton of a C-4'-hydroxylated 2-phenylchromene, existing primarily as a flavylium cation. Secondary structure such as quinoid bases and carbinol or chalcone pseudobases occur in aqueous solution in dependence on pH value (as shown below).[41]



3.5.4 Conclusions

The coloring matter of roselle could be extracted in both aqueous and aqueous alcoholic solution. However, only the electrocoagulation in an aqueous solution gave a coagulum. Furthermore, after filtering off the unexpected dark-green precipitate formed, the remaining colorless electrolysed solution was obtained.

3.6 Electrocoagulation of beet root

3.6.1 Introduction

Red beet (*Beta* v*ulgaris* L.) is an excellent source of nitrogenous pigments, the betalains, mainly being composed of two red-violet betacyanins, betanin and isobetanin and minor yellow betaxanthins. Betanin is also known for its nontoxic properties, and red beet has been the subject of much experimental interest in using the red pigment in the pharmaceutical and food industries.[42]

Betalains are of great taxonomic significance in higher plants. The presence of betalains in members of the order Caryophyllales has been an important criterion for their classification.[43]

In this experimental section, electrocoagulation of beet root in aqueous and aqueous alcoholic solution was investigated.

3.6.2 Experiment

3.6.2.1 Plant material

Fresh beet root sample was purchased from local market in Chiang Mai, Thailand.

3.6.2.2 Chemicals

(1) *n*-Butanol $C_4H_{10}O$ (AR grade); Fisher Scientific UK Ltd, UK.

(2) Hydrochloric acid solution HCl (37%); Carlo Erba Reagent Co. Ronando, MI, Italy.

(3) Ethanol C_2H_5OH (absolute); E. Merck Darmstadt, Germany.

(4) Sodium chloride NaCl (99.9%, AR grade); Ajax Chemical Co., Sydney, Australia.

3.6.2.3 Procedure

(a) Electrocoagulation of beet root; ethanolic extraction

Fresh beet root (85 g) was blended and placed in a conical flask. Absolute ethanol (500 mL) was added and the mixture was stirred overnight at room temperature. After filtering, the ethanolic solution was adjusted to 500 mL by absolute ethanol and prepared for electrolysis experimentation.

Distilled water was added to a 125 mL of the sample solution to make a final volume of 250 mL and 50% aqueous ethanolic concentration. sodium chloride (0.5 g) was added and a small quantity of this solution was taken for UV-Vis absorbance (190-800 nm) measurement.

Direct current (0.8 A, 21.0 – 28.6 V) was then passed through the solution via the two aluminium plates electrodes (15 x 4 x 0.05 cm, spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred solution) for 2 hr. Every 15 min during a period of electrolysis, a 4 mL sample of the solution was withdrawn, centrifuged and taken for an absorbance measurement at an appropriate wavelength (475 and 530 nm) and then plotted with electrocoagulation time.

The resulting mixture was filtered through a Buchner funnel. The filtrate was evaporated and dried in vacuum. The precipitate was dissolved with 10% hydrochloric acid and then extracted with *n*-butanol. Alcoholic solution was evaporated and the solid residue was collected.

A small quantity of obtained compounds was dissolved in the appropriate solvent and taken for TLC test compared with the crude extract.

(b) Electrocoagulation of beet root; aqueous extraction

Distilled water (400 mL) was added to 148 g of fresh blended beet root. The mixture was stirred for 1 hour at room temperature. After filtering the filtrate was collected and beet root sample was re-extracted with 450 mL of distilled water. The bright red filtrate from both extractions was combined.

Sodium chloride (0.5 g) was added into 250 mL of the sample solution. A small quantity of solution was taken for UV-Vis absorbance measurement. The solution was placed in a 400 mL beaker, direct current (1.8 A, 20.7 – 28.3 V) was then passed through the solution via the two aluminium plates electrodes (15 x 4 x 0.05 cm spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred solution) for 2 hr. Every 15 min during a period of electrolysis, a 4 mL sample of the solution was withdrawn, centrifuged and taken for an absorbance measurement at an appropriate wavelength (535 nm) and then plotted with electrocoagulation time.

3.6.3 Results and discussion

When the extracted solution of fresh beet root sample was adjusted to a 250 mL, 50% ethanolic solution by distilled water, the 475 and 530 nm absorption wavelength were obtained from this bright red solution. The absorbances of the solution during electrolysis are shown in Table 3.9. The plot between absorbance and electrolysis time is shown in Figure 3.23

Table 3.9 Absorbance of beet root aqueous alcoholic solution during elctrocoagulation process.

	Electrolysis Time (min)								
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Absorbance (475 nm)	2.15	0.80	0.54	0.40	0.29	0.32	0.31	0.09	0.06
Absorbance (530 nm)	1.17	0.25	0.14	0.11	0.19	0.22	0.24	0.06	0.04

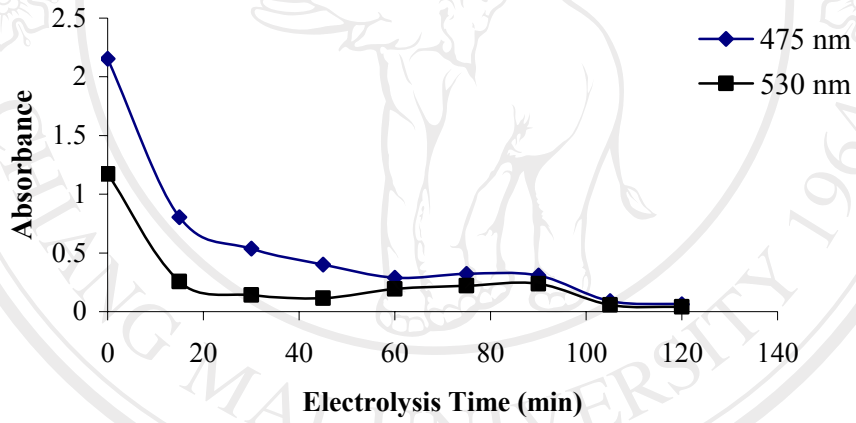
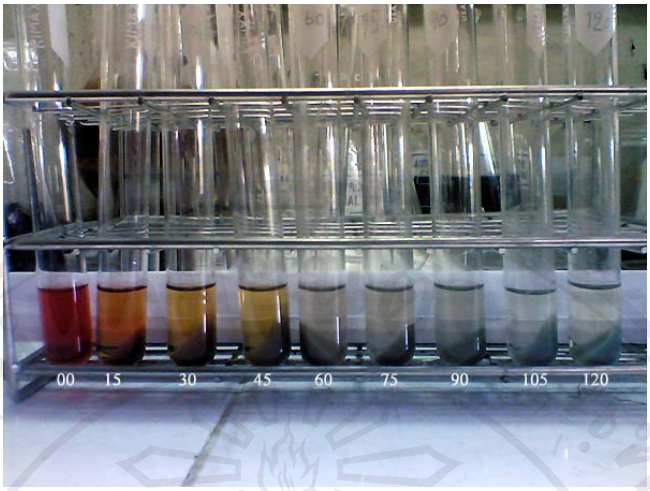


Figure 3.23 Electrocoagulation curve of beet root solution (50% EtOH, 2 hr)



Picture 3.15 EC fractions of beet root (50% EtOH, 2 hr)

Picture 3.15 shows electrolysis fractions of beet root in 50% alcoholic solution. As illustrated in this picture, a dark-brown compound was obtained from the precipitate. Furthermore, the filtrate was changed to colorless after electrocoagulation process.

In aqueous solution, dark-red color of beet root sample was obtained. This solution shows the maximum absorption wavelength at 535 nm. The absorbance at this wavelength was measured during electrolysis process as shown in Table 3.10. The plot between absorbance and electrolysis time is shown in Figure 3.24 and fractions taken during electrolysis are shown in Picture 3.16.

Table 3.10 Absorbance of beet root aqueous solution during elctrocoagulation process.

	Electrolysis Time (min)								
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Absorbance (535 nm)	0.93	0.35	0.21	0.18	0.15	0.13	0.13	0.12	0.14

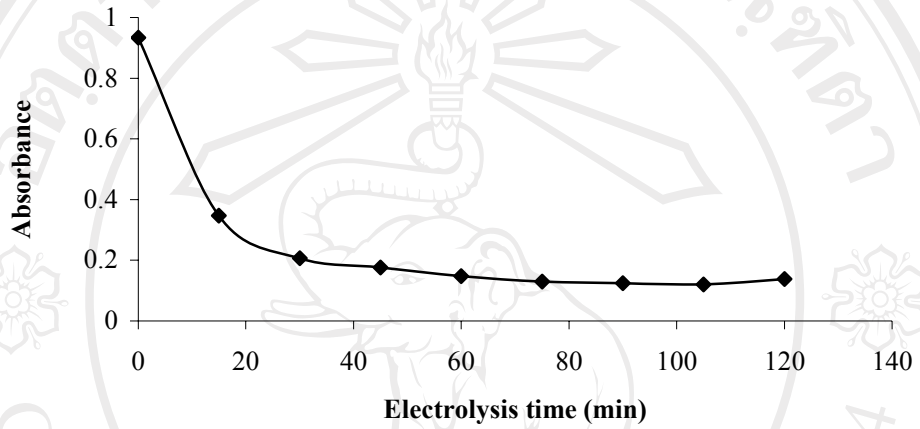
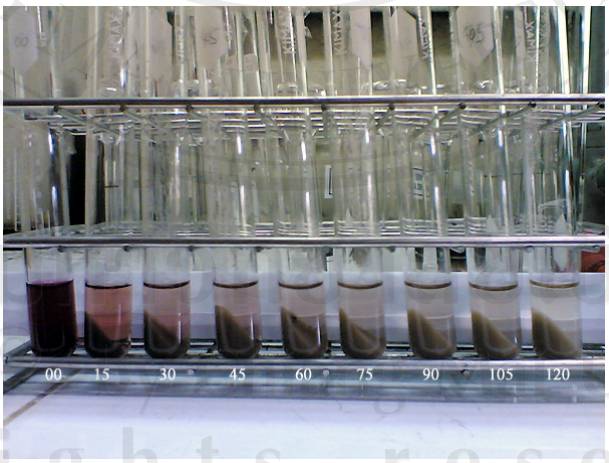


Figure 3.24 Electrocoagulation curve of beet root (aqueous, 2 hr)



Picture 3.16 EC fractions of beet root aqueous solution during 2 hr of EC process.

3.6.4 Conclusions

When electrocoagulation process of beet root solution was investigated, the coloring matter can be coagulated within 60 min of electrolysis both in aqueous and aqueous-alcoholic solution. After filtering off the precipitate, the remaining filtrate appeared as colorless solution.

3.7 Isolation of crocin from gardenia by electrocoagulation.

3.7.1 Introduction

Gardenia fruit (*Gardenia jasminoides* Ellis.) is widely used in Asian countries as a natural colorant, and as a traditional Chinese medicine for its homeostatic, antiphlogistic, analgesic and antipyretic effects. The extracts of gardenia fruit can give yellow, red and blue colors, and is used in many foods such as noodles and confectioneries.[44]

The major colorant in gardenia fruit was isolated and characterized by Choi, H.J and his coworkers in 2001.[45] This study reported the extraction of ground dried gardenia fruit by methylene chloride and methanol and the identification of the yellow colorant by FAB-MS, UV/visible and NMR data. The result showed that crocin was obtained from this gardenia's yellow major colorant.

Crocin (Figure 3.25) is a carotenoid–glycosyl esters used as a colorants for textiles and food. This substance shows antioxidant quenchers and antitumor activities.

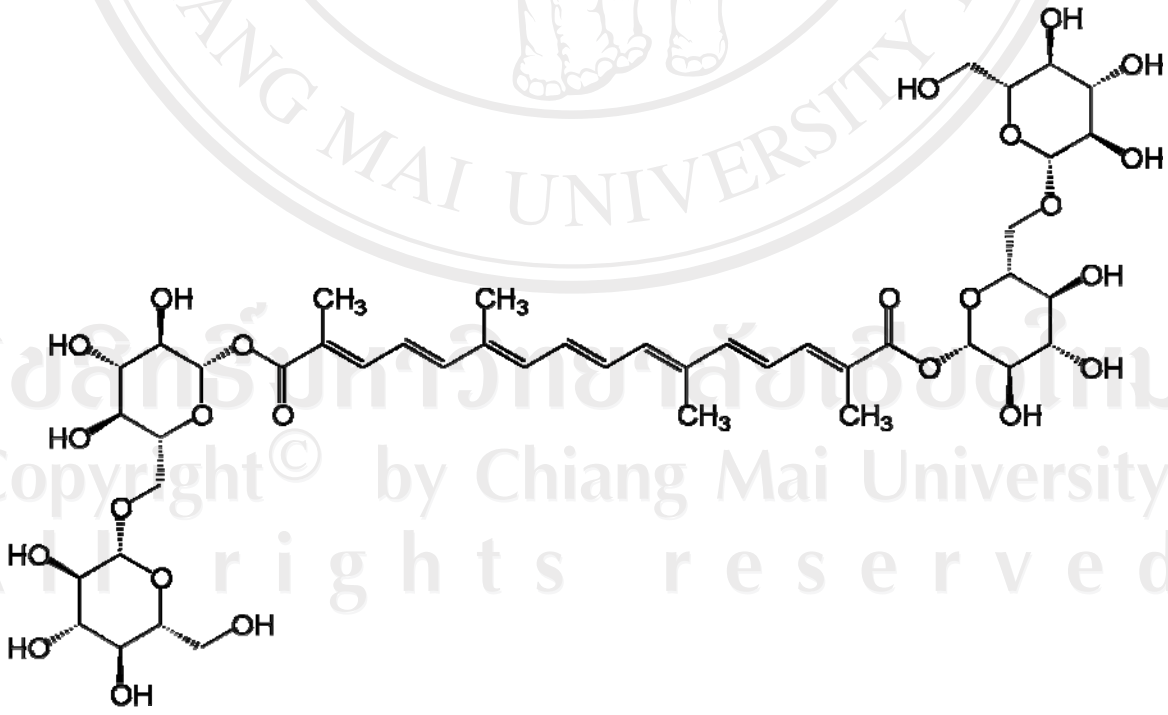


Figure 3.25 Crocin structure

In this study, electrocoagulation was applied to the isolation of crocin from gardenia fruit. The final recovery extract was compared with the reference compound

3.7.2 Experiment

3.7.2.1 Plant material

Dried gardenia fruit was obtained from local market in Chiang Mai, Thailand.

3.7.2.2 Instrument and apparatus

As indicated in 3.1.1.2

3.7.2.3 Chemicals

(1) *n*-Butanol $C_4H_{10}O$ (AR grade); Fisher Scientific UK Ltd., UK.

(2) Hydrochloric acid solution HCl (37%); Carlo Erba Reagent Co. Ronando, MI, Italy.

(3) Ethanol C_2H_5OH (Absolute); E. Merck Darmstadt, Germany.

(4) Sodium chloride NaCl (99.9%, AR grade); Ajax Chemical Co., Sydney, Australia.

(5) Isopropanol $C_5H_{11}O$; E. Merck Darmstadt, Germany.

3.7.2.4 Procedure

(a) Electrocoagulation of gardenia; aqueous solution

(a-1) 1.0 A current

Dried gardenia sample (60.01 g) was extracted by 500 mL of hot distilled water (60 ^{o}C). After 30 min the mixture was filtered and the sample was re-extracted with the same condition. When the extraction was completed a 985-mL solution was obtained. The solution was divided into 2 parts. The first 250 mL was studied by electrocoagulation while the rest was evaporated and dried in vacuum. The obtained crude extract was used in experiment a-2, a-3 and b.

Sodium chloride (0.5 g) was added into 250 mL of the sample solution. The solution was placed in a 400 mL beaker, direct current (1.0 A, 23.0 V) was then passed through the solution via the two aluminium plates electrodes (15 x 4 x 0.05 cm spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred solution) for 40 min.

The resulting mixture was filtered through a Buchner funnel. The filtrate was evaporated and dried in vacuum. The precipitate was dissolved with 10% hydrochloric acid and then extracted with *n*-butanol. The alcoholic solution was evaporated and the residue was collected.

A small quantity of the crude extract, filtrate and precipitate from EC process were studied and compared with standard crocin by TLC technique (silica gel, ethyl acetate: isopropanol: water = 13: 5: 2 v/v) [29b] as shown in Figure 3.19

(a-2) 0.4 A current

Gardenia crude extract (5.1 g; from experiment a-1) was dissolved and adjusted to final volume 250 mL by distilled water. Sodium chloride (0.52 g) was added to the sample solution. The solution was placed in a 400 mL beaker, direct current (0.4 A, 7.0-7.3 V) was then passed through the solution via the two aluminium plates electrodes (15 x 4 x 0.05 cm spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred solution) for 20 min. During the process a 2-3 drops of reducing bubble agent was used.

The resulting mixture was filtered through a Buchner funnel. The filtrate was evaporated. The residue was dissolved in absolute ethanol and the remaining undissolved inorganic matter was then filtered off from the solution.

(a-3) 0.6 A current

Gardenia crude extract (5.05 g; from experiment a-1) was dissolved and adjusted to a final volume of 250 mL by distilled water. Sodium chloride (0.52 g) was added to the sample solution. The solution was placed in a 400 mL beaker, direct current (0.6 A, 12.1 V) was then passed through the solution via the two aluminium plates electrodes (15 x 4 x 0.05 cm spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred solution) for 20 min. During the process a 2-3 drops of reducing bubble agent was used.

The resulting mixture was filtered through a Buchner funnel. The filtrate was evaporated. The residue was dissolved in absolute ethanol and the remaining undissolved inorganic matter was then filtered off from the solution.

The crude extract of gardenia, filtrates from experiment a part a-2, a-3 and experiment b were separated on TLC plate compared with standard crocin.

(b) Electrocoagulation of gardenia;ethanolic solution

Gardenia crude extract (5.02 g; from experiment a-1) was dissolved and adjusted to a final volume of 250 mL by 25% ethanolic solution. Sodium chloride (0.5 g) was added to the sample solution. The solution was placed in a 400 mL beaker, direct current (0.9 A, 20.2-29.2 V) was then passed through the solution via the two aluminium plates electrodes (15 x 4 x 0.05 cm spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred solution) for 30 min. During the process a 2-3 drops of reducing bubble agent was used.

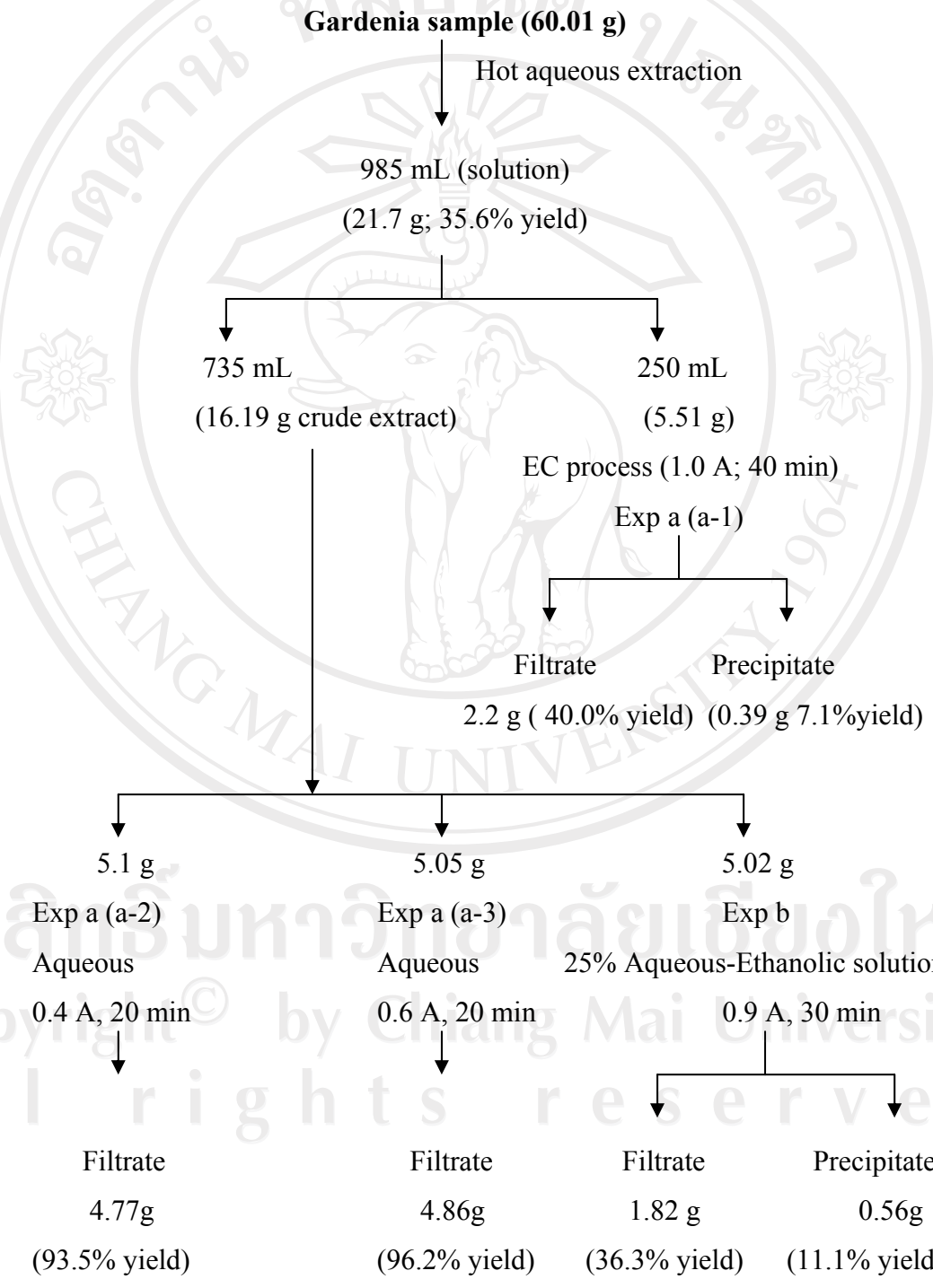
The resulting mixture was filtered through a Buchner funnel. The filtrate was evaporated. The residue was dissolved in absolute ethanol and the remaining undissolved inorganic matter was then filtered off from the solution.

The precipitate was dissolved with 10% hydrochloric acid and then extracted with *n*-butanol. Alcoholic solution was evaporated and the residue was collected.

The filtrate and precipitate from EC process was dissolved in 85% ethanol and separated on a TLC plate compared with the crude aqueous extract and standard crocin.

3.7.3 Results and discussion

Gardenia sample was extracted and separated by EC process. The weight and percentage yield of compounds from various studied conditions are shown in a flow chart below (Flowchart 1).



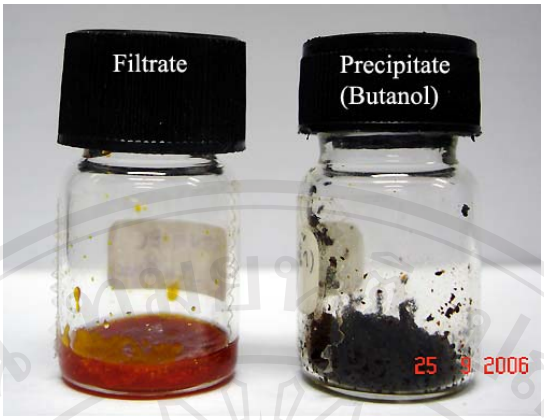
Flowchart 1 Weight and percentage yield of compounds extracted from gardenia.

Dried sample and crude extract , experimental set up of gardenia are shown in picture 3.17 and 3.18 respectively.

Picture 3.17 Gardenia samples and crude extract.

Left; gardenia sample (with peel), right; crude extract (aqueous).

Picture 3.18 EC of gardenia in aqueous solution.



Picture 3.19 Compounds obtained from filtrate (left) and precipitate (right) after EC process of gardenia in aqueous solution (1.0 A, 40 min).

When gardenia sample was extracted then separated by EC process in aqueous solution (1.0 A 40 min), the bright brown resinous compound (Picture 3.19; left) was obtained from the filtrate. For the precipitate part, a dark - high moisture solid compound (Picture 3.19; right) was obtained. These compounds were separated on TLC plate (silica gel; ethyl acetate: isopropanol: water = 13: 5: 2 v/v) compared with crude extract and authentic crocin (Figure 3.26)

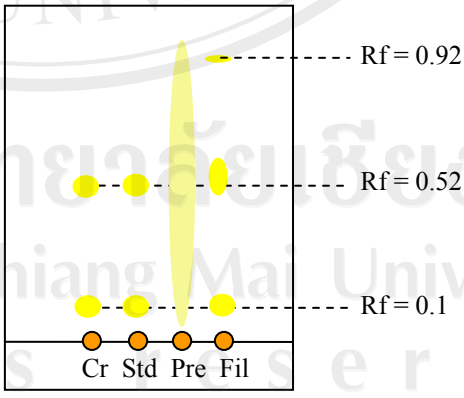
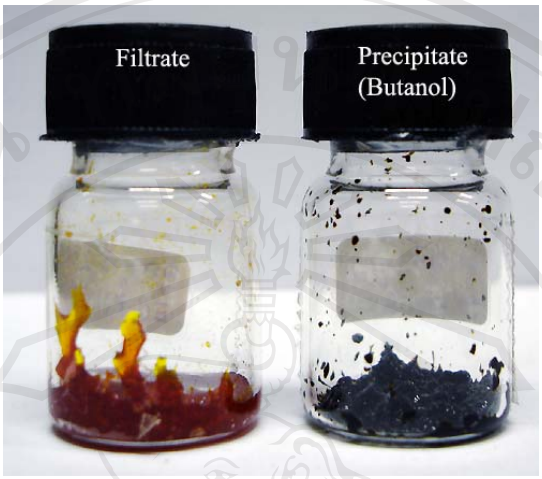


Figure 3.26 TLC chromatogram of gardenia (from left to right) crude extract, authentic crocin, EC precipitate and filtrate (aqueous solution,1.0 A, 40 min) [silica gel; ethyl acetate : isopropanol : water = 13 : 5 : 2 v/v]

The results show that crocin was found in the crude extract and filtrate after EC process also. When EC process was studied in another solvent system (25% ethanol, 0.9 A 30 minute) the results are almost the same (Picture 3.20 and Figure 3.27).



Picture 3.20 Compounds obtained from filtrate (left) and precipitate (right) after EC process of gardenia in 25% ethanolic solution.

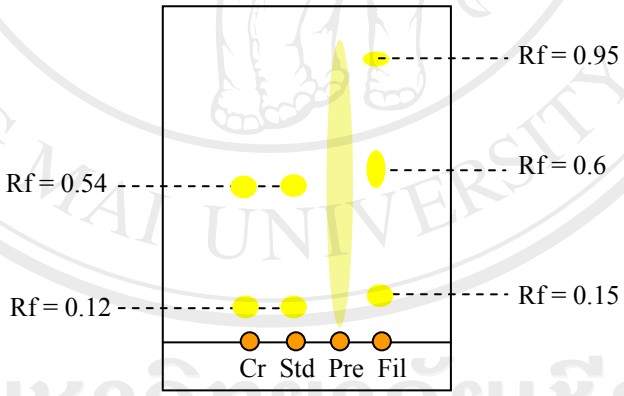


Figure 3.27 TLC chromatogram of (from left to right) gardenia crude extract, authentic crocin, EC precipitate and filtrate (ethanolic solution, 0.9 A, 30 min)

When the lower current, 0.6 and 0.4 A, was applied to the ec process of aqueous solution (experimental 1 part II and III) a small quantity of coagulum was obtained (could not weighted). Recovered compound come from filtrate part only (Picture 3.21). However, TLC chromatogram showed that crocin was founded in crude extract and filtrate from all ec conditions (Figure 3.28).

Picture 3.21 Compounds obtained from filtrate after EC process of gardenia in aqueous solution; at 0.6 A (left) and 0.4 A (right).

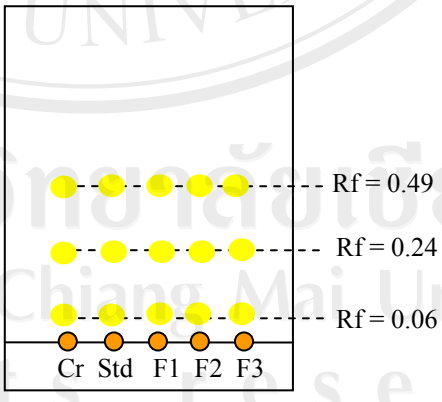


Figure 3.28 TLC chromatogram of (from left to right) gardenia crude extract, authentic crocin, EC filtrate in ethanolic solution (F1), aqueous solution 0.4A (F2) and 0.6 A (F3).

3.7.4 Conclusions

Crocin could not be coagulated by EC process in an aqueous solution except using a high current. The isolation of crocin from natural source by EC should be done by removing undesirable compounds by using a low current electrocoagulation and then recovering crocin from the filtrate.

3.8 Electrocoagulation of betel nut

3.8.1 Introduction

Areca catechu, commonly known as betel nut, is known since the pre-Christian era. It is the most commonly used drug in the world after tobacco, ethanol and caffeine. It enjoys the liking and preference of millions of people worldwide from East Africa, South Asia, Southern China and Tibet, the whole of the East and South–East Asia and down to Australia and the surrounding islands. Betel nut has, since antiquity, been used both medically and psychosomatically as a breath freshener, digestive aid, worm expellant, aphrodisiac and to maintain stamina. Betel nut has also been used in different bowel complaints and to stimulate the relaxed bowel. The chewing of the nut is known to have a sialagogue effect and is also associated with a depressive effect on the heart and a fall in the blood pressure.

The betel nut has been widely studied phytochemically and some alkaloids have been found present; those commonly known include arecoline, arecaidine, guvacoline, guvacine, arecolidine and choline. Besides, it is also known to contain phenolic compounds, such as hydroxychavicol and safrole. The presence of tannins, gallic acid, catechin, betasitosterol, gums and amino acids have also been reported. Betel nut has been extensively studied pharmacologically and has been reported to possess multiple activities. [36,37]

In this study, electrocoagulation was applied to isolate coloring matter from betel nut.

3.8.2 Experiment

3.8.2.1 Plant material

Dried betel nut sample was purchased from a local market in Chiang Mai, Thailand

3.8.2.2 Instrumental and apparatus

As indicated in 3.1.1.2

3.8.2.3 Chemicals

(1) *n*-Butanol $C_4H_{10}O$ (Analytical Reagent Grade); Fisher Scientific UK Ltd., UK.

(2) Hydrochloric acid solution HCl (37%); Carlo Erba Reagent Co. Ronando, MI, Italy.

(3) Ethanol C_2H_5OH (absolute); E. Merck Darmstadt, Germany.

(4) Sodium chloride NaCl (99.9%, AR grade); Ajax Chemical Co., Sydney, Australia.

3.8.2.4 Procedure

(a) Aqueous extraction of betel nut; High temperature

Distilled water was (250 mL) added to 12.53 g of dried and crushed betel nut. The mixture was stirred for 1 hour at 50-55 ^{o}C. After filtering the filtrate was collected then evaporated under vacuum, the brown solid residue was obtained.

(b) Aqueous extraction (room temperature) and electrocoagulation of betel nut.

Distilled water was (250 mL) added to 12.50 g of dried and crushed betel nut. The mixture was stirred for 24 hour at room temperature. After filtering the filtrate was collected.

Sodium chloride (0.5 g) was added to 250 mL of the sample solution (filtrate). The solution was placed in a 400 mL beaker, direct current (1.5 A, 28.9 V) was then passed through the solution via the two aluminium plates electrodes (15 x 4 x 0.05 cm spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred solution) for 45 min.

The resulting mixture was filtered through a Buchner funnel. The filtrate was evaporated and dried in vacuum. The precipitate was dissolved with 10% hydrochloric acid and then extracted with *n*-butanol. The alcoholic solution was evaporated and the red solid residue was collected.

(c) Aqueous extraction (high temperature) and electrocoagulation of betel nut.

Distillate water (250 mL) was added to 12.53 g of dried and crushed betel nut. The mixture was stirred for 1 hour at 50-55 ^{o}C. After filtering the filtrate was collected and extracted by the same EC procedure as in experiment b.

3.8.3 Results and discussion

Three extraction experiments of dried blended betel nut were studied and the weights of obtained compounds and percentage yields are shown in Table 3.11

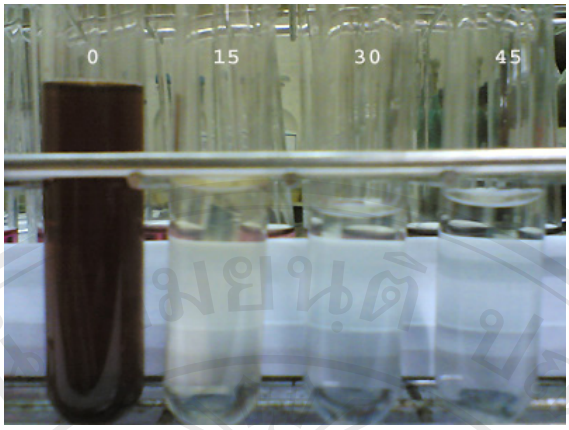
Table 3.11 Weight and percentage yield of products obtained from three extraction conditions of betel nut.

Extraction condition	Weight of product	% yield
Aqueous extraction at 50-55 ^{o}C	4.84	38.6
Aqueous extraction (T_{room}) and EC process for 45 min (precipitate part)	1.60	12.8
Aqueous extraction (50-55 ^{o}C) and EC process for 45 min (precipitate part)	1.7	13.6

Picture 3.22 shows the experimental setting for EC process of betel nut sample solution, the filtrate and the recovered compounds from electrolysis coagulum. fractions taken during electrolysis are shown in Picture 3.23.

(a) (b) (c)

Picture 3.22 Experimental setting for EC process of betel nut (a), filtrate (b) and recovered compounds from coagulum after EC process (c).



Picture 3.23 Fractions taken from EC process of betel nut (from left to right) before, 15, 30 and 45 min of electrolysis.

The results show that electrocoagulation of betel nut solution very successive to remove the coloring matter from the aqueouse solution. The recovered compounds from coagulum appeared as a bright-red concentrate color.

3.8.4 Conclusions

The coloring matter from betel nut could be completely coagulated by electrocoagulaton process in aqueous solution within 15-30 min of electrolysis time.