Detection of Pigments and Natural Colorants from Thai Herbal Plants for Possible Use as Coloring Dyes

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Abstract. Fourteen Thai herbal plants were extracted using methanol, ethanol, and acetone. Comparison of the various extraction methods for pigments and polyphenols (colorants) showed that methanol provided a more complete extraction than ethanol and acetone. Percentage yield ranged from 1.7 to 40.7 and was dependent on the plant extracted and solubility of pigments and polyphenols (colorants) extracted by the various solvents. The absorption spectra of pigments and polyphenols (colorants) in the plant extract were present in the visible region (351 to 665 nm) and the absorption spectra of polyphenols were present in the ultraviolet region (200 to 349 nm). Color of plant extracts varied and included violet–blue, yellow–green, red, orange–red, gray–purple, blue–green, gray–orange, gray–yellow, and black. High-performance liquid chromatography (HPLC) was used to separate and identify compounds of plant pigments and polyphenols (colorants). Ya-nang had the greatest number of peaks and chlorophyll A and chlorophyll B were found only in Ya-nang. Numerous pigments and polyphenols were detected. Such compounds have the potential to be used as coloring dyes.

Synthetic hair coloring products are widely used and are known to cause problems such as breakage of hair stands, loss of hair, dry scalp, risk of cancer (Zheng et al., 2002), allergic, and mutagenic and carcinogenic effects (Thun et al., 1994). Compounds considered harmful include 4-methoxy m-phenylenediamine (4MMPD) or 4-methoxy-m-phenylenediamine sulfate (4MMPD sulfate), two coal-tar ingredients (Hecht, 1978). Currently, there is a popular trend in using pigments and colorants from plant extracts as substitutes for synthetic colorants, because natural pigments and colorants are non-toxic and non-mutagenic, have desirable pharmacological properties (Lazzé et al., 2003), and are friendly to the environment. The horticulture industry in countries such as Thailand that have an abundance of herbal plants may benefit economically from culturing and marketing such plants for the extraction of plant pigments and colorants for use in the production of coloring products. The main pigment compounds of plants are carotene (golden), pheophytin (olive green), chlorophyll a (blue–green), chlorophyll b (yellow–green), lutein (yellow), xanthophylls (yellow), betalains (yellow, orange, red, purple), and anthocyanins (red). In addition, polyphenols such as tannins, flavonoids,

and anthocyanins are major plant secondary metabolites and represent the most studied phytochemicals. Harnafi and Amrani (2008) presented a simple technique to determine plant extracts such as polyphenols and used spectrophotometric assays to measure antioxidants. Plant extracts were mainly polynuclear compounds: polyphenols, tannins, flavonols and flavonols–catechin, taxopholin, and quercetin. Panayotov et al. (2006) reported that the quantities of flavonols (large group of yellow-pigmenting polyphenol compounds) contained in extracts of oak (*Quercus robur*), chestnut (*Castanea sativa*), mulberry (*Morus alba*), acacia (*Robinia pseudoacacia*), teak (*Tectona grandis*), tany (*Tanacetum vulgare*), and common rue (*Ruta graveolens*) were determined with ultraviolet spectrophotometry. Solovchenko et al. (2001) studied pigment extraction of apple peel using polar organic solvents (acetone or methanol) and a chloroform–methanol mixture and found that polar organic solvent extraction did not result in complete extraction of intact pigments. Such extracts contained amounts of light-absorbing impurities. The chloroform–methanol extraction, however, reduced the content of such impurities and increased the accuracy of the content of chlorophylls and carotenoids. In addition, the chloroform–methanol extraction method proved useful for the analysis of phenolic substances (anthocyanins and flavonoids) in the water–methanol fraction of the extracts. Although laboratory equipment and methods exist to

identify pigments and polyphenols (colorants) in plants, a limited amount of research has been conducted using herbal plants.

The objective of this study was to detect the presence of pigments and polyphenols (colorants) in herbal plants using ultraviolet-visible spectrophotometry and HPLC. This research will provide information on the presence, color, and number of pigments and polyphenols (colorants) in plant extracts for possible use as hair coloring dyes.

Materials and Methods

Chemicals used for extraction. Chemicals used for extraction of various compounds were organic solvents of analytical grade (acetone, methanol, and ethanol) from Sigma Chemicals (NJ). Silica gel 60 (70 to 230 mesh ASTM) from Merck (Germany) was used for separation by column chromatography. Chlorophyll A, and chlorophyll B standards from Sigma (Switzerland) (purities greater than 98%) were used to compare with plant extracts by HPLC analysis. Distilled and deionized water was used throughout this study.

Plants used in the study. The following 14 Thai herbal plants were used; butterfly pea (*Clitoria ternatea* L.), turmeric (*Curcuma longa* L.), krachaidum (*Kaempferia parviflora* Wall. ex Baker), kae-lae [*Machura cochinchinensis* (Lour.) Corner.], pradu (*Pterocarpus macrocarpus* Kurz), black rice (*Oryza sativa* L.), safflower (*Carthamus tinctorius* L.), ebony tree (*Diospyros mollis* Griff.), marigold (*Tagetes erecta* L.), sappan wood (*Caesalpinia sappan* L.), samor-pipek (*Terminalia Belerica* Roxb.), roselle (*Hibiscus sabdariffa* L.), Ya-nang [*Tiliacora triandra* (Colebr.) Diels], and dragon fruit (*Hylocereus undatus* L.). Plants were purchased from a local retail shop in Bangkok, Thailand. Before analysis, the plants were washed and dried by hot air oven at 50 °C for 24 h and stored dry at 25 °C.

Analysis of extracts. A rotary evaporator (Büchi Rotavapor R200; Büchi Labortechnik AG, Switzerland) was used for solvent removal. An ultraviolet-visible spectrophotometer (ultraviolet-1601; Shimadzu Corporation, Japan) was used for analyzing spectral properties of pigments and polyphenols (colorants). Royal Horticultural Society (RHS) Color Charts (The Royal Horticultural Society, London, UK) was used for measuring color of plant extracts. HPLC (1200 Series; Agilent Technologies) was used for localization and characterization of plant extract.

Pigment extraction. For pigment extraction, 100 g of plant material was ground in a blender and held at room temperature for maximum color development. Plant material was macerated with 400 mL of 100% solvents (acetone, methanol, and ethanol) and placed at room temperature for 1 d. Solvents were then removed and extraction was repeated once with fresh solvents (400 mL) under the same conditions as described previously. The pigment solution was separated from plant tissue using a Buchner funnel with Whatman No. 1 filter paper. To achieve complete pigment extraction from the plant

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material, the filtered residue was rinsed twice with the extraction solution. The combined extracts were filtered and solvent removed with a rotary evaporator (Büchi Rotavapor R200; Büchi Labortechnik AG). Fats and wax were removed with petroleum ether. All extracts were kept at 4 °C after preparation until analysis. A yield percentage of plant extract product was calculated by:

% yield (v/w)

$$= \frac{\text{volume of plant extracts (ml)}}{\text{weight of raw materials (g)}} \times 100\%$$

Statistical analysis. A randomized complete block design with three replications was

used for statistical analysis using SAS (SAS Institute, Cary, NC). Extraction yield percentage data were transformed using arcsine. Extraction yield means were separated by Duncan's multiple range test at $P = 0.05$.

Identification of pigments and colorants. Plant extracts were placed into a quartz cuvette for spectral measurement. The ultraviolet-visible spectrophotometer (ultraviolet-1601; Shimadzu Corporation) was used to analyze by spectrum mode (scanning visible range, 350 nm to 800 nm; range, -3.99A to 3.99A). Color of plant extract was measured using the RHS Color Charts. HPLC conditions were as follows: methanolic extracts (10 µL) were used to identify pigments and

colorants by HPLC (1200 Series; Agilent Technologies) with an Eclipse XDB-C18 column (4.6 mm i.d. × 250 mm, 5 µm). Water and methanol (HPLC-grade) were used in the solvent system. The column was eluted with a mixture of water-methanol (1:1) for 15 min and then for 50 min with a linear gradient of methanol-water (1:1) to 100% methanol. The flow rate was 1 mL/min and a variable wavelength diode array detector set at 445 nm; column temperature was 25 °C.

The solvent extracted pigments were separated using column chromatography for a brief study of plant extract compounds using the wavelength of maximum absorption (λ_{max}). Silica gel 60 (70 to 230 mesh

Table 1. The effects of different solvents on extraction yield, wavelength of maximum absorption, and color in the visible region of the light spectrum of 14 Thai herbal plants.

Plants	Solvents	Percent yield	λ_{max} (nm)	Color
Black rice (<i>Oryza sativa</i> L.)	Acetone	5.4 qrs ²	665	Gray 201B
	Methanol	6.0 qp	370, 400, 420, 445, 485, 510, 535, 545, 570	Violet-blue 97A
	Ethanol	4.6 tu	545	Blue 98B
Butterfly pea (<i>Clitoria ternatea</i> L.)	Acetone	11.0 ij	365, 570	Yellow-green 148C
	Methanol	21.5 c	360, 585	Yellow-green 147B
	Ethanol	6.8 no	365, 585	Yellow-green 148D
Dragon fruit (<i>Hylocereus undatus</i> L.)	Acetone	4.1 u	350, 465, 535	Red 53C
	Methanol	4.1 u	355, 470, 535	Red 51B
	Ethanol	7.7 m	375, 490, 540	Red 42D
Ebony tree (<i>Diospyros mollis</i> Griff.)	Acetone	7.7 m	365	Gray-orange 176A
	Methanol	4.9 st	355	Gray-orange 166A
	Ethanol	3.3 v	355	Gray-orange 166A
Kae-lae [<i>Maclura cochinchinensis</i> (Lour.) Corner.]	Acetone	2.5 w	380	Gray-orange 166D
	Methanol	6.5 nop	370, 390	Gray-orange 173A
	Ethanol	1.7 x	385	Gray-red 178A
Krachaidum (<i>Kaempferia parviflora</i> Wall. ex Baker)	Acetone	9.3 k	360, 380, 390, 405	Gray-orange 165B
	Methanol	13.3 h	360, 375, 390, 425	Violet-blue 89D
	Ethanol	5.9 qp	360, 370, 380, 390, 400, 420, 545	Gray-red 180A
Marigold (<i>Calendula officinalis</i> L.)	Acetone	8.4 l	355	Yellow-green 147C
	Methanol	6.4 op	360	Gray-yellow 160A
	Ethanol	5.5 qrs	360	Gray-orange 167B
Pradu (<i>Pterocarpus macrocarpus</i> Kurz)	Acetone	8.4 l	360, 370, 380, 420	Gray-red 178A
	Methanol	13.4 h	360, 375, 385, 420	Orange-red 34A
	Ethanol	5.5 qr	360, 370, 385	Gray-red 178A
Roselle (<i>Hibiscus sabdariffa</i> L.)	Acetone	5.3 rs	360, 605, 665	Gray-green 188C
	Methanol	40.7 a	360, 370, 390, 420, 535, 665	Gray-purple 187A
	Ethanol	14.2 g	365, 545, 665	Gray-purple 187B
Safflower (<i>Carthamus tinctorius</i> L.)	Acetone	6.7 no	370, 510	Gray-brown 199B
	Methanol	26.3 b	370, 520	Gray-orange 166B
	Ethanol	10.7 j	365, 520	Gray-brown 199C
Samor-pipek (<i>Terminalia Belerica</i> Roxb.)	Acetone	9.7 k	365, 380, 395, 420, 605, 665	Gray-brown 199A
	Methanol	20.9 c	360, 380, 395, 410, 425, 435, 605, 665	Gray-orange 174D
	Ethanol	11.3 i	360, 390, 610, 665	Yellow-green 153D
Sappan wood (<i>Caesalpinia sappan</i> L.)	Acetone	5.6 qr	435	Gray-orange 176A
	Methanol	14.5 g	445	Gray-orange 176A
	Ethanol	7.1 mn	445	Gray-orange 176A
Turmeric (<i>Curcuma longa</i> L.)	Acetone	16.6 e	365, 380, 390, 405, 420, 445, 460, 485	Orange-red 34B
	Methanol	21.4 c	360, 370, 380, 390, 425, 445, 460, 485, 520	Orange-red 34B
	Ethanol	18.2 d	365, 375, 395, 425, 450, 460, 470, 485, 515	Orange-red 34B
Ya-nang [<i>Tiliacora triandra</i> (Colebr. Diels)]	Acetone	15.5 f	380, 395, 405, 445, 475, 505, 535, 560, 610, 660, 675	Black 202A
	Methanol	13.3 h	380, 405, 425, 475, 505, 535, 565, 615, 675	Black 202A
	Ethanol	14.2 g	365, 380, 395, 425, 450, 530, 555, 605, 660	Black 202A

²Means in column separated by Duncan's multiple range test. Values with different letters are significantly different at the 0.05 level.

Table 2. Ultraviolet-visible absorption data of fractions from 14 Thai herbal plants.

Plants	Total fractions	No. of fractions	λ_{max}		
			Acetone	Methanol	Ethanol
Black rice (<i>Oryza sativa</i> L.)	10	1	203	203	201, 228
		2	202	206	208, 229, 281
		3	208, 227, 279	206	207
		4	205	205	205
		5	204	205	204
		6	202	203	201
		7	203	201	203
		8	200	206	200
		9	208	201	200
		10	207	200	201
Butterfly pea (<i>Clitoria ternatea</i> L.)	10	1	201, 272	200	201
		2	202, 270	200, 278	202, 227, 275
		3	201, 268, 349	207, 281	202, 227, 274
		4	202, 267, 349	205, 280	206, 270
		5	201, 267, 349	209, 271	209, 269
		6	204, 266, 346	213, 268, 351	208, 267
		7	201, 266	208, 267, 348	205, 266
		8	202, 267	208, 267, 349	200
		9	201, 266	206, 268	204
		10	202, 267	205, 269	200
Dragon fruit (<i>Hylocereus undatus</i> L.)	7	1	203, 270	200	202, 273
		2	204, 269	205	205, 271
		3	205, 268	207, 270	203, 269, 355
		4	206	201, 269	201, 255
		5	200	204, 269	204, 269
		6	203	204, 269	202, 475
		7	201	204, 267	201, 476
Ebony tree (<i>Diospyros mollis</i> Griff.)	10	1	226	219	226
		2	227	225	225
		3	226	227	229
		4	224	227	228
		5	227	226	221
		6	226	226	226
		7	225	225	225
		8	224	224	224
		9	222	218	222
		10	225	218	223
Kae-Lae [<i>Maclura cochinchinensis</i> (Lour.)]	9	1	203, 331	201, 282	200, 283
		2	206, 283, 331	209, 282	202, 283, 329
		3	207, 282, 330	209, 282	201, 283, 330
		4	207, 282, 329	208, 280, 329, 389	203, 282, 327
		5	202, 283, 328	202, 284, 326, 391	202, 327, 390
		6	200, 328	202, 287, 326, 389	203, 265, 327, 392
		7	202, 389	200, 288, 326, 385	201, 391
		8	204, 254, 330, 387	203, 287, 326	202
		9	201, 254, 329	202, 286, 327	200
Krachaidum (<i>Kaempferia parviflora</i> Wall. ex Baker)	12	1	201	202	201
		2	202	205	201
		3	201	204	202
		4	202, 267, 329	205, 266, 327	201, 267, 330
		5	203, 265, 331	203, 265, 329	202, 265, 330
		6	203, 265, 331	209, 266, 329	200, 265, 330
		7	207, 265, 331	200, 265, 330	210, 265, 329
		8	200, 265, 332	212, 265, 331	201, 265, 332
		9	200, 265, 333	200, 265, 332	202, 265, 332
		10	210, 265, 333	201, 265, 333	200, 265, 333
		11	203, 265, 333	202, 265, 332	201, 265, 333
		12	202, 265, 333	203, 265, 333	203, 265, 332
Marigold (<i>Calendula officinalis</i> L.)	9	1	202, 280	202	202, 243, 282
		2	203, 279	203, 281, 364	201, 279
		3	202, 278	200, 260, 362	201, 275, 355
		4	201, 275, 359	204, 262, 360	206, 275, 361
		5	203, 261, 359	201, 275, 361	201, 275, 358
		6	202, 262, 358	201, 275, 362	202, 275, 358
		7	204, 276, 359	202, 264, 363	202, 277, 357
		8	202, 275, 359	202, 276, 361	200, 278, 356
		9	203, 276, 360	203, 276, 361	203, 279, 358

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Table 2. (Continued) Ultraviolet-visible absorption data of fractions from 14 Thai herbal plants.

Plants	Total fractions	No. of fractions	λ_{max}		
			Acetone	Methanol	Ethanol
Pradu (<i>Pterocarpus macrocarpus</i> Kurz)	10	1	200	201, 282	201
		2	202, 282	202, 282	202, 282
		3	203, 282	202, 282	205, 283, 335
		4	202, 283	207, 284, 328	201, 283
		5	209, 283	202, 284, 329	203, 285
		6	206, 284	207, 284, 329	204, 285
		7	206, 286	208, 285, 328	207, 285
		8	205, 285	204, 285, 326	205, 285
		9	203, 283	201, 284	202, 282
		10	208, 285	200, 285	204, 285
Roselle (<i>Hibiscus sabdariffa</i> L.)	10	1	200	208	205
		2	201	208	201
		3	201	211	201
		4	207, 291, 324	205	202
		5	208, 285, 324	215, 291	207, 289
		6	207, 236	207, 290, 325	217, 296, 324
		7	203, 236	206, 286, 326	217, 290, 325
		8	204, 239	209, 284, 326	209, 284
		9	202, 241	207, 283, 325	207, 283
		10	203, 240	205, 282, 326	208, 283
Safflower (<i>Carthamus tinctorius</i> L.)	12	1	201	202	203
		2	200, 270	200, 272	202, 275
		3	201, 282	200, 273	205, 290
		4	202, 292	202, 271	204, 292
		5	204, 270	203, 270	208, 270
		6	202, 270	200, 269	201, 270
		7	202, 269	209, 269	203, 269
		8	201, 269	201, 269	201, 269
		9	203	202, 269	200, 269
		10	203	200, 269	202, 269
		11	203	202, 269	201
		12	202	202	201
Samor-pipek (<i>Terminalia Belerica</i> Roxb.)	12	1	203	206, 282	279
		2	219, 277	220, 280	219, 278
		3	219, 278	220, 279	218, 278
		4	217, 278	222, 277	218, 278
		5	220, 277	218, 277	218, 278
		6	217, 275	218, 278	217, 277
		7	217, 277	220, 277	216, 277
		8	218, 278	218, 278, 355	219, 278
		9	219, 277	254, 277, 356	220, 277
		10	219, 277	254, 277, 357	219, 278
		11	219, 277	254, 278, 357	220, 277
		12	220, 277	254, 278, 356	221, 277
Sappan wood (<i>Caesalpinia sappan</i> L.)	16	1	204	204	204
		2	203	204	206, 282
		3	205	203	211, 283
		4	205, 284	210, 284	204, 284
		5	211, 284	210, 285	208, 285
		6	207, 285	208, 285	208, 285
		7	210, 254, 285	214, 286	215, 285
		8	208, 254, 285	211, 252, 286	206, 284
		9	209, 254, 284	209, 252, 286	214, 284
		10	206, 253, 285	211, 252, 286	210, 284
		11	212, 252, 284	210, 253, 285	207, 284
		12	205, 283	207, 285	207, 284
		13	205	203	206
		14	202	200	203
		15	204	201	203
		16	205	204	203
Turmeric (<i>Curcuma longa</i> L.)	20	1	202	202	203
		2	201	203	204
		3	200, 417	200, 421	203, 416
		4	201, 422	202, 418	205, 421
		5	201, 358	201, 236, 421	201, 236, 422
		6	200, 233	202, 240, 422	202, 233, 421
		7	200, 238, 422	203, 238, 422	201, 236, 422

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Table 2. (Continued) Ultraviolet-visible absorption data of fractions from 14 Thai herbal plants.

Plants	Total fractions	No. of fractions	λ_{max}		
			Acetone	Methanol	Ethanol
		8	202, 237, 421	202, 238, 422	203, 238, 422
		9	202, 237, 421	201, 238, 423	203, 239, 422
		10	202, 238, 420	200, 238, 423	202, 237, 357
		11	203, 238, 352	202, 239, 423	202, 237
		12	205, 241, 340	201, 238, 419	203, 238
		13	202, 235, 346	203, 236, 353	203, 238
		14	202, 236, 346	203, 235, 356	202, 237
		15	200, 235, 350	203, 235	203, 238
		16	200, 235, 248	203, 234	203, 237
		17	202, 234, 347	203, 234	201, 237
		18	200, 233, 347	203, 233	203, 238
		19	201, 233, 348	202, 233	202, 237
		20	201, 233, 352	202, 233	201, 236
Ya-Nang [<i>Tiliacora triandra</i> (Colebr. Diels)]	7	1	202	200	201
		2	202, 413, 665	201	201, 271, 663
		3	202, 412, 664	201	202, 271, 664
		4	203, 401, 664	204, 272	207, 271, 663
		5	200, 665	206, 274, 451	202, 272, 662
		6	201, 665	201, 273	202, 664
		7	201, 664	201	201, 268, 400, 664

ASTM) 40 g was slurred in 60% methanol in water and held 1 h. A glass column (28 × 2.5 cm i.d.) was filled with silica gel. The sample (2 mL) was applied to the silica gel and then eluted with 60% methanol in water at a flow rate of 1 mL/min. The eluent was allowed to flow down through the column and a total of 5 mL/fraction was collected for analysis. The fraction was analyzed by ultraviolet-visible spectrophotometer (scanning range, 190 nm to 800 nm; range, −3.99A to 3.99A).

Results and Discussion

Pigments extracted with methanol gave the highest percentage yield of pigment and polyphenols (colorants) (Table 1). Examples are roselle with 40.7, safflower with 26.3, butterfly pea with 21.5, and turmeric with 21.4. Anthocyanins are usually extracted with organic solvent, most commonly methanol. Methanol destroys cell membranes and simultaneously dissolves the anthocyanins (Shahidi and Naczki, 2003). Ethanol extraction gave the highest yield of pigment and polyphenols (colorants) in dragon fruit with 7.7, and acetone extraction gave the highest yield of pigment and polyphenols (colorants) in Ya-nang with 15.5, marigold (*Tagetes erecta* L.) with 8.4, and ebony tree with 7.7. Overall, results of the solvent extraction can be explained by the relationship between the polarity of solvents and the structure of pigments and polyphenols (colorants); methanol extraction gave the highest yield because pigments and polyphenol compounds such as flavonoids (anthocyanins, flavonol) and nonflavonoids (phenolic acid) are soluble in methanol. Khanavi et al. (2009) compared the effect of different solvent extraction methods on antioxidant activity and content of total phenolics in *Stachys* species and reported significant difference among solvents used; methanolic extraction gave the highest antioxidant activity and total phenolic content.

Measuring the absorbance at different wavelengths provides an absorption spectrum for the pigments and colorants in the extracts. Because different pigments and colorants have different absorption spectra, pigments and colorants can be tentatively identified by their absorption spectra (Ross, 1974). In this study, black rice absorbed the wavelengths of violet, blue, green, and yellow, thus reflecting the yellow, orange, red, and violet color. Butterfly pea absorbed the wavelengths of violet and yellow, thus reflecting the yellow and violet color. Dragon fruit absorbed the wavelengths of violet, blue, and green, thus reflecting the yellow, orange, and red color. Ebony tree, kae-lae, marigold, and pradu absorbed the wavelengths of violet, thus reflecting the yellow color. Krachaidum absorbed the wavelengths of violet and green, thus reflecting the yellow and red color. Roselle absorbed the wavelengths of violet, green, orange, and red, thus reflecting the yellow, red, blue, and green color. Safflower absorbed the wavelengths of violet and cyan, thus reflecting the yellow and red–orange color. Samor-pipek absorbed the wavelengths of violet, orange, and red, thus reflecting the yellow, blue, and green color. Sappan wood absorbed the wavelengths of blue, thus reflecting the orange color. Turmeric absorbed the wavelengths of violet, blue, and cyan, thus reflecting the yellow, orange, and red–orange color. Ya-nang absorbed the wavelengths of violet, blue, cyan, green, orange, and red, thus reflecting the yellow, orange, red–orange, red, blue, and green color (Table 1). In a previous study, anthocyanins absorb mainly in the green and yellow regions of the spectrum (Karageorgou and Manetas, 2006). Chlorophyll a and b can absorb visible light in the red region and blue region (Lee and Schwartz, 2006). Carotenoids absorb light in the violet and blue region of the spectrum (Glimn-Lacy and Kaufman, 2006). Therefore, anthocyanins, chlorophylls, and carotenoids are potentially present in plant extracts analyzed in this study.

Color of the plant extracts is listed in Table 1. Color varied depending on the plants extracted and the solvent used for extraction. Examples are butterfly pea extract (yellow–green color), dragon fruit extract (red color), pradu extract (gray–red and orange–red color), sappan wood and ebony tree extract (gray–orange color), turmeric extract (orange–red color), kae-lae extract (gray–orange and gray–red color), roselle extract (gray–green and gray–purple color), safflower extract (gray–brown and gray–orange color), and Ya-nang extract (black color). The different colors of plant extract with various solvents may be the result of different physical properties (solubility) and different types of pigments and polyphenols (colorants) in the plants.

Spectrophotometric analysis of the various fractions of each plant extract had similar major absorption peaks at wavelengths in the areas of the ultraviolet region and some fractions also peaked at the visible region (Table 2). Each fraction had a different shade of color; thus, the wavelengths of maximum absorption changed in each fraction. Comparison of the absorption spectra and various absorbance ratios revealed differences in the extract of pigments sampled from different solvents (Tietjen, 1970). The ultraviolet spectrum of the same groups and same derivatives such as flavonol aglycones, anthocyanidin, phenolic acid, and their derivatives have a similar spectrum for each group, which can be used for spectrum analysis for qualitative and selective evaluation of classification of plant phenolics (Maisuthisakul et al., 2005).

In this study, results using HPLC showed that each plant extract had several peaks in the chromatogram, and different peaks (compounds) had different retention times (Fig. 1). Ya-nang had the greatest number of peaks (10) followed by krachaidum with six and ebony tree with five. The remaining herbal plant extracts had one to four peaks depending on the plant. Chlorophyll A and chlorophyll B

Fig. 1. High-performance liquid chromatograms of pigments isolated from 14 Thai herbal plants showing absorbance in milli absorbance units (mAU) with time in minutes.

were found only in Ya-nang. In a previous study, it was reported that turmeric had phenolic compounds (curcuminoids) as main compounds, which give the yellow color. Curcuminoids contain three different diarylheptanoids, curcumin (diferuloylmethane), demethoxycurcumin (*p*-hydroxy-cinnamoylferuloylmethane), and bisdemethoxycurcumin (bip-hydroxy-cinnamoylmethane). The strong coloring pigment in turmeric contain curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, and two other cur-

cuminoids (Stankovic, 2004). Sappan wood has been found to be a source of flavonoids such as 7-hydroxy-3-(4'-hydroxy benzylidene)-chroman-4-one, 8-methoxybouducellin, quercetin, rhamnetin, and ombuin and sterols such as β -sitosterol, campesterol, stigmasterol, brazilin, brazilein, protosappanin E, and taraxerol (Oh et al., 1998). Saito et al. (1985) reported butterfly pea had pigments such as kaempferol, kaempferol 3-glucoside, kaempferol 3-rutinobioside-7-rhamnoside, quercetin, and quercetin 3-glucoside. Roselle gives

a brilliant red color such as Dp 3-sambubioside (70.9%, reddish violet color). Cyanidin 3-sambubioside (29.1%) is also present with lesser amounts of Dp and Cy 3-glucoside (Pouget et al., 1990). Black rice gives a dark purple color as a result of anthocyanin pigments such as cyaniding 3-glucoside and peonidin 3-glucoside identified by liquid chromatography—mass spectroscopy (Hu et al., 2003). Based on color and λ_{max} of the pigments and polyphenols detected in this study, it is possible that such compounds are

Fig. 1. (Continued)

similar and related to compounds previously identified in the literature and cited in this report.

Conclusions

Methanol extraction gave the highest yield of pigment in roselle, safflower, butterfly pea, samor-pipek, sappan wood, pradu, krachaidum, kae-lae, turmeric, and black rice. Ethanol extraction gave the highest yield of pigment in dragon fruit. Acetone extraction gave the highest yield of pigment in Ya-nang, marigold, and ebony tree. Pigments and polyphenols (colorants) of each plant extract produced a different wavelength of maximum absorption depending on the compound and concentration ratio of pigment and colorants in each plant extract. The absorption spectra of pigments and colorants in the plant extract were present in the visible region and the ultraviolet region. HPLC separation of pigments and colorants in plant extracts showed that each extract had several compounds. Ya-nang had the highest number of compounds. Pigments that can be used as coloring dyes and identified in this study are

carotenoids, chlorophylls, and polyphenols such as tannins and anthocyanins. These pigments and colorants coat and penetrate hair fibers (Alpert et al., 2002). These pigments and natural colorants are widely used and are in increased demand in several industries, because the advantages of the natural dyes are harmless, non-toxic, non-carcinogenic, non-pollutant, and induce less wastewater problems. Therefore, the use of such compounds would prove to be a viable economic enterprise. Moreover, these natural colorants can be used in the textile industry, printing industry, and handicraft industry. The present study results strongly indicate that pigments and natural colorants from Thai herbal plants could have great value in cosmetic coloration and in export markets. Further studies should involve developing pigments and colorants from extracts of these Thai herbal plants for use as hair-coloring dyes in the cosmetic industry.

Literature Cited

- Alpert, A., M. Altenburg, and D.C. Bailey. 2002. *Milady's standard cosmetology*. Delmar Thomson Learning, New York, NY.
- Glimn-Lacy, J. and P.B. Kaufman. 2006. *Botany illustrated: Introduction to plants, major groups, flowering plant families*. Springer, New York, NY.
- Harnafi, H. and S. Amrani. 2008. Spectrophotometric methods for determination of plant polyphenols content and their antioxidant activity assessment: An overview. *Phcog. Rev.* 2:20-22.
- Hecht, A. 1978. Hair dyes: A look at safety and regulation. 311-254/53. HEW Publication No. (FDA) 78-5010. U.S. Government Printing Office, Rockville, MD.
- Hu, C., J. Zawistowski, W. Ling, and D.D. Kitts. 2003. Black rice (*Oryza sativa* L. *indica*) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. *J. Agr. Food Chem.* 51:5271-5277.
- Karageorgou, P. and Y. Manetas. 2006. The importance of being red when young: Anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. *Tree Physiol.* 26:613-621.
- Khanavi, M., M. Hajimahmoodi, M. Cheraghi-Niroomand, Z. Kargar, Y. Ajani, A. Hadjiakhoondi, and M.R. Oveisi. 2009. Comparison of the antioxidant activity and total phenolic contents in some *Stachys* species. *Afr. J. Biotechnol.* 8:1143-1147.
- Lazzé, M.C., R. Pizzala, M. Savio, L.A. Stivala, E. Prosperi, and L. Bianchi. 2003. Anthocyanins

- protect against DNA damage induced by tert-butyl-hydroperoxide in rat smooth muscle and hepatoma cells. *Mutat. Res.* 535:103–115.
- Lee, J. and S.J. Schwartz. 2006. Pigments in plant foods. In: Hui, Y.H. (ed.). *Handbook of food science, technology and engineering*. Vol. 1. CRC-Taylor & Francis, New York, NY.
- Maisuthisakul, P., M. Suttajit, and R. Pongsawatmanit. 2005. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chem.* 100:1409–1418.
- Oh, S.R., D.S. Kim, I.S. Lee, K.J. Jund, J.J. Lee, and H.K. Lee. 1998. Anticomplementary activity of constituents from heartwood of *Caesalpinia sappan*. *Planta Med.* 64:456–458.
- Panayotov, P., D. Stoyanov, S. Kjulanova, and M. Panayotov. 2006. Entomology to public health 2006: Determination of the quantity of flavonoids in plant extracts by UV-spectrophotometry. Jan. 2009. <<http://www.cababstractsplus.org/abstracts/Abstract.aspx?AcNo=20013014883>>.
- Pouget, M.P., B. Vennat, B. Lejeune, and A. Pourrat. 1990. Extraction analysis and study of the stability of Hibiscus anthocyanins. *Lebenson. Wiss. Technol.* 23:103–105.
- Ross, C.W. 1974. *Plant physiology laboratory manual*. Wadsworth Publishing Company, Inc., Belmont, CA.
- Saito, N., K. Abe, T. Honda, C.F. Timberlake, and P. Bridle. 1985. Acylated delphinidin glucosides and flavonols from *Clitoria ternatea*. *Phytochemistry* 24:1583–1586.
- Shahidi, F. and M. Nacz. 2003. *Phenolics in food and nutraceuticals*. CRC Press, Boca Raton, FL.
- Solovchenko, A.E., O.B. Chivkunova, M.N. Merzlyak, and I.V. Reshetnikova. 2001. A spectrophotometric analysis of pigments in apples. *Russ. J. Plant Physiol.* 48:693–700.
- Stankovic, I. 2004. Curcumin: Chemical and Technical Assessment (CTA): First draft in JECFA on line publication. Oct. 2008. <http://ftp.fao.org/esn/jecfa/cta_61_curcumin.pdf>.
- Thun, M.J., S.F. Altekruze, M.M. Namboodiri, E.E. Calle, D.G. Myers, and C.W. Heath, Jr. 1994. Hair dye use and risk of fatal cancers in U.S. women. *J. Natl. Cancer Inst.* 86:210–215.
- Tietjen, J.H. 1970. Studies on the absorption spectra of plant pigments in estuaries. *Hydrobiologia* 35:420–430.
- Zheng, T., T.R. Holford, P.H. Mayne, P.H. Owens, P. Boyle, B. Zhang, Y.W. Zhang, and S.H. Zahm. 2002. Use of hair coloring products and breast cancer risk: A case-control study in Connecticut. *Eur. J. Cancer* 38:1647–1652.