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Original Article

Characterization and chemical composition of epicuticular wax from banana leaves grown in Northern Thailand

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Abstract

This study aimed to investigate the physicochemical properties and chemical composition of epicuticular wax extracted from leaves of Kluai Namwa, a banana cultivar which is widely grown in Northern Thailand. Its genotype was identified by a botanist. The wax was extracted using solvent extraction. The fatty acid profiles and physicochemical properties of the wax namely melting point, congealing point, crystal structures and polymorphism, hardness, color, and solubility were examined and compared to those of beeswax, carnauba wax and paraffin wax. The results showed that the genotype of Kluai Namwa was *Musa acuminata X M. balbisiana* (ABB group) cv. *Pisang Awak*. The highest amount of wax extracted was 274 μg/cm^2 surface area. The fatty acid composition and the physicochemical properties of the wax were similar to those of carnauba wax. It could be suggested that the banana wax could be used as a replacement for carnauba wax in various utilizing areas.

Keywords: epicuticular wax, banana wax, Kluai Namwa, *Musa* spp. carnauba wax

1. Introduction

Banana (*Musa spp*.) is one of the most widely cultivated plants in Thailand. All parts of this plant are used in daily life (Debabandya *et al.*, 2010). Unripe and ripe fruits which can be harvested year-round are known as functional fruits, providing nutritional and medicinal values (Sampath Kumar *et al.*, 2013). Banana leaves are commonly used for food wrapping and decoration because they are large, flexible and waterproof. However, they are less frequently used nowadays and largely replaced by plastic. After harvesting the crop, the mature leaves are considered as agricultural waste and they are often destroyed by burning that leads to air pollution. However, it is interesting that, the outermost part of banana leaf is covered with lipid substance, which is so-called the epicuticular wax. This wax is solid mixtures, composed of esters of long chain fatty acids and long chain fatty alcohols, free fatty acids, fatty dialcohols (diols), aldehydes and n-alkanes (Freeman & Turner, 1985; Yanagida *et al.*, 2005a). This surface wax serves as a mechanical barrier which protects the plant tissues against UV radiation and bacterial or fungal attacks and also reduces water loss in summer time (Riederer & Schreiber, 2001). Nowadays, carnauba wax is the most commonly used natural wax in various industries especially, for polishing leathers, glasses, wooden furniture and automobiles. Interestingly, it is also a common ingredient in pharmaceutical and cosmetic products. This point of view leads us to generate the idea of using banana leaves as a natural source of waxy substance. From the extensive literature review, studies involving extraction

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and characterization of the epicuticular wax from banana leaves have been scarcely reported. Those noteworthy ones were reported by Freeman and Turner (1985), and Yanagida *et al.* (2003; 2005b). According to Freeman and Turner (1985), epicuticular waxes from leaf, flower bracts and fruits of 12 banana (*Musa spp*.) varieties were extracted and examined their chemical compositions. Fruits gave higher wax content followed by bracts and leaf respectively. The wax from leaf in field-grown banana was about 230 mg/cm^2 was 60% higher than those planted in a glasshouse. In addition, Thailand has numerous cultivars of banana, while the yield, characterization and chemical composition of epicuticular wax from banana leaves grown in Thailand has never been investigated The objectives of this study were to investigate and characterize the chemical composition and the properties of wax isolated from leaves of Kluai Namwa, a banana cultivar which is widely grown in Northern Thailand and to compare its properties to those of other waxy substances including carnauba wax.

2. Materials and Methods

The following waxy substances were used to compare their physicochemical properties with the banana wax namely, beeswax (S. Tong Chemicals Co. Ltd. Lot. P011814, Bangkok, Thailand), carnauba wax (Union Sciences Co. Ltd. Lot. 9776, Chiang Mail, Thailand) and paraffin wax (B. L. Hau & Co. Ltd. Lot. 7117L14, Bangkok, Thailand).

2.1 Sample preparation

Kluai Namwa grown in Northern Thailand particularly in Chiang Mai, Lampang, Pitsanulok and Tak provinces were collected as samples for wax extraction. Fresh leaves were used for wax extraction. After measuring their surface areas, banana leaves were hung in the air to keep them partially dried and dust was removed using an air blower. All reagents and chemicals used were of analytical grade.

2.2 Extraction of epicuticular wax

The epicuticular wax was extracted from banana leaves by sohxlet apparatus using n-hexane as a solvent and the solvent was removed by using a rotary evaporator. The extracted wax was purified and decolorized using activated charcoal. Briefly, 1% w/v of activated charcoal was added to the hot hexane extract. The mixture was frequently stirred for a few minutes then filtered through filter papers (Whatman® No. 5, Sigma-Aldrich St. Louis, MO, USA) using vacuum filtration. The decolorization process was repeated 3-5 times depended on the color intensity of the hexane extract. The residual of hexane in the wax was quantified by gas chromatography. The codes were assigned to the extracted waxes according to their origins i.e., BNS, BNP, BNT, BNTk which represented banana leaves cultivated in Chiang Mai, Pitsanulok, Lampang and Tak provinces, respectively.

2.3 Identification of banana cultivars

Kluai Namwa from different origins were identified and authenticated by the botanist at Queen Sirikit Botanical Garden, Department of Forest, Ministry of Natural Resources and Environment in Chiang Mai.

2.4 Verification and determination of chemical compositions in the wax

2.4.1 Thin layer chromatography

Thin layer chromatography (TLC) technique was used to verify the chemical composition of the wax. The stationary phase was Silica Gel 60 F254 and the mobile phase was the mixture of hexane: ethyl alcohol: acetic acid 50:50:1.

2.4.2 Gas chromatography

Fatty acid composition of the extracted wax was determined by using gas chromatography (GC), Shimadzu GC 2010 equipped with DB-23 column and a flame ionization detector. The sample was injected in a split mode with a split ratio of 1:50 and an injector temperature of 250°C. The temperature program started with an initial temperature of 80°C, increased at a rate of 10°C/min up to 180°C, hold for 15 min, increased with a ramp rate of 4°C/min to reach a final temperature of 220°C with a final hold time of 7 min; the duration of the total temperature program was 42 min. The detector temperature was set at 300°C. Helium was used as a carrier gas at a flow rate of 62.9 ml/min.

2.5 Acid number determination

The acid value of the samples was determined using the method previously described (Suzanne, 2010). Briefly, the wax was dissolved in ethanol at 50°C. The mixture was titrated with standardized solution of potassium hydroxide using phenolphthalein as an indicator. A blank was also undertaken in the same manner. Acid value was calculated from the amount of standardized solution of potassium hydroxide used for wax and blank titration. The experiment was performed in triplicate.

2.6 Saponification number determination

The determination of saponification number was performed according to the following procedures (Suzanne, 2010); the wax was mixed with alcoholic 0.4 M KOH and refluxed for 30 min. The resultant solution, after the addition of phenolphthalein, was titrated with standardized solution of 0.5 M hydrochloric acid. A blank was also carried out. Saponification value was calculated from the amount of standardized hydrochloric acid solution used for sample and blank titration. The experiment was performed in triplicate.

2.7 Characterization of physical properties

2.7.1 Determination of melting point, congealing point and polymorphism by DSC

The melting point and the congealing point of the wax was determined using differential scanning calorimeter DSC 7 (Perkin Elmer, USA). The sample weighed 3.0-3.5 mg was placed in an aluminum pan with holes. The heating experiment was studied from 50°C to 100°C at a heating rate of 5°C/min and followed by the cooling experiment from 100°C to 50°C under the same cooling rate. Nitrogen was used as purge gas at a flow rate was 20 ml/min.

2.7.2 Examination of internal crystal structure

X-ray diffractometer (Siemens-D 500, Germany) was used to examine the internal structure of the wax crystals at room temperature using the following conditions: voltage, 20 kV; current, 20 mA; time constant, 0.5 second; scan speed, 4 q/min and scattering angle range (2q) of 5-30°.

2.7.3 Examination of crystal modifications (Habits)

The crystal modification of wax crystals was examined using scanning electron microscope (JSM-5910LV, Jeol, Japan).

2.7.4 Hardness measurement

The hardness of wax was determined using Texture Analyzer TA.XT2i (stable micro system). This method was modified from the standard method used for testing the hardness of waxes in industry (*American Society for Testing and Materials* International [ASTM] D1321, 2015). The hardness was presented in terms of the force (Newton) acting against the penetration of the needle into the wax matrix at the definite distance (in mm.) at a fixed temperature. The experiment was performed in triplicate.

2.7.5 Color measurement

The color of the wax was examined using Minolta Color Reader CR-10. The CIE color parameters of L*, a* and b* were recorded.

2.7.6 Density and specific gravity determination

The density of the wax was determined using the density factor, i.e., a ratio by weight of wax to water of an exactly known volume. The wax was melted and poured into the suppository mold. After solidified, the excessive wax above the mold surface was removed. The solid wax in the mold was taken out and weighed. The volume inside each suppository mold was determined using purified water. The specific gravity was calculated by dividing the density value obtained by the density of water at same temperature. The experiment was performed in triplicate.

2.7.7 Solubility determination

The solubility of the extracted waxes in various solvents was determined using the method previously described by Yanagida *et al.* (2003a) with some modifications. The solvents used were hexane, dichloromethane, chloroform, ethanol and methanol. The excess amount of wax sample was placed in a well-closed flask containing a specific solvent. After the mixture was stirred using a small magnetic bar for 12 hours at room temperature, it was centrifuged to obtain clear supernatant. The known volume of the supernatant was sampling and the solvent was evaporated. The solid residue of the wax was weighed. The experiment was performed in triplicate.

3. Results and Discussion

3.1 Identification of banana cultivars

All banana samples collected from different origins were identified as Kluai Namwa (*Musa acuminata* X *M. balbisiana* (ABB group) cv. *Pisang Awak*. The samples were kept as a voucher specimen at Queen Sirikit Botanical Garden in Chiang Mai. Kluai Namwa is a diploid cultivar and a common edible banana widely grown in Northern Thailand as well as all parts of Thailand.

3.2 Extraction of banana wax

The yield of the wax extracted from Kluai Namwa's leaves depended on cultivating areas. They were 273.8 μg/cm^2, 212.6 μg/cm^2, 167.4 μg/cm^2 and 129.1 μg/cm^2, from Chiang Mai, Lampang, Pitsanulok and Tak provinces respectively. The yield value of BNS (273.8 μg/cm^2) was higher than the values reported by Freeman and Turner (1985) with the highest one found in field-grown banana (230 μg/cm^2) which was 60% higher than those planted in a glasshouse. Yanagida *et al.* (2003b) reported the yields of wax extracted from banana leaves of different species on dried weight basis i.e., Ito bashou (Japanese name) or *M. liukiuensis* (0.58%), kluai Pa or *M.acuminata* (1.05 %) and kluai Roi Wee or *M. chiliocarpa* (1.41%). Based on the percentage of moisture content reported, the maximum yield of 1.41% from *M. chiliocarpa* can be calculated as only 0.15 g/100 g of fresh leaves which was significantly lower than the result of our study (0.92 g/100 g of fresh leaves). Thus, Kluai Namwa's leaves are the most abundant source of banana wax reported until now. According to the higher yield percentage, the BNS and BNT were selected to be the samples for characterization and physicochemical properties determination.

In order to obtain purified, white-colored wax, the extracted wax was subjected to decolorization process using activated charcoal. After decolorization, the color of banana

wax became whiter as shown in Figure 1. Although loss of wax content was observed, its main composition as revealed by TLC analysis did not change as shown in Figure 2 and the melting point and congealing point determined by DSC did not altered (data not shown).

3.3 Chemical compositions of banana wax

3.3.1 Chemical compositions of by TLC and GC

TLC chromatogram shown in Figure 3 demonstrated that the major compositions of the wax extracted from banana leaves and carnauba wax were similar. This was also supported by the fatty acid profiles obtained by gas chromatographic technique after saponification as shown in Table 1. The saturated fatty acids presented in both waxes were behenic acid (C-22), lignoceric acid, palmitic acid and stearic acid. For unsaturated fatty acids; linolenic acid, linoleic acid and oleic acid were found predominantly in banana wax. These results were in agreement with those reported by Yanagida *et al.* (2003a), which demonstrated that C-22 fatty acid was the most abundant component of banana wax.

Figure 1. Epicuticular wax extracted from banana leaves, BNT and BNS; (A) before decolorization, (B) after decolorization.

3.3.2 Acid number and saponification number

The acid number and/or saponification number is meaningful parameters providing information about the chemical functionality of the wax that contains carboxylic groups. As shown in Table 2, the acid number and saponi-

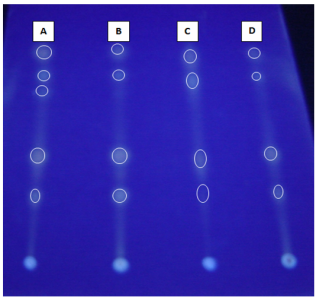

Figure 2. TLC chromatograms of wax from banana leaves: (A), (B) - BNT, before and after decolorization; (C), (D) BNS, before and after decolorization respectively.

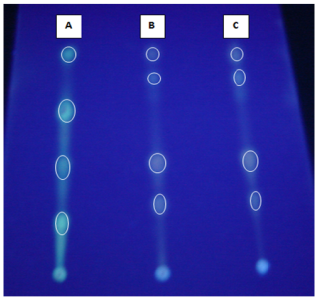

Figure 3. TLC chromatograms of (A) carnauba wax (B) BNT, (C) BNS.

Table 1. Composition of wax from banana leaves and the reference waxes.

Compounds	% composition			
	BNT	BNS	Carnauba wax	Beeswax
Myristic acid (C14:0)	ND	ND	28.75	ND
Palmitic acid (C16:0)	12.19	22.14	10.23	34.03
Stearic acid (C18:0)	2.14	3.69	11.72	53.49
Oleic acid (C18:1)	2.17	5.84	1.42	ND
Linoleic acid (C18:2)	8.59	4.51	0.77	0.20
Linolenic acid (C18:3)	33.79	8.65	0.24	2.56
Arachidic acid (C20:0)	ND	ND	28.75	ND
EPA(C20:5)	7.86	7.39	ND	0.185
Behenic acid (C22:0)	14.30	23.55	16.75	0.144
Lignoceric acid (C24:0)	9.45	13.93	28.66	0.86

Note: ND (undetected); EPA: Eicosapentaenoic acid.

Table 2. Physicochemical parameters (mean ± SD, n = 3) of wax from banana leaves and those of reference waxes.

Wax samples	Acid number	Saponification number	Hardness (N)	Specific gravity
BNT	1.68 ± 0.26^{a}	81.66 ± 4.39^{a}	39.17 ± 4.36^{b}	0.9266 ± 0.0173^{d}
BNS	3.58 ± 0.92^{a}	94.72 ± 5.18^{a}	$29.30 \pm 3.42^{b,c}$	$0.9025 \pm 0.0101^{d,e}$
Beeswax	17-24*	87-104*	35.35 ± 2.16	$0.9543 \pm 0.0022^{d,e}$
Carnauba wax	2-7*	78-95*	38.68 ± 3.47^{c}	0.9373 ± 0.0010^{e}
Paraffin wax	NS	NS	25.24 ± 7.47^{b}	0.9308 ± 0.0039^{e}

Note: * Reference values specified in PhEur, USP; NS –not specified in USP. Significant differences were tested at $p < 0.05$.

fication number of banana waxes, BNT and BNS were significantly different; however, they were in the range of carnauba wax's values specified in Pharmacopeias. This supported the similarity in terms of the components containing carboxylic acids of the two natural waxes.

3.4 Characterization of the physicochemical properties of banana wax

3.4.1 Melting point, congealing point and polymorphism

The DSC thermogram of banana wax obtained from different sources; BNS and BNT were compared to that of carnauba wax as shown in Figure 4. The melting point (peak temperature) and congealing point of BNT were 81.38°C and 73.81°C and those of BNS were 79.01°C and 74.11°C respectively. They were not much different. Whereas carnauba wax exhibited the major melting peak at 78.98°C and the congealing point peak at 73.91°C. They were more similar to those of BNS. However, these properties are more vital for wax utilization than chemical compositions. Despite of some differences in their chemical composition observed, the comparable melting points of both waxes revealed the identical internal strengths of wax crystals. The measured melting point of carnauba wax was in agreement with that specified in USP30 NF30, i.e., 80-86°C. The extracted banana wax was of high purity as evidenced by a single sharp and approaching symmetrical melting peak, whereas the thermogram of carnauba wax showed two distinct melting peaks. Moreover, the thermogram of banana wax did not signify the existence of polymorphism

3.4.2 Examination of internal crystal structure

The X-ray powdered diffractograms in Figure 5 illustrated the internal crystal structure of banana wax as compared to carnauba wax. The sharp peaks demonstrated that banana waxes as well as carnauba wax were in crystalline state. It also indicated that the internal structure of banana wax and carnauba wax were similar which denoted by the same peak positions of Bragg's angle (2q) at of 21°, 23° and 19° respectively. These observations supported the similarity

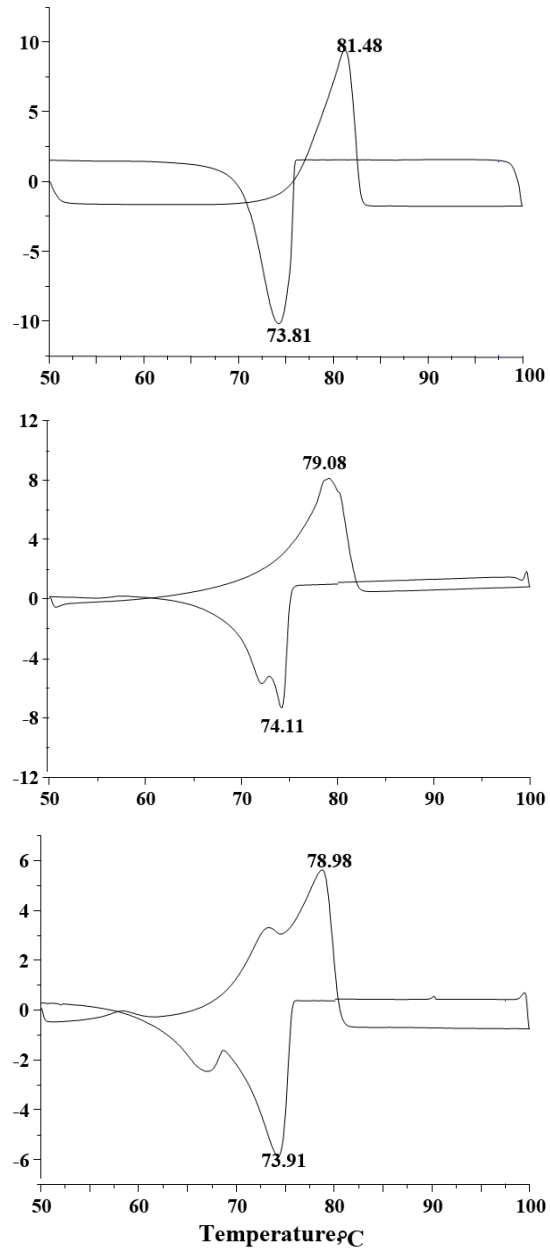

Figure 4. DSC thermograms wax from banana waxes; BNT-upper, BNS-middle and carnauba wax-lower.

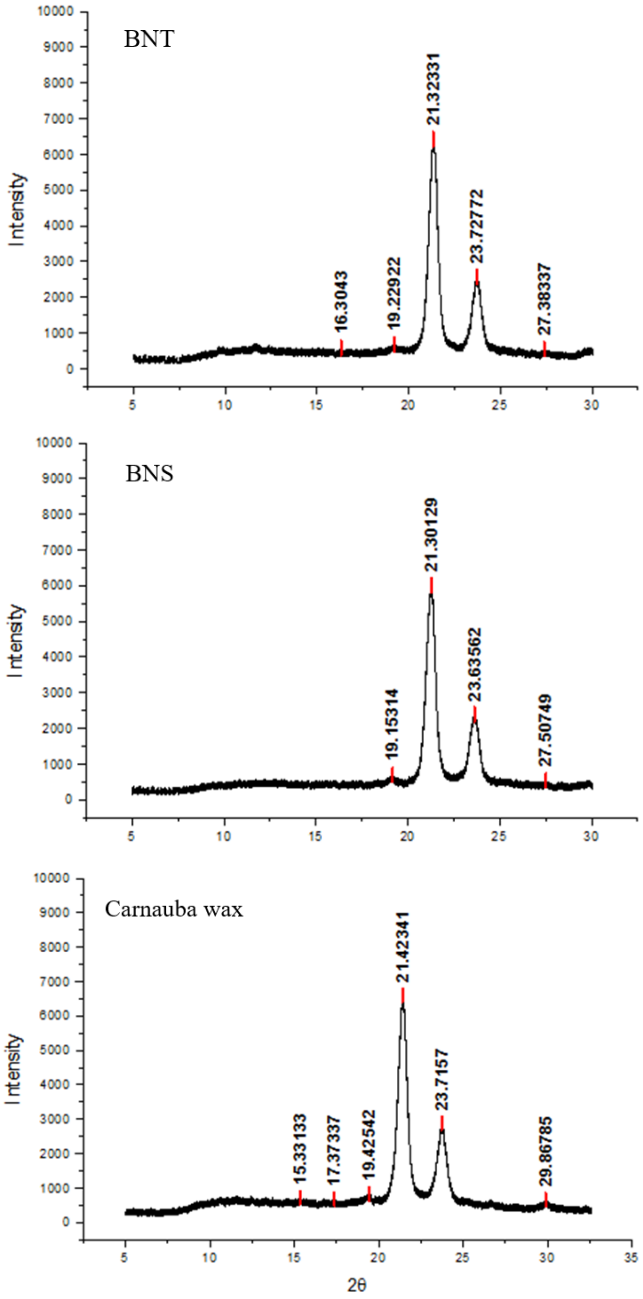

Figure 5. X-ray powdered diffractograms of wax from banana leaves: BNT, BNS and carnauba wax.

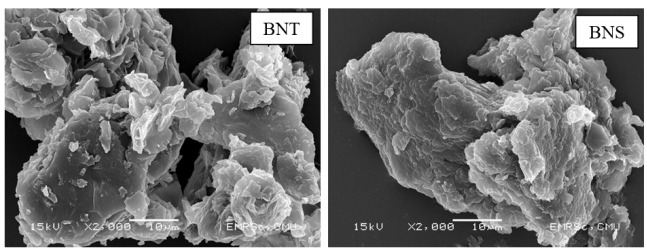

Figure 6. SEM micrograph of banana waxes, BNT- left and BNS- right.

of the two waxes as previously demonstrated by the composition and DSC studies.

3.4.3 Examination of crystal modifications (crystal habits)

The SEM micrograph in Figure 6 illustrated the crystal habits of banana waxes obtained from different sources, BNT and BNS. They habits were quite similar exhibited the aggregated plate-liked crystals of varying sizes. Despite of the irregular habits, the internal structure exhibited high degree of crystallinity as shown by X-ray diffractogram. The thin and uniform thickness of these plate-liked habits contributes to sharp melting point peak as demonstrated by DSC study.

3.4.4 Hardness measurement

According to the modified method by using Texture analyzer TA.XT2i, the hardness of the wax presented in terms of the resistant forces (Newton) against the penetration of the needle into the solid wax was shown in Table 2. The hardness of BNT and carnauba were insignificantly different, whereas BNS was softer than BNT, beeswax and carnauba wax the lower hardness value of BNS was significantly different from those waxes at $p < 0.05$.

3.4.5 Color measurement

The color of the wax was determined according to CIE (International Commission on Illumination, 1976) color parameters (L*, a*, b*). The L* value represents the lightness; the maximum L* value of 100 represents pure white color whereas zero value indicates black color. Each a* and b* represents two color components. The positive value of a* indicates red color whereas the negative value represents for green color. For b* value, its positive value represents yellow color and the negative one represents blue color. As shown in Table 3, paraffin wax had the highest L* value followed by banana wax (non-decolorized) and carnauba wax respectively. This demonstrated the intensity of white color of waxes in descending order. As banana wax was whiter than carnauba wax, the decolorization process might not be necessary for some cases of utilization. In addition, when a* and b* values are considered altogether, the color of banana wax can be summarized as white with greenish yellow color, whereas carnauba wax had white color with slightly red-yellow color tone.

3.4.6 Density and specific gravity determination

The specific gravity of banana wax and those of the reference waxes were shown in Table 2. Although the method used was adopted from density factor determination, the results obtained from the reference, beeswax was in agreement with that specified in the reference book (Rowe, 2012). The measured values of the samples were then reliable.

Table 3. CIE Color parameters (L*, a*, b*) of wax from banana leaves (before decolorization) and the reference waxes.

Wax samples	Color parameters (L, a, b) (mean ± SD)		
	L (white-black)	**a** (red-green)	**b** (yellow-blue)
BNT	88.2 ±0.56	-2.8 ±0.19	+29.0 ±0.87
BNS	85.9 ±0.63	-3.8 ±0.40	+23.5 ±0.71
Beeswax	85.7 ±1.70	-2.4 ±0.29	+2.7 ±0.48
Carnauba wax	77.9 ±0.34	+1.7 ±0.16	+1.7 ±0.16
Paraffin wax	93.4 ±1.14	-1.7 ±0.15	+0.5 ±0.26

The banana waxes from different origins had significantly different specific gravity and they were significantly lower than that of carnauba wax ($p < 0.05$).

3.4.7 Solubility determination

The solubility of wax from banana leaves, BNT and BNS in various solvents was shown in Table 4. The wax was soluble in chloroform, hexane, dichloromethane, ethyl alcohol and methyl alcohol in descending order. According to USP descriptive terms of solubility, the solubility of banana wax in all solvents can be classified as slightly soluble i.e., 100-1,000 parts of solvent are needed to dissolve one part of the wax (USP 35/NF30). However, the significant differences in the solubility of BNT and BNS in hexane and methanol were observed at $p < 0.05$.

4. Conclusions

All Kluai Namwa samples collected from different planting areas in Northern Thailand were *Musa acuminata* X *M. balbisiana* (ABB group) cv. *Pisang Awak*. The percentage yield and the physicochemical properties of the extracted wax depended on planting area. The sample which gave the highest yield of wax was Kluai Namwa grown in Chiang Mai province (0.92 g/100 g of fresh leaves) whereas that grown in Lampang province was more similar to carnauba wax. However, the reported yield of unpurified carnauba wax obtained from natural source was approximately five percent (Duke & duCellier, 1993). From extensive investigation by using different characterized methods, we could confirm that the wax extracted from Kluai Namwa's leaves grown in Northern Thailand was similar to carnauba wax in terms of chemical composition and physicochemical properties. This could bring about the idea of replacing banana wax for carnauba wax in pharmaceutical, cosmetic and the other wax utilizing areas.

Table 4. Solubility of wax from banana leaves in various solvents.

Solvents Solubility	(g/100 ml) (mean ± SD, n = 3)	
	BNT	BNS
Chloroform	0.64 ±0.18	0.69 ±0.10
Dichloromethane	0.34 ±0.03	0.38 ±0.08
Ethyl alcohol	0.15 ±0.03	0.14 ±0.04
Hexane	0.50 ±0.11*	0.80 ±0.05*
Methyl alcohol	0.10 ±0.02*	0.15 ±0.01*

* Significant difference at $p < 0.05$ (SPSS version 17).

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