Screening of Cosmeceutical Activities of *Musa sapientum* L Blossom Extracts

การศึกษาฤทธิ์เวชสำอางเบื้องต้นของสารสกัดปลีกล้วยน้ำว้า

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ABSTRACT

The cosmeceutical activities of ethanolic and aqueous extracts of banana (*Musa sapientum*) blossom were investigated including total phenolic contents using Folin-ciocalteau complexation and cosmeceutical properties focusing on the anti-oxidative effects using DPPH assay, anti-tyrosinase effects using tyrosinase enzymological assay and anti-acne effects using agar disc diffusion to detect the inhibitory zone diameter for anti-bacterial effects against *P. acne*, *S.epidermidis*, and *S. aureus*. The results revealed that the extract yields were 27.12% and 6.32% for aqueous and ethanolic extracts, respectively. Despite lower yield, the content of phenolic compounds and cosmeceutical effects of the ethanolic extract were more obvious than those of the aqueous extract, giving the GAE at 61.9±1.33 mg/g, the EC_{50} as low as 147.62±1.19 µg/ml in DPPH assay and the inhibitory zone diameters of 6.33-15.00 mm. Actually, the aqueous extract showed no anti-bacterial activity at all. In terms of anti-tyrosinase activity, both aqueous and ethanolic extracts revealed poor results. As the result, the ethanolic extract of banana blossom was found as the potential fraction containing some cosmeceutical active agents, particularly, against oxidative reaction and acne-bacteria.

บทคัดย่อ

การศึกษาฤทธิ์ทางเวชสำอางของส่วนสกัดชั้นน้ำและเอทานอลของตัวอย่างปลีกล้วยน้ำว้าโดยเบื้องต้นหาปริมาณสุทธิสารฟินอลิก โดยใช้วิธี ‘Folin-ciocalteau complexation’ และแสดงค่าเป็นปริมาณสมมูลกับกรดแกลิก และศึกษาฤทธิ์เวชสำอางต่างๆ ได้แก่ ฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH assay ฤทธิ์ต้านเอนไซม์ไทโรซิเนส และฤทธิ์ต้านเชื้อแบคทีเรียที่ก่อสิว *P. acne*, *S.epidermidis*, และ *S. aureus* โดยวิธี ‘agar disc diffusion’ ผลการทดสอบแสดงให้เห็นความแตกต่างของคุณสมบัติของส่วนสกัดชั้นน้ำและเอทานอลของตัวอย่างปลีกล้วย ผลการทดสอบพบว่าส่วนสกัดชั้นน้ำและส่วนสกัดชั้นเอทานอลให้สารสกัดที่ ร้อยละ 27.12 และ 6.32 ตามลำดับ แต่จากการศึกษาหาปริมาณสุทธิฟินอลิกและฤทธิ์เวชสำอางต่างๆ พบว่ามีปริมาณสุทธิฟินอลิกและฤทธิ์เวชสำอางที่ดีกว่าส่วนสกัดชั้นน้ำ โดยได้ค่าปริมาณสมมูลกับกรดแกลลิก เท่ากับ 61.9±1.33 mg/g ของสารสกัด มีฤทธิ์ต้านออกซิเดชันที่ให้ค่า IC_{50} ต่ำ (147.62±1.19 µg/ml) และ มีฤทธิ์ต้านเชื้อแบคทีเรียก่อสิวที่ดี โดยมีเส้นผ่านศูนย์กลางพื้นที่ยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียที่อยู่ในช่วง

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6.33-15.00 mm อย่างไรก็ตามพบฤทธิ์ยับยั้งการทำงานของเอนไซม์ไทโรซิเนสที่อ่อนมากในสารสกัดปลีกล้วย จากผลการศึกษาแสดงให้เห็นว่าส่วนสกัดชั้นเอทานอลของปลีกล้วยมีฤทธิ์เวชสำอางที่ดีกว่าส่วนสกัดชั้นน้ำ โดยเฉพาะฤทธิ์ต้านออกวิเดชันและฤทธิ์ต้านเชื้อแบคทีเรีย

Key Words: Cosmeceutical effects, *Musa sapientum* L blossom

คำสำคัญ: เวชสำอาง ปลีกล้วยน้ำว้า

Introduction

Musa sapientum L. (*M. sapientum*) or banana is a tropical fruit crop grown in many countries around the world (Arias et al, 2003). It is the world's fourth most important food crop after rice, wheat and maize in terms of gross value of production (Tepsorn, 2009). In Thailand, various cultivars of banana are grown and one of the most popular cultivars is *M. sapientum* L., locally known as "Kluay Naam Wa." It is generally known as a valuable Thai traditional herbal which all parts of banana tree are used for multiple purposes including food and illness treatment. Roots are used for the treatment of strangury; resin for hemorrhage control; raw fruits for diarrhea and peptic ulcers (Eriyamremu et al, 2007), and ripen fruits for constipation, and blossom for promoting lactation (Udomkan and Parichart, 2006). Banana blossom is a byproduct of interest in which only a few of scientific data were currently found regarding chemical compositions and biological activities: it was reported containing of phenols, flavanoids, tannins, and saponins (Decena, 2010; Somsub et al, 2008; Monthathip, et al, 1995) and possessing of antimicrobial (Tepsorn, 2009; Mumtaz et al, 2010) and anti-oxidation properties (Pari and Umamaheswari, 2000; Sheng et al, 2011). From this evidence, some other health-beneficial effects including cosmeceutical effects of banana blossom are suggested, but none of the evidences was found. Therefore, this present study aims to screen the cosmeceutical potentials of banana blossom extracts: determination of total phenolic content, whitening effects by investigating of anti-tyrosinase enzyme activity, anti-oxidation, and anti-bacteria causes of acne. The obtained results will be useful supporting data for further studies and applications of banana blossom in the aesthetic sciences and health.

Materials and methods

Materials

Musa sapientum L blossoms were collected from the banana tree (age 6-8 months) cultivated in Loei province, Thailand, 95%Ethanol was purchased from GPO of Thailand, methanol and absolute ethanol were purchased from Fisher scientific, Vitamin C, Folin reagent and 2,2-diphenyl-2-picry hydrazine (DPPH) were purchased from Fluka, Aluminium chloride was purchased from APS, Sodium carbonate was purchased from Ajax Finechem, Di-potassium hydrogen orthophosphate phosphate & Potassium dihydrogen orthophosphate were purchased from Univar, 3-(3, 4-dihydroxyphenylalanine)-l-alanine (L-Dopa), mushroom tyrosinase enzyme, Gallic acid and Kojic acid were purchased from Sigma, Trypticase Soy Agar (TSA) and Mueller Hinton Agar (MHA) were purchased from Himedia Laboratories Pvt. Ltd, Ready-to-use Blood agar, Amoxicillin & Erythromycin were purchased from Oxoid, *Propionibacterium acne* (*P.acne*) (DMST14916), *Staphylococcus aureus* (*S.aureus*) (DMST8013), *Staphylococcus epidermidis* (*S.epidermidis*) (DMST15505) were purchased from Department of medical Sciences of Thailand.

Methods

1. Preparation of extracts

The collected *Musa sapientum* blossoms were thoroughly washed in running water, cut into small pieces and dried in a hot air oven under 50°C

for 12 hours. The ethanolic extract was prepared by macerating 1 kg dried banana blossom in 4 liters of ethanol for 24 hours at room temperature, whereas, the aqueous extract was prepared by boiling of 1 kg dried banana blossom in 4 liters of deionized water for 5-10 minutes. Then, filtrates of ethanolic and aqueous extract were separately collected and concentrated by the filtration (Whatman No. 1 filter paper), rotary evaporating and lyophilization techniques to yield dried ethanolic and aqueous-fraction extracts, respectively. Finally, the obtained extracts were stored at -20 °C until use.

2. Determination of total phenolic contents

Using 96-well plate colorimetric reaction model through coloring complex forming of phenolic compounds and Folin–Ciocalteu reagent (Chotimarkorn et al, 2010), the total phenolic contents were determined with having gallic acid as the reference. Firstly, a calibration curve of the gallic acid was prepared to be used for extrapolation of the total phenolic contents in banana blossom extract samples.

To establish the calibration curve, various concentrations of ethanolic gallic acid solutions were prepared and used for reaction. With triplication, 50 µl of each concentration of gallic acid solution was mixed with 125 µl 20% Na_2CO_3 and 25 µl 1 N Folin reagent. Then, mixture was incubated at 30 °C for 2 hours in order to measure for their optical absorbencies at 650 nm wavelength. Finally, the calibration curve was generated plotted between concentrations (x-axis) versus corresponding optical absorbencies (y-axis).

With three independent experiments, the total phenolic contents of each sample were determined. The 1 mg/ml concentrated ethanolic solution of each sample was prepared and used for reaction following the aforementioned processes. Finally, the optical absorbencies at wavelength 650 nm were measured. Then, by extrapolation from gallic acid calibration curve, total phenolic content of each sample was delivered and expressed as gallic acid equivalent weight (mg/g of crude extract).

3. Determination of the anti-oxidative activity

Using modified DPPH assay (Chotimarkorn et al, 2010) and having vitamin C as the reference (IC_{50} = 10.96 µg/ml), the stock solutions of all samples were prepared and diluted using methanol as the solvent to get various concentrations in a 100-µl volume in each well of 96-well plate.

Then, 50 µl of 1 mM methanolic DPPH solution was added and incubated for 20 minutes at 30 °C. In parallel, due to concerning of interference from extract color, the background of each sample was prepared by replacement of the DPPH solution volume with methanol. After incubation, the absorbencies at the wavelength 520 nm were measured in order to calculate for IC_{50} (the concentration causes 50% oxidative inhibition).

4. Determination of the anti-tyrosinase activity

The 1500 unit/ml tyrosinase solution, 2.5mM of L-DOPA solutions and stock solution of samples were separately prepared in 1 mM phosphate buffer pH 6.8, kept in light protected container at -20 °C temperature until use. In the experiment, having Kojic acid as standard reference, the various concentration of samples were prepared by using 1 mM phosphate buffer pH 6.8 as solvent to make a 100-µl volume in each well of 96-well plate. Then, the 50 µl of L-DOPA solution was added, mixed well and incubated at 4°C for 20 minutes before adding of 50 µl tyrosinase solution. The reaction was set under temperature 25 °C for 5 minutes and followed with absorbance measuring

at 450 nm using ultraviolet-visible microplate reader. The obtained absorbencies were used to calculate for %inhibitions of corresponding that the liner relationship was delivered and used to predict the concentration causes 50% tyrosinase inhibition (IC_{50}).

5. Screening of the anti-microbial activity

5.1) Preparation of bacterial innoculum

The bacteria were cultured in suitable agar plates: Blood Agar for *P.acne* and Trypticase Soy Agar (TSA) for *S. aureus* and *S. epidermidis*. Then, the bacterial colonies of each were separately innoculated in 0.85% NaCl to get the bacterial innoculums with approximately density 10^8 bacterial cells/ml which the optical density and turbidity were equivalent to standard McFarland No. 0.5. These were used for anti-microbial activity assay.

5.2) Antimicrobial activity assay

In the experiment, the bacterial agar plates of each bacterial type were separately prepared by spreading over the surface of suitable solid nutrient agar with 100 µl bacterial inoculums, then, waited until getting dried. After that the sterile filter paper disc containing various concentrations of sample extracts 1-5 mg/disc and standard drugs (and solvent background for ethanolic extract) were prepared and placed onto the surface of bacterial agar plates. Erythromycin is the standard drug used for *P.acne* and Amoxicillin (30 µg/disc) for *S. aureus* and *S. epidermidis*. In preparation of ethanolic extract solution, the 95% ethanol in aqueous solution was used as solvent. Then, the disc contained The plates were incubated at temperature of 35 ° C with specific conditions: The TSA agar plates for anti-*P.acne* testing were incubated under anaerobic for 72-96 hours, while, the MHA agar plate for anti-*S. aureus* and *S. epidermidis* testing were incubated under aerobic condition for 16-18 hours incubation.

Results and discussion

1. Yield of extracts

The dried banana blossom material 10 g was obtained from 100 g fresh banana blossom that gave the ethanolic and aqueous extracts as dark green matter with different physical characteristics and %yielded: the viscous mass of ethanolic extract with %yield of 6.23 and dried aqueous extract with %yield of 27.12 were obtained (Table 2).

2. Phenolic content

From gallic acid calibration curve, the liner relationship ($y = 0.294x$; $r^2 = 0.995$) was obtained (Figure 1). The highest content of phenolic compounds was found in ethanolic extract with gallic acid equivalent weight of 61.9±1.33 mg/g followed by aqueous extract 19.73±0.09 (Table 2).

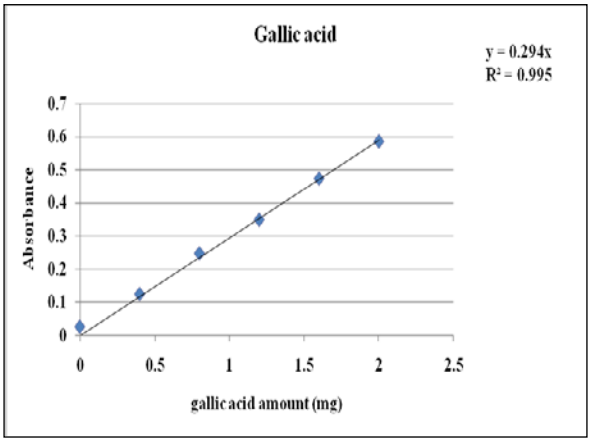


Figure 1 Gallic acid calibration curve

3. Antioxidative activity

Compared to vitamin C with IC_{50} of 10.96±0.65 µg/ml ethanolic extract showed good anti-oxidative effect with the IC_{50} as low as 147.62±1.19 µg/ml, in

contrast, poor activity was found in aqueous extract (IC_{50} = 519.75±13.62 µg/ml) (Figure 2), (Table 3).

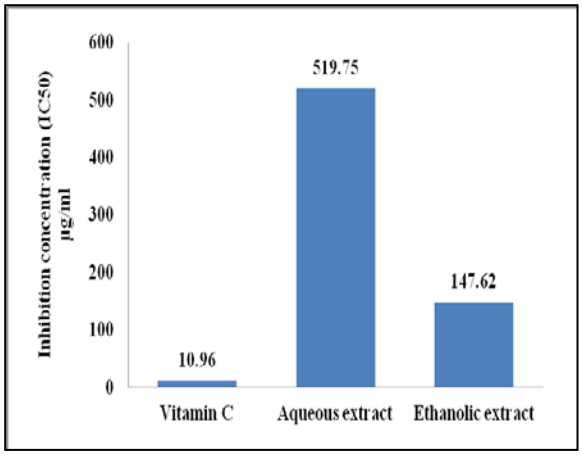


Figure 2 IC_{50} of ethanolic and aqueous extract in DPPH assay

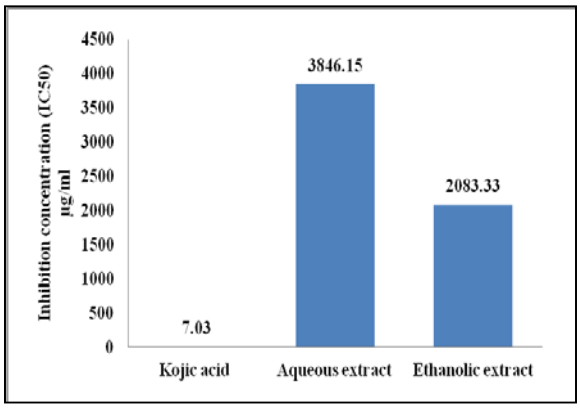


Figure 3 IC_{50} of ethanolic and aqueous extract in tyrosinase assay

4. Anti-tyrosinase activity

Compared to kojic acid with EC_{50} of 7.03 µg/ml the banana blossom extracts showed poor anti-tyrosinase activity with giving EC_{50} range between

5. Anti-bacterial activity

The ethanolic extract showed good anti-bacterial activity against studied bacterial strain as showing of inhibition zone with diameters range between 6.33-12.16 mm in anti-*P.acne*, 6.33-8.50 mm in anti-*S. aureus* and 6.83-15.00 mm in anti-*S. epidermidis* 2083.33±104.66- 3846.15±273.34 µg/ml (Figure 3), (Table 4). testing (Table 5). In contrast, there was no activity found in aqueous extract (Table 1).

Table 1 Anti-bacterial activity of aqueous and ethanolic extracts

Extract	*P. acne*	*S. aureus*	*S. epidermidis*
aqueous extract	-	-	-
Ethanol extract	+	+	+

Table 2 Yields, total phenolic ,anti-oxidative and antiyrosinase activity of banana blossom ethanolic and aqueous extracts.

Fraction	Characteristics	%Yield Total phenolics [a]
Aqueous extract		27.12% 19.73±0.09*
Ethanolic extract		6.23% 61.9±1.33*

[a] expressed as gallic acid equivalent weight (mg/g of dried extract)

*statistic significance (P-value < 0.001)

Table 3 Anti-oxidative activity of vitamin C, banana blossom ethanolic and aqueous extracts .

Extract	anti-oxidative activity		
	Linear regression	**R_2**	**IC_{50}(Mean±SD)**
Vitamin C	Y= 4.5585x	0.9975	10.96±0.33 [a]
Aqueous extract	Y= 0.0962x	0.9679	519.75±13.62 [a,b]
Ethanolic extract	Y= 0.3387x	0.9729	147.62± 1.19 [a,b]

a. statistic significance (P-value < 0.005) compared to vitamin C

b. statistic significance (P-value < 0.001) compared among samples.

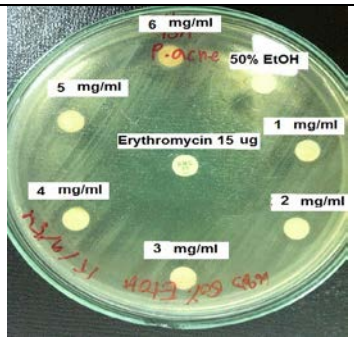
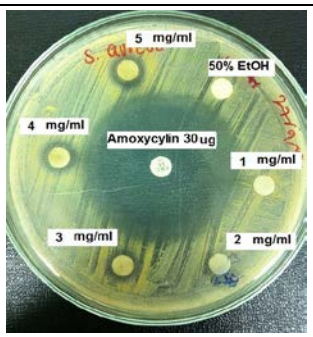
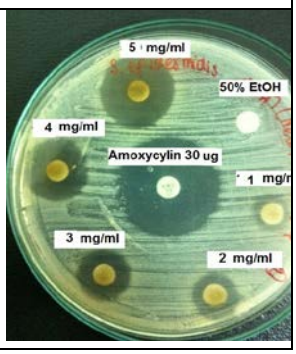
Table 4 Anti-tyrosinase activity of kojic acid , banana blossom ethanolic and aqueous extracts .

Extract	antiyrosinase activity		
	Linear regression	**R_2**	**IC_{50}(Mean±SD)**
Kojic acid	Y= 7.1088x	0.9522	7.03±0.12 [a]
Aqueous extract	Y= 0.0139x	0.9809	3846.15±273.34 [a,b]
Ethanolic extract	Y= 0.024x	0.9804	2083.33± 104.66 [a,b]

a. statistic significance (P-value < 0.005) compared to Kojic acid

b. statistic significance (P-value < 0.001) compared among samples.

Table 5 Inhibition zone diameter of ethanolic extracts and standard drugs

Concentration of extraction (mg/disc)	Diameter (inhibition Zone) (mean± SD; mm)		
	P.acne	*S. aureus*	*S.epidermidis*
1	6.33 ± 0.28	ND	6.83 ± 0.28
2	7.16 ± 0.28	6.33 ± 0.28	7.33 ± 0.57
3	8.33 ± 0.57	7.16 ± 0.28	10.33 ± 0.57
4	9.33 ± 0.28	8.12 ± 0.35	13.66 ± 0.57
5	12.16± 0.28	8.50 ± 0.50	15.05 ± 0.72
95%EtOH	ND	ND	ND
Amoxicillin (30 µg/disc)	-	47.66 ± 2.51	30.33 ± 2.51
Erythromycin (15 µg/disc)	42.66 ± 6.42	-	-
			

EtOH = ethanol

ND = not detected inhibition zone

Discussion

The results obtained from this study demonstrated the differences between aqueous and ethanolic extract of banana blossom which may be attributed to different extraction conditions and chemical compositions. Although on this point, the high temperature used in aqueous extract preparation may partly cause the degradation of bioactive agents in the aqueous extract and resulted in poor efficacy of action (Decena, 2010). As the results, the association between the total phenolic content and biological activities was suggested; the phenolic compounds were highly found in the ethanolic extract and it showed good anti-oxidative and anti-bacterial effects. In contrast, the aqueous extract contained less phenolic compound showed no activity. Compared to aqueous extract, the

better anti-bacterial effects of the ethanolic extract of banana blossom obtained from this present study was demonstrated and complied with previous reports of Tepsorn (2009) and Mumtaz et al (2010); however, different types of bacteria were used in those previous studies. Poor anti-tyrosinase effect in both ethanolic and aqueous extracts of banana blossom was found and may be attributed to (1) the molecular factors of chemical constituents in banana blossom and (2) the selected study model weren't suitable for the bioactive agents contained banana blossom extracts which was reported to contain the tyrosinase-substrate amino acid as tyrosine. Therefore, the whitening effect should be confirmed by using other study models. Moreover, the in-depth studies regarding chemical compositions and pharmacological effects as well as involving mechanism should be done to be as supporting data for utilization of banana blossom in aesthetic and healthcare with safe and effective manners.

Conclusion and suggestion

The data showed differences in biological activity between two fractions of banana blossom extract. The results revealed that the aqueous extract showed higher yield than the ethanolic extract. However, the ethanolic extract revealed higher contents of phenolics and better anti-oxidative and anti-bacterial activities. The banana blossom extracts in all fractions showed poor anti-tyrosinase activity. It may be concluded that the ethanolic extract of banana blossom is a potential fraction containing anti-bacterial anti-oxidative agents. However, furture studies regarding other cosmeceutical effect, mechanisms of action should be done to gain useful and complete information for the most appropriate applications.

Acknowledgement

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