

Antimicrobial, Antioxidant Activities and Total Phenolic Content of Thai Medicinal Plants Used to Treat HIV Patients

Jitpisute Chunthornng-Orn MSc*,
Sumalee Panthong BSc**, Arunporn Itharat PhD**

* Student of Master Degree on Medical Science Program, Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand

** Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand

Background: Opportunistic infections in AIDs patients is the leading cause of death in among them. HIV infection was reported as causes of increasing oxidative stress which may lead to progress of many syndrome. Thus medicinal plants as demonstrated antimicrobial and antioxidant activities would be therapeutic values to treat opportunistic infections of AIDs patients.

Objective: To investigate antibacterial, antioxidant activities and total phenolic contents of five Thai medicinal plants using by Thai traditional doctors to treat opportunistic infections of AIDs patients such as *Dioscorea bulbifera* L. (DB), *Momordica charantia* L. (MC), *Carica papaya* L. (female and male trees, CPF and CPM) and *Phyllanthus amarus* Schum & Thonn. (PA).
Material and Method: The ethanolic and water extracts of those herbs were tested. For antioxidant method was measured using DPPH radical scavenging assay, anti-microbial activity using disc diffusion assay and minimal inhibitory concentration (MIC) was determined by using the modified resazurin assay against four species of micro-organisms: *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Total phenolic content was determined by Folin-Ciocalteu colorimetric method.

Results: For water extract of PA showed the highest antibacterial activity against *S. aureus* (MIC value = 0.625 mg/ml). The ethanolic extract of MC showed the highest activity against *B. subtilis* (MIC = 0.625 mg/ml). Only ethanolic extract of DB inhibited growth of *E. coli* (MIC = 5 mg/ml) it also inhibited growth of gram positive bacteria such as *S. aureus* and *B. subtilis* with the same MIC values (2.5 mg/ml). No plant extracts showed activity against *C. albicans*. The ethanolic extract of CPM, PA and DB and the water extract of PA showed high antioxidant activity (EC_{50} of 8.48, 9.54, 11.07 and 11.37 μ g/ml, respectively). The water extract of PA and the ethanolic extract of DB, CPM showed high total phenolic content of 262.54, 106.26 and 83.78 mg/g, respectively. The total phenolic content of these extracts correlated with DPPH radical scavenging activity, while only ethanolic extract of PA showed high antioxidant activity (9.54 μ g/ml) but it contain low phenolic content (45.50 mg/g).

Conclusion: Our findings support the usage of the these plants to treat opportunistic infection of Thai traditional doctors in AIDs patients. Thus, it is recommended that the isolation of pure active antibacterial and antioxidant from these plant extracts should be carried on.

Keywords: Antimicrobial, Antioxidant, DPPH, *Dioscorea bulbifera* L., *Momordica charantia* L., *Carica papaya* L., *Phyllanthus amarus* Schum & Thonn

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Opportunistic infections among AIDs patients are due to invasive fungal and bacterial infection, the Thai traditional doctors used *Dioscorea bulbifera* L.

Correspondence to:

Itharat A, Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasart University, Rangsit Campus, Klongluang, Pathumthanee 12120, Thailand.
Phone: 0-2926-9749, Fax: 0-2926-9705
E-mail: iarunporn@yahoo.com

(DB), *Momordica charantia* L. (MC), *Carica papaya* L. (female and male trees, CPF and CPM) and *Phyllanthus amarus* Schum & Thonn. (PA) for antifungal and antibacterial infections of AIDs patients. There is the evidence suggesting that the faster progression in AIDs patients is due to increasing oxidative stress⁽¹⁾. Furthermore antioxidant can prevent the pathology of many diseases and biological damage in living tissues which reactive oxygen species (ROS)

or free radical are implicated⁽²⁾. The Thai traditional doctors used these plants for antioxidant of AIDs patients. Thus antimicrobial and antioxidant activities of these plants should be carried on. The objective of this research is to investigate five Thai plants *i.e.* *Dioscorea bulbifera* L. (DB), *Momordica charantia* L. (MC), *Carica papaya* L. (female and male trees, CPF and CPM) and *Phyllanthus amarus* Schum & Thonn. (PA) for antimicrobial and antioxidant activities.

Material and Method

Plant materials

The bulbils of *Dioscorea bulbifera*, the whole part of *Momordica charantia* L., the roots of *Carica papaya* L. (female and male trees) and the whole part of *Phyllanthus amarus* Schum & Thonn were collected from Srakeaw Province. The voucher specimen were deposited at herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkla University, Songkla, Thailand.

Preparation of the plant extracts

Plant materials were washed, sliced thinly, dried in an oven at 50°C, powdered and extracted similar to those practiced by Thai traditional doctors, *i.e.* each dried plant material (300 g) was macerated in 95% ethanol for 3 days, 3 times, filtered and dried filtrate by using an evaporator. For decoction, each residue from maceration (100 g) or each dried plant material (100 g) was boiled in distilled water for 30 minutes, filtered and dried using a lyophilizer. The percentage of yield is shown in Table 1.

Antimicrobial activity

Four microorganisms were used as test organisms *i.e.* *Staphylococcus aureus* (ATCC25923), *Bacillus subtilis* (ATCC6633) *Escherichia coli* (ATCC25922) and *Candida albicans* (90028). The cultures of bacteria and fungi were maintained at 4°C throughout the study and used as stock cultures. The cultivation/assay medium for *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* was Mueller Hinton Agar (MHA) and for *Candida albicans* was Sabouraud Dextrose Agar (SDA) as base medium for the screening of antibacterial and antifungal activity, respectively. Mueller-Hinton broth (MHB) used for preparation of inoculums and was bought from Merck, Germany and all other chemical reagents were analytical reagent grades.

Bacterial cultures for antimicrobial testing were prepared from colonies 16-18 hrs-old MHA plate (for bacteria), 48 hrs-old SDA plate (for fungi) where it was suspended in an appropriate medium (5 ml). Cultures were grown for 2 hrs while continuously shaken at 100 rpm at 37°C. For antibacterial and antifungal activity assays, 1 ml of each culture was diluted with MHB medium to 5×10^5 cfu/ml using densitometer (GrandBio, England).

Disc diffusion assay⁽³⁾ was used for screening according to standard method of NCCLS (2004) and using microtiter plate for determination minimum inhibitory concentration (MIC) of each plant extract⁽⁴⁾ against bacteria and fungi. The concentration of plant extract per disc was 5 mg/disc. The MIC test was modified by adding resazurin after incubating at 37°C

Table 1. Percentage of yield, antioxidant activity and total phenolic content of plant extracts used to treat AIDs patients

Botanical name /Family	Part used	Extraction method	% yield	Antioxidant activity EC ₅₀ (µg/ml) ± SEM	Total Phenolic Content (GAE) (mg/g) ± SEM
<i>Dioscorea bulbifera</i> L. (DB) /DIOSCOREACEAE	Bulbils	Water (H)	8.65	79.02 ± 6.07	17.18 ± 0.15
		Ethanol (E)	2.02	11.07 ± 0.49	106.26 ± 2.23
<i>Momordica charantia</i> L. (MC) /CUCURBITACEAE	Whole plant	Water (H)	15.21	> 100 ± 0	32.28 ± 0.64
		Ethanol (E)	5.08	> 100 ± 0	20.33 ± 0.30
<i>Carica papaya</i> L. (Female tree) (CPF)/CARICACEAE	Roots	Water (H)	16.23	> 100 ± 0	16.71 ± 0.17
		Ethanol (E)	4.12	> 100 ± 0	6.86 ± 0.25
<i>Carica papaya</i> L. (Male tree) (CPM)/CARICACEAE	Roots	Water (H)	11.98	41.66 ± 0.53	32.53 ± 3.05
		Ethanol (E)	0.53	8.48 ± 0.62	83.78 ± 0.76
<i>Phyllanthus amarus</i> Schum & Thonn. (PA)/EUPHORBIACEAE	Whole plant	Water (H)	15.75	11.37 ± 0.95	262.54 ± 3.87
		Ethanol (E)	6.27	9.54 ± 1.47	45.50 ± 2.53
BHT				14.54 ± 0.39	NT

***NT = No test

for 18-20 hrs and incubating further another 2 hrs: following by Sarker et al⁽⁴⁾.

The antimicrobial test were performed in triplicate. Ampicillin and DMSO were used as positive and negative control, respectively.

DPPH radical scavenging assay⁽⁵⁾

Scavenging effect of extracts on DPPH radical were examined based on the method of Yamasaki⁽⁵⁾. This antioxidant activity testing method based on chemical testing. Butylated hydroxytoluene (BHT) was used as reference standard and positive control. Samples for testing were prepared by dissolution in absolute ethanol for ethanolic extracts and dissolution in sterile water for aqueous extracts. Samples were assayed at various concentrations in ranging of 1-100 µg/ml (100, 50, 10 and 1 µg/ml). A portion of sample solution (0.1 ml) was mixed with the same volume of 6×10^{-5} M DPPH in absolute ethanol. After the mixture had been allowed to stand for 30 minutes at room temperature, its absorbance was measured at 520 nm using a spectrophotometer. All tests were determined in triplicate.

The values were reported as means \pm SEM of three determinations. The percentage of inhibition was calculated as follows: % Inhibition = $[(OD_{\text{control}} - OD_{\text{sample}}) / OD_{\text{control}}] \times 100$

EC₅₀ value (effective concentration of sample required to scavenge DPPH radical by 50%) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

Total phenolic content^(6,7)

Phenolic content was determined using colorimetric measurement. The Folin-Ciocalteu reagent used for determination of total phenolic content in plant extracts and they were expressed as gallic acid equivalents (GAE) and the method used was described in Folin and Ciocalteu (1927).

Results and Discussion

Antioxidant activity and total phenolic content

DPPH radical scavenging assay of all extracts showed that the ethanolic extract from the roots of *Carica papaya* L. (Male tree) possessed the strongest antioxidant activity (EC₅₀ = 8.48 ± 0.62 µg/ml), followed by the ethanolic extracts of *Phyllanthus amarus* Schum & Thonn., *Dioscorea bulbifera* L. and the aqueous extracts of *Phyllanthus amarus* Schum & Thonn. (EC₅₀ = 9.54, 11.07 and 11.37 µg/ml, respectively). The activity

of these extract were greater than that of the synthetic antioxidant, BHT (EC₅₀ = 14.54 µg/ml). The aqueous extract of *Phyllanthus amarus* Schum & Thonn. showed the highest total phenolic content (GAE = 262.54 mg/g), followed by the ethanolic extracts of *Dioscorea bulbifera* L. and *Carica* L. (Male tree) (GAE = 106.26 and 83.78 mg/g, respectively). These results are shown in Table 1. All results of antioxidant and total phenolic content of the ethanolic extract of *Dioscorea bulbifera* L. showed relatively high antioxidant activity, also having high content of GAE.

Antimicrobial activity

The screening for antibacterial activity of all plant extracts were evaluated as the inhibition zone (IZ) and the active extract was further determined for MIC values and the results showed in Table 2. The ethanolic extract of *Dioscorea bulbifera* L. showed the highest antimicrobial activity against three strains *i.e.* *S. aureus*, *B. subtilis* and *E. coli* having MIC values of 2.5, 2.5 and 5 mg/ml, respectively. These results are reported for the first time for *Dioscorea bulbifera*. However, the data related with the previous report on antimicrobial study of *Dioscorea membranacea*⁽⁸⁾ (which is in the same family of *Dioscorea bulbifera*). These results are no activity on *Candida albicans*.

The other three plant extracts inhibited only one strain of bacteria *i.e.* MCE, CPFE showed against *B. subtilis*, CPME and PAH showed against *S. aureus* but none of them had activity against *C. albicans*.

Conclusion

The present study demonstrated that the ethanolic extracts of *Dioscorea bulbifera* L., *Momordica charantia* L., *Carica papaya* L. (female and male trees), *Phyllanthus amarus* Schum & Thonn and the aqueous extract of *Phyllanthus amarus* Schum & Thonn. had both antibacterial and free radical

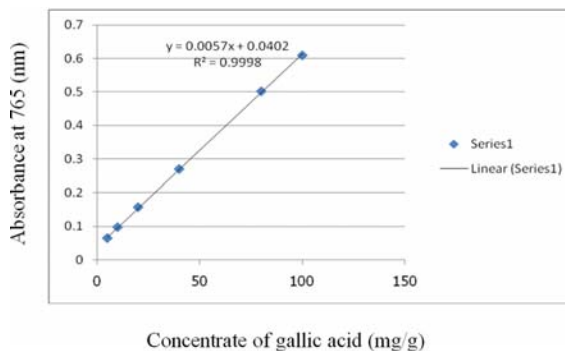


Fig. 1 Standard curve of Gallic acid

Table 2. Antimicrobial activity of nine plant extracts expressed as Inhibition zone diameter (mm) by disc diffusion assay and MIC (mg/ml) determination using the modified resazurin assay

Plant extracts	<i>S. aureus</i>		<i>S. aureus</i>		<i>B. subtilis</i>		<i>B. subtilis</i>		<i>E. coli</i>		<i>E. coli</i>		<i>C. albicans</i>		<i>C. albicans</i>	
	Inhibition zone diameter(mm) Mean \pm SD	MIC (mg/ml) Mean \pm SEM	Inhibition zone diameter(mm) Mean \pm SEM	MIC (mg/ml) Mean \pm SEM	Inhibition zone diameter(mm) Mean \pm SD	MIC (mg/ml) Mean \pm SEM	Inhibition zone diameter (mm) Mean \pm SD	MIC (mg/ml) Mean \pm SEM	Inhibition zone diameter (mm) Mean \pm SD	MIC (mg/ml) Mean \pm SEM	Inhibition zone diameter (mm) Mean \pm SEM	MIC (mg/ml) Mean \pm SEM	Inhibition zone diameter (mm) Mean \pm SEM			
DB H	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI			
MC H	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI			
CPF H	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI			
CPM H	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI			
PA H	10.0 \pm 0.5	0.625	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI			
DB E	8.0 \pm 1	2.5	8.67 \pm 0.58	2.5	11.33 \pm 1.15	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0			
MC E	NI	NI	10.0 \pm 1	0.625	NI	NI	NI	NI	NI	NI	NI	NI	NI			
CPF E	NI	NI	7.0 \pm 0	2.5	NI	NI	NI	NI	NI	NI	NI	NI	NI			
CPM E	7.33 \pm 0.58	> 5	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI			
PA E	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI			
Ampicillin	31 \pm 0	-	35	-	19	-	-	-	-	-	-	-	-			

NI = No Inhibition

scavenging activities while the ethanolic extract of *Phyllanthus amarus* Schum & Thonn. and the aqueous extract of *Dioscorea bulbifera* L. showed only free radical scavenging activity and while the ethanolic extract of *Momordica charantia* L. and *Carica papaya* L. (female trees) showed only antibacterial activity. The ethanolic extract of *Dioscorea bulbifera* L. showed the best antimicrobial activity, highest antioxidant and high total phenolic content. These activities of five these plants were reported for the first time. The ethanolic extract of *Carica papaya* L. (Male trees) possess the best antioxidant activity, high total phenolic content and also showed antibacterial against *S. aureus* where this reported data are also for the first time. These results support the use *Carica papaya* root of male trees as infectious drug by Thai traditional doctors.

The authors findings support the usage of the these plants to treat opportunistic infection of Thai traditional doctors in AIDs patients. Thus, it is recommended that the isolation of pure active antibacterial and antioxidant from these plant extracts should be carried on.

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Potential conflicts of interest

None.

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ฤทธิ์ต้านเชื้อจุลชีพ ฤทธิ์ต้านอนุมูลอิสระและการหาปริมาณ total phenolic ของสมุนไพรไทยที่ใช้ในการรักษาผู้ป่วยติดเชื้อเอชไอวี

จิตพิสุทธิ์ จันทรวงออ่อน, สุมาลี ปานทอง, อรุณพร อธิรัตน์

ภูมิหลัง: ภาวะติดเชื้อฉวยโอกาสของผู้ป่วยเอดส์เป็นสาเหตุหลักที่นำมาสู่การตาย การมีอนุมูลอิสระในร่างกายมากเกินไปในผู้ติดเชื้อเอชไอวีจะกระตุ้นให้ผู้ติดเชื้อมีอาการป่วยต่างๆเกิดขึ้นเร็วกว่าปกติ ดังนั้นสมุนไพรที่มีการพิสูจน์ว่ามีฤทธิ์ต้านเชื้อจุลชีพ และมีฤทธิ์ต้านอนุมูลอิสระ มีคุณค่าแก่การนำมาใช้รักษาผู้ป่วยเอดส์ได้

วัตถุประสงค์: เพื่อตรวจสอบวิเคราะห์หาฤทธิ์ต้านเชื้อจุลชีพ, ฤทธิ์ต้านอนุมูลอิสระ และการหาปริมาณ total phenolic ของสมุนไพรไทยที่หมอพื้นบ้านใช้รักษาผู้ป่วยเอดส์ คือ ว่านคางคก (DB), มะระขี้นก (MC), มะละกอ (ตัวเมีย และตัวผู้, CPF และ CPM) และลูกใต้ใบ (PA) วิธีการสกัดสารเพื่อใช้ในการทดสอบมีทั้งการสกัดชั้นน้ำและการสกัดชั้นเอทานอล ฤทธิ์ต้านอนุมูลอิสระใช้วิธี DPPH radical scavenging assay, ฤทธิ์ต้านเชื้อจุลชีพใช้วิธี disc diffusion assay และ minimal inhibitory concentration (MIC) โดยเชื้อจุลชีพที่ใช้ทดสอบทั้งหมดมี 4 ชนิด คือ *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* และ *Candida albicans* การวิเคราะห์หาปริมาณ total phenolic ใช้วิธี Folin-Ciocalteu colorimetric

ผลการศึกษา: สารสกัดชั้นน้ำของลูกใต้ใบมีฤทธิ์ต้านเชื้อ *S. aureus* ดีที่สุด (MIC = 0.625 mg/ml) สารสกัดชั้นเอทานอลของมะระขี้นกมีฤทธิ์ต้านเชื้อ *B. subtilis* ดีที่สุด (MIC = 0.625 mg/ml) สารสกัดชั้นเอทานอลของว่านคางคกเป็นสารสกัดเพียงชนิดเดียวที่มีฤทธิ์ต้านเชื้อ *E. coli* (MIC = 5 mg/ml) และสารสกัดนี้ยังมีฤทธิ์ยับยั้งแบคทีเรียแกรมบวก ได้แก่ *S. aureus* และ *B. subtilis* โดยมีค่า MIC เท่ากัน เท่ากับ 2.5 mg/ml แต่ไม่มีสารสกัดใดเลยที่มีฤทธิ์ต้านเชื้อ *C. albicans* สารสกัดชั้นเอทานอลของ CPM, PA, และ DB และสารสกัดชั้นน้ำของ PA มีฤทธิ์ต้านอนุมูลอิสระสูง (EC_{50} = 8.48, 9.54, 11.07 และ 11.37 μ g/ml ตามลำดับ) สารสกัดชั้นน้ำของ PA และสารสกัดชั้นเอทานอลของ DB, CPM มีปริมาณ total phenolic สูง (ปริมาณ total phenolic = 262.54, 106.26 และ 83.78 mg/g ตามลำดับ) ปริมาณโททอลฟีนอลิกของสารสกัดเหล่านี้มีความสัมพันธ์ กับความสามารถในการต้านอนุมูลอิสระด้วยวิธี DPPH radical scavenging ขณะที่สารสกัดชั้นเอทานอลของ PA เป็นสารสกัดเพียงตัวเดียวที่มีปริมาณ total phenolic ต่ำ (45.50 mg/g) และไม่สอดคล้องกับความสามารถ ในการต้านอนุมูลอิสระด้วยวิธี DPPH radical scavenging (EC_{50} = 9.54 μ g/ml)

สรุป: จากผลการทดลองทั้งหมดที่ได้สามารถสนับสนุนการใช้สมุนไพรเหล่านี้ในการรักษาผู้ป่วยติดเชื้อเอชไอวีต่อไป และควรจะแยกหาสารสำคัญที่ออกฤทธิ์ต้านเชื้อจุลชีพ และต้านอนุมูลอิสระเพื่อพัฒนาเป็นยารักษาผู้ป่วยติดเชื้อเอชไอวีในอนาคต