Antibacterial, Total Phenolic Content and Antioxidant Activities of Medicinal Plant Extracts

ฤทธิ์ต้านแบคทีเรีย ปริมาณสารประกอบฟีนอลิค และฤทธิ์ต้านออกซิเดชัน ของสารสกัดพืชสมุนไพร

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ABSTRACT

Medicinal plant has many benefits for treatment of disease for long time through the traditional medicine and become the world commonly used as a traditional treatment and modern western medicine. The purpose of this study was to observe antibacterial activities of medicinal plant extracts for inhibition of pathogenic bacteria. The result showed that aqueous extract of *Chromolaena odorata* was the most effective extract, which could inhibit all tested bacteria with inhibition zone ranging from 8.0-14.2 mm. The aqueous extracts of *Curcuma longa* against *S. aureus, S. epidermidis* and MRSA showed minimal inhibitory concentration and minimal bactericidal concentration of 19.38 mg/ml. Moreover, the extract of *C. odorata* showed the highest phenolic compound content of 68.697±3.189 mg GAE/g extract. Antioxidant activity was also determined by DPPH assay and the highest activity of 266.084±1.749 mg GAE/g extract was from the extract of *C. longa*.

บทคัดย่อ

พืชสมุนไพรหลายชนิดมีประโยชน์ในการรักษาโรคมายาวนาน โดยสืบทอดความรู้ทางการแพทย์มาจากภูมิปัญญาชาวบ้าน ดังนั้นงานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลการยับยั้งการเจริญของสารสกัดพืชสมุนไพรต่อแบคทีเรียก่อโรค โดยพบว่าสารสกัดน้ำของสาบเสือให้ผลการยับยั้งแบคทีเรียก่อโรคได้อย่างมีประสิทธิภาพที่สุด ซึ่งสามารถยับยั้งการเจริญของแบคทีเรียทดสอบได้ทุกชนิดโดยมีขนาดเส้นผ่านศูนย์กลางวงใสอยู่ที่ 8.0-14.2 มิลลิเมตร และพบว่าสารสกัดน้ำของขมิ้นฤทธิ์ต่อเชื้อ *Staphylococcus aureus, S. epidermidis* และ methicillin resistant *S. aureus* ดีที่สุดโดยมีค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งและฆ่าเชื้อแบคทีเรียที่ 19.38 มิลลิกรัมต่อมิลลิลิตร นอกจากนั้นยังพบว่าสารสกัดจากสาบเสือให้ปริมาณสารประกอบฟีนอลิคสูงที่สุดที่ 68.697±3.189 mg GAE/g Extract ส่วนฤทธิ์ต้านออกซิเดชันโดยวิธี DPPH พบว่าสารสกัดจากขมิ้นให้ผลต้านออกซิเดชันสูงที่สุดคือ 266.084±1.749 mg GAE/g Extract

Key Words: Pathogenic bacteria, medicinal plant extract, antioxidant activity

คำสำคัญ: แบคทีเรียก่อโรค สารสกัดพืชสมุนไพร ฤทธิ์ต้านออกซิเดชัน

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Introduction

Bacteria on skin are normally normal flora bacteria but they can be opportunistic pathogens and can cause the disease by infection of host skin such as wound (Brown et al., 2012). However, treatment of these pathogens by antibiotics led to an increase of drug resistant pathogenic bacteria that could cause the infectious disease and becomes a worldwide health problem. Moreover, using some modern drugs might cause side effect to human body (EL-Zawahry et al., 2013)

According to folk wisdom, medicinal plants could be used for treatment and prevention infectious disease. Medicinal plants have played an important role in being a source of new drugs since the plants had been used to treat the severe disease. Some chemicals in medicinal plant could inhibit drug resistant pathogenic bacteria, (EL-Zawahry et al., 2013). Moreover, medicinal plants also promoted health benefit (Mazid et al., 2012). Therefore, medicinal plants were used worldwide and being one of most important sources of medicines (Mazid et al., 2012; Nitha et al., 2012).

Antimicrobial activities of medicinal plant were presented in many studies and a wide range of medicinal plant had been reported that different parts of medicinal plant demonstrated properties for various treatments. Although, there were several medicinal plants had been tested for antimicrobial activities but many of them had not been investigated (Mahesh, Satish, 2008).

Free radicals demonstrated an important role in some serious disease such as cancer, inflammation and diabetes (Liu et al., 2008). Recently, many reports have shown that phenolic compounds were known as good natural antioxidants (Srinivasa et al., 2010). The phenolic compounds that normally found in many plants, fruits, vegetables and cereals extracts showed strong antioxidant activity and might promote cell protection against the oxidative reaction caused by free radicals (Liu et al., 2008; Wang et al., 2013).

Objective of the study

The aim of this study was to observe the antibacterial activities, total phenolic content and antioxidant activity of some medicinal plant extracts.

Methodology

Preparation of medicinal plant extracts

Medicinal plants were extracted by distilled water at 45ºC for 3 hours and the extraction was repeated once. Next, the extracts were filtered using steriled Whatman No.1 and the solvent was evaporated by using evaporator. Finally, the extracts were dried by lyophilizer and the crude extracts were kept at -20ºC. The extracts were dissolved with dimethyl sulphoxide to a concentration of 500 mg/ml before use (Mahesh, Satish, 2008).

Antibacterial activities

Medicinal plant extracts were tested against *Staphylococcus aureus, Staphylococcus epidermidis,* Methicillin Resistant *S. aureus* (MRSA), *Micrococcus luteus, Pseudomonas aeruginosa, Shigella dysenteriae, Salmonella typhi* and *Escherichia coli* by agar disc diffusion method (Pitinidhipat, Yasurin, 2012) and broth dilution method by culturing tested bacteria for 18-24 hours in broth media and adjusted the turbidity of tested bacteria with McFarland No. 0.5, which approximately corresponds to 1.5×10^8 CFU/ml (Murray et al., 2007). The McFarland standard No. 0.5 contained 1% barium chloride (0.05 ml) and 1%

sulfuric acid (9.95 ml). Then tested bacteria were swabbed over Mueller Hinton agar (MHA). Then, paper discs were dipped in medicinal plant extracts and placed on MHA plates. The plates were incubated at 37ºC for 18-24 hours and the inhibition zones were observed.

Furthermore, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by broth dilution method (Pitinidhipat, Yasurin, 2012). The turbidity of tested bacteria was adjusted to McFarland No. 0.5 and incubated with medicinal plant extracts at different concentrations ranging from 0.49 - 250 mg/ml. The MIC was determined as the lowest concentration of medicinal plant extract that could inhibit tested bacteria. The tubes that showed no growth of bacteria after treatment with medicinal plant extract were chosen to streak plate. The plate that showed the bacterial growth inhibition by 99.99% after treatment with the lowest concentration of medicinal plant extract was recorded as the MBC.

Total phenolic content

Total phenolic content of medicinal plant extracts were measured by Folin–Ciocalteu method (Chandler and Dodds, 1983). Medicinal plant extracts were prepared at a concentration of 1 mg/ml. The extracts (500 µl) was mixed with deionized water (1,250 µl), 95% ethanol (250 µl), and 50% folin-ciocalteu (125 µl). After that, the mixture was incubated in the dark at room temperature for 5 minutes. Finally, 5% Na_2CO_3 (250 µl) was added to stop the reactions and incubated in the dark at room temperature for 1 hour. Total phenolic content were determined using gallic acid as a standard compound in concentration of 0.01-0.10 mg/ml and the absorbance was measured at 725 nm. Content of total phenolic compounds have been expressed as mg of gallic acid equivalent per 1 g of sample extracts (mg gallic acid/g extract).

Antioxidant activity

Antioxidant activity of medicinal plant extracts was determined by DPPH radical scavenging assay (Hou *et al*., 2001). Medicinal plant extracts (500 µl) at different concentrations ranging from 0.005-10 mg/ml were mixed with 1,500 µl of 0.1 mM DPPH (Sigma-Aldrich) reagent in methanol. The mixture was incubated in the dark for 20 minutes at room temperature, then the absorbance was observed at 517 nm. Standard solution of gallic acid (0.001-0.01 mg/ml) was prepared in methanol. The radical-scavenging activities of the samples were expressed as percentage of inhibition and calculated using $[(A_0 – A_1)/A_0] \times 100$. A_0 was the absorbance of the methanol control and A_1 was the absorbance of the samples. The IC_{50} value demonstrated the effective concentration of sample that used to reduce DPPH radicals by 50%. The antioxidant activity of the sample was calculated by IC_{50} gallic acid / IC_{50} sample x 1000 and expressed as mg of gallic acid equivalent (GAE) per 1 g of extract.

Results

Antibacterial activities of medicinal plant extracts (500 mg/ml) were determined by agar disc diffusion method. The results showed that *Chromolaena odorata* aqueous extract could inhibit all tested bacteria; *S. aureus, S. epidermidis,* MRSA, *M. luteus, P. aeruginosa, S. dysenteriae, S.* typhi and *E. coli* with inhibition zones ranging from 8.0 ± 0.5 to 14.2 ± 0.8 mm. Moreover, *Centella asiatica* aqueous extract inhibited all tested bacteria except *S. aureus* with inhibition zones ranging from 7.8 ± 0.8 to 13.5 ±

0.5 mm. *Curcuma longa* aqueous extract inhibited only *S. aureus, S. epidermidis,* MRSA, *M. luteus, P. aeruginos* with inhibition zones ranging from 7.2 ± 0.3 to 9.3 ± 0.6 mm. *Centella asiatica, Chromolaena odorata*, *Curcuma longa* medicinal plant extracts could inhibit methicillin resistant *S. aureus* (Table 1).

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of aqueous extracts were shown in Table 2. The highest activity of *C. longa* aqueous extracts was observed after tesing *C. longa* extracts against *S. aureus, S. epidermidis* and MRSA with MIC and MBC of 19.38 mg/ml.

Phenolic content of the plant extract was shown in Figure 1. Total phenolic content of *Erythrina variegata, Moringa oleifera, Centella asiatica, Rhinacanthus nasutus,Amomum krervanh, Chromolaena odorata, Thunbergia laurifolia, Curcuma longa, Chrysanthemum indicum* and *Acacia cincina* were 47.685±2.911, 31.133±2.091, 27.408±1.148,25.620±3.237, 17.448±0.497, 68.697±3.189, 26.208±2.499, 22.054±0.890, 26.747±0.511 and 51.422±1.780 mg GAE/g extract, respectively. The extract of *C. odorata* gave the highest activity followed by the extract of *A. concinna* and *E. variegata*.

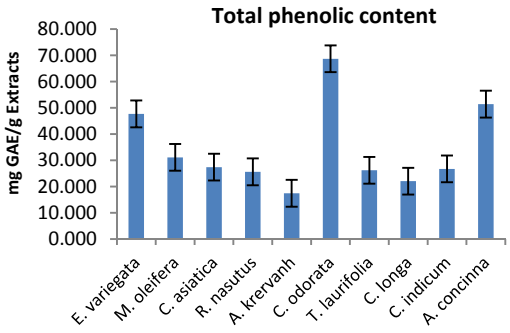

Figure 1 Total phenolic content of aqueous plant extracts

The result of antioxidant activities of aqueous extract was expressed as mg of gallic acid equivalent (GAE) per 1 g of extract (Figure 2). Therefore, antioxidant activities of *Erythrina variegata, Moringa oleifera, Centella asiatica, Rhinacanthus nasutus, Amomum krervanh, Chromolaena odorata, Thunbergia laurifolia, Curcuma longa, Chrysanthemum indicum* and *Acacia cincina* were 13.595±0.013, 10.342±0.440, 16.569±1.248, 11.522±0.345, 9.884±0.474, 10.257±0.353, 39.725±2.508, 266.084±1.749, 11.536±0.167 and 1.268±0.058 mg GAE/ g extract, respectively. The extract of *C. longa* gave the highest antioxidant activity followed by the extracts of *T. laurifolia* and *C. asiatica.*

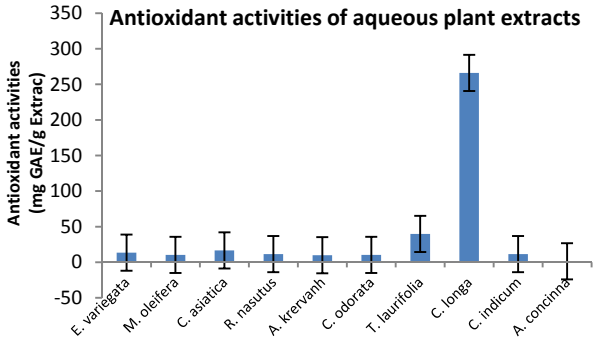

Figure 2 Antioxidant activities of aqueous plant extracts

Table 1 Antibacterial activities of aqueous plant extracts by agar disc diffusion method

Aqueous medicinal plant extracts	Inhibition zone (millimetre)							
	S. aureus	*S. epidermidis*	MRSA	*M. luteus*	*P. aeruginosa*	*S. dysenteriae*	*S.* Typhi	*E. coli*
Erythrina variegata	-	-	-	-	-	8.3 ± 0.8	-	-
Moringa oleifera	-	-	-	-	-	-	-	-
Centella asiatica	-	7.8 ± 0.8	11.3 ± 1.0	13.5 ± 0.5	8.3 ± 0.6	11.3 ± 0.8	11.7 ± 0.8	11.3 ± 0.8
Rhinacanthus nasutus	-	-	-	-	-	-	-	-
Amomum krervanh	-	-	-	-	-	-	-	-
Chromolaena odorata	8.0 ± 0.5	10.2 ± 0.8	11.0 ± 0.5	14.2 ± 0.8	9.8 ± 0.3	11.0 ± 1.0	11.3 ± 0.3	12.3 ± 0.8
Thunbergia laurifolia	-	-	-	-	14.3 ± 0.6	-	-	-
Curcuma longa	7.3 ± 0.3	9.3 ± 0.6	7.5 ± 0.0	9.3 ± 0.3	7.2 ± 0.3	-	-	-
Chrysanthemum indicum	-	-	-	-	-	-	-	-
Acacia concinna	-	7.2 ± 0.3	-	-	7.3 ± 0.3	-	-	-

Discussion and conclusions

Medicinal plants could be used for the source of new drugs development. Some medicinal plant extracts could be used to inhibit the growth of bacteria and multi-drug resistant pathogenic bacteria (EL-Zawahry et al., 2013). Theses pathogenic bacteria could infect to human body and caused the disease (Burton et al., 2011)

Aqueous extract of *Chromolaena odorata* was the most effective extract, which could inhibit all tested bacteria with inhibition zone ranging from 8.0-14.2 mm. MIC and MBC of the plant extracts were investigated and the highest anti-bacterial activity of *C. longa* aqueous extracts was observed against *S. aureus, S. epidermidis* and MRSA with MIC and MBC of 19.38 mg/ml. *Centella asiatica, Chromolaena odorata*, *Curcuma longa* medicinal plant extracts could inhibit methicillin resistant *S. aureus*. Moreover, *C. longa* extract gave the highest DPPH radical scavenging activity by 266.084±1.749 mg GAE/g extract assay.

Curcumin was demonstrated as an active compound that produced from the rhizome of *C. longa* and it had several properties such as antioxidant, anti-inflammation and antiviral activities. Moreover, the study showed that curcumin was not toxic to human cells and could inhibit growth of a variety of bacteria and pathogenic fungi (Akram et al., 2010).

The highest amount of total phenolic content was from the extract of *C. odorata* (68.697±3.189 mg GAE/g extract). *C. odorata* also showed the efficacy of antibacterial activities against all tested bacteria. The results were in agreement with the study of phenolic content, which mostly played an important role in the inhibition the growth of various microorganism and mycotoxin production as well as suppressing harmful toxic effects (Prakash et al., 2012)

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Table 2 The antibacterial activities of aqueous plant extracts by broth dilution method

Aqueous medicinal plant extracts	Concentration of aqueous medicinal plant extracts (mg/ml)															
	S. aureus		***S. epidermidis***		**MRSA**		***M. luteus***		***P. aeruginosa***		***S. dysenteriae***		***S.* Typhi**		***E. coli***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. variegata	-	-	-	-	-	-	-	-	-	-	31.25	250.0	-	-	-	-
M. oleifera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. asiatica	-	-	62.50	125.0	62.50	125.0	15.63	125.0	62.50	62.50	62.50	125.0	62.50	125.0	62.50	125.0
R. nasutus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. krervanh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. odorata	62.50	125.0	31.25	62.50	31.25	62.50	15.63	62.50	31.25	62.50	31.25	125.0	31.25	62.50	31.25	125.0
T. laurifolia	-	-	-	-	-	-	-	-	125.0	250.0	-	-	-	-	-	-
C. longa	19.38	19.38	19.38	19.38	19.38	19.38	38.75	77.50	38.75	38.75	-	-	-	-	-	-
C. indicum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. concinna	-	-	31.25	125.0	-	-	-	-	62.50	62.50	-	-	-	-	-	-