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Antibacterial Activities of The Extracts of *Mimosa pudica* L. An in-vitro Study

Nguyen Thi Le Thoa, Pham Cam Nam and Dang Minh Nhat

[#] *Chemical Engineering Faculty, Danang University of Science and Technology, 54 Nguyen Luong Bang, Dannang, 550000, Vietnam*
E-mail: lethoatcv@yahoo.com

Abstract*— *Mimosa pudica L.*, also called sensitive plant or touch-me-not, belongs to the genus Mimosa (Family: *Mimosaceae*). This plant grows as a weed in nearly every parts of Vietnam and is used as a traditional medicine for the treatment of some diseases. This study aims to evaluate the antibacterial activity of the water and ethanol extracts of this plant by using disc diffusion method. The total flavonoid as quercetin equivalent (QE) per gram (dry weight) of these two extracts was also estimated. The result of tests for in-vitro antibacterial activity indicates that the ethanol extract showed significant activity against *E.coli, S.aureus, B.subtilis and S.typhi* with the zone of inhibition was 11mm, 19mm, 17mm and 16mm respectively. The water extract only inhibited the growth of *S.aureus* (14mm) and *B.subtilis* (15mm) and there was no resistance against *E.coli* and *S.typhi*. The analysis of total flavonoid content found that the ethanol extract contains higher amount of flavonoid than water extract and flavonoid is responsible mainly for the antibacterial activity of *Mimosa pudica L.

***Keywords*— *Mimosa pudica L.*; antibacterial; inhibition zone; *E.coli; S.aureus; B.subtilis; S.typhi;* flavonoids.**

I. INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health. Especially, in recent years, plants with various biological properties such as antioxidant, antimicrobial, and anti-inflammatory have been introduced and investigated increasingly in pharmaceutical and food industries since some reports showed that medicine derived from plant sources is free from side effects on human health compared to synthetic substances [1]. In fact, around 80% of the population from developing countries still use medicinal plants for their primary health care [2]. Therefore, there is a requirement to explore properties, safety and efficiency of plant as an alternative to synthetic compounds.

The antimicrobial property of compounds extracted from medicinal plants is of great significance for medical and food application. These compounds are synthesized in the secondary metabolism of the plant such as flavonoids, tannin and essential oils [1].

Flavonoids belong to phenolic group. Based on chemical structure, flavonoids are classified into flavonols, flavones, flavonones, isoflavones, catechin, anthocyanidins and chalcones [3]. Flavonoids can resist to microbial because they inhibit the synthesis of nucleic acid, function of cytoplasmic membrane and energy metabolism [4]. Flavonoids are found in various plants and *Mimosa Pudica* is an example.

Mimosa pudica L., also called sensitive plant or touch-me-not, belongs to the genus Mimosa (Family: *Mimosaceae*). This plant grows as a weed in nearly every parts of Vietnam. *M.pudica* has been used as a traditional medicine for the treatment of some diseases and conditions including diarrhea, insomnia, tumor, headache, skin conditions, fever and blood pressure. Some studies showed the presence of various bioactive compounds in this plant like tannins, steroids, flavonoids, glycosides, non-protein amino acid leucenine (mimosine), alkaloids [5, 6].

On the basis of these backgrounds, this study was conducted to investigate the *in-vitro* antimicrobial activities of the *M. pudica* extracts from water and ethanol solvents against clinically important pathogens namely *E.coli*, *S.aureus*, *B.subtilis* and *S.typhi.* It also determined the total flavonoids amount in these two extracts.

II. MATERIAL AND METHODS

A. Collection and preparation of plant material

The leaves and stem of *M.pudica* was collected from suburb of Danang city, Vietnam. They then were thoroughly rinsed under water, dried to the moisture content of 8-10% and stored in an air tight container.

B. Preparation of bacteria cultures

The Gram-negative and –positive bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus, Salmonella typhi* were provided by Nation Agro-Forestry-Fisheries Quality Assurance Department Zone 2 in Danang and Tamky Preventive Medicine Center, Quangnam Province. These cultures were grown on nutrient agar for 24h at 37^0C and then cultured in nutrient broth for next experiments.

C. Preparation of extracts:

It is assumed that flavonoids are responsible for the antibacterial of *M.pudica*. Therefore, the water and ethanol extracts of *M.pudica* were conducted at optimal conditions to obtain the highest content of flavonoids. Based on the research in the lab (that is not presented here), the water and ethanol extracts were prepared as follow:

+ Water extract: 2g of the dry leaves and sterm of *M.pudica* and 84 mL of water were added into the round bottom flask. The extraction was conducted at 92^0C and 35 minutes. After that, the extract was filtered and evaporated to the final volume of 25 mL

+ Ethanol extract: 2g of the dry leaves and sterm of *M.pudica* and 72 mL of ethanol 90% (v/v) were added into the round bottom flask. The extraction was conducted at 72^0C and 37 minutes. After that, the extract was filtered and solvent was totally removed in rotary evaporator. The selected sample was then diluted in distilled water to the final volume of 25 mL.

D. Agar well diffusion method

Antibacterial activity was carried out using agar well diffusion method. Firstly, 100µl of tested bacteria suspension was spread on the nutrient agar plate. Then, it was placed at room temperature to dry. A sterile cork borer of 8mm diameter was used to make wells on the medium. 100 µl of the extract was dropped into the well. The plate then was left for 30 minutes at room temperature to diffuse the extract and incubated at 37^0C for 24 h. The extract has antimicrobial activity if a clear, distinct zone of inhibition appear surrounding the medium. The antimicrobial activity of the extract was determined by measuring the diameter of zone of inhibition in terms of millimeter. Water and chloramphenicol were used as negative and positive control. These control samples were prepared as the same procedure but water and chloramphenicol was replaced tested extract respectively. The experiment was performed in triplicate.

E. Test for the presence of flavonoid

Shinoda test was used to determine the presence of flavonoid. Particularly, 1mL of the extract, few drops of *conc.* HCl and magnesium were added into test tube. The solution turned into pink coloration indicating the presence of flavonoids [3]

F. Determination of total flavonoid content:

Total flavonoid content was determined using aluminium chloride colorimetric method. 1 mL of the extract, 3 mL of methanol, 0.2 mL of aluminum chloride, 0.2 mL of 1 M sodium acetate and 5.6 mL of distilled water were mixed together in the tube. Then it was placed at room temperature for 30 minutes. After that, the absorbance of the reaction mixture was measured at 415nm with spectrophotometer against blank. The blank sample was prepared by using methanol instead of water. The total content of flavonoid compounds was expressed as quercetin equivalents and was calculated using the standard curve of quercetin (QE). The experiment was performed in triplicate.

III. Result and Discussion

A. Total flavonoid content of the water and ethanol extract

The Shinoda test revealed Flavonoid in the water and ethanol extract of *M.pudica L.* Table 1 shows the total flavonoid content of the water and ethanol extract using aluminium chloride colorimetric method. The total flavonoid content values (y) were obtained from the standard curve of quercetin: $y = 1.529x - 0.005$, $R^2 = 0.9975$ (figure 1) where x is the absorbance and y is the concentration of quercetin (mg/mL).

TABLE I
THE TOTAL FLAVONOID CONTENT OF WATER AND ETHANOL EXTRACTS

Solvent	Flavonoid content (mg/mL)
Water	0.847
Ethanol	2.436

It is clear that the total flavonoid content in ethanol extract of *M.pudica* was about 3-fold more than in water extract. This result indicated that ethanol was better solvent for the extraction of flavonoid in *M.pudica* than water. This means that flavonoid compounds in *M.pudica* have polarity and chemical characteristics that are much more soluble in ethanol than in water. Ethanol has been known as a good solvent for flavonoid extraction and is safe for human consumption [7].

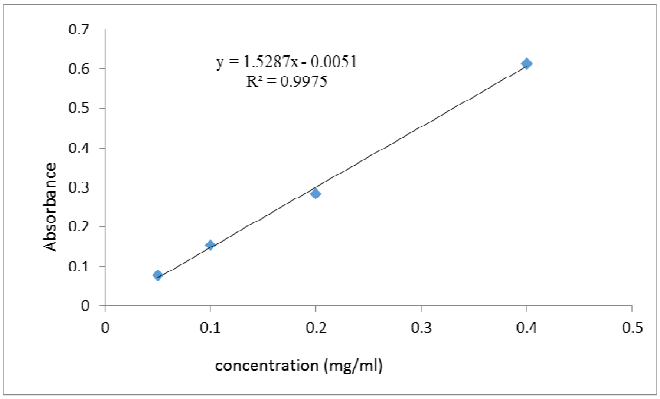

Fig. 1 The standard curve of quercetin

The result is coincident with the extraction of flavonoid from *Limnophila aromatic* using various extraction solvent[7], and from *Azadirachta indica* [8]

B. Antibacterial activities of the water and ethanol extract of M.pudica

The water and ethanol extracts of *M.pudica* were tested again some selected bacteria using agar well diffusion method as described in 2.4. The antibacterial activity of these two extract is presented in the Table 2.

The antibacterial activity of *M.pudica* extracts on the agar plates varied in water and ethanol solvents ranging from 11 to 19 mm as shown in Table 2. The positive controls showed significantly inhibition zone against the selected bacteria, but there was no inhibitory effect against selected bacteria of negative control.

TABLE II
ANTIBACTERIAL ACTIVITY OF THE WATER AND ETHANOL EXTRACTS OF M.PUDICA

Bacteria / Extract	Zone of inhibition (mm)			
	E.coli	***S.aureus***	***B.cereus***	***S.typhi***
Water	0	14	15	0
Ethanol	11	19	17	16
Positive control	33	34	38	38
Negative control	-	-	-	-

The ethanol extract exhibited higher antibacterial property than water extract. In fact, the ethanol extract resisted four tested pathogens (figure 2) while water extract revealed no inhibition of growth of *E. coli* and *S. typhi*. Moreover, the inhibition zone produced by the ethanol solvent against selected bacteria was larger than water solvent. Therefore, the microorganisms' responses were different to the different extracts of *M.pudica*.

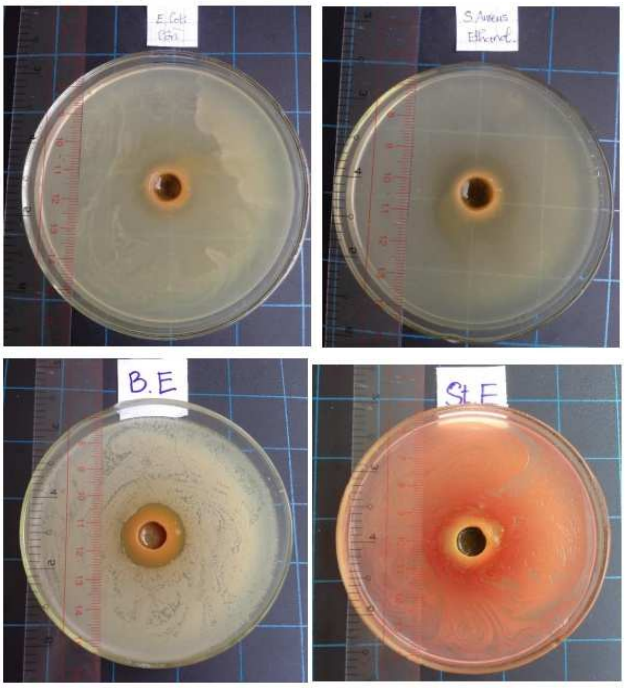

Fig. 2 Inhibition zone of ethanol extract against (A) *E.coli*; (B) *S.aureus*; (C) *B.cereus* and (D) *S.typhi*

This study showed that ethanol extracts of *M.pudica* gave stronger inhibition to Gram positive bacteria compared to Gram negative bacteria and no resistant activity against Gram negative bacteria was observed in water extract. This result is similar to various research reported. Rudi Hendra *et al* (2011) indicated that the extracts from pericarp, medocarp, and seed of *Phaleria macrocarpa* have higher inhibition zone to Gram positive bacteria than to Gram negative bacteria. Another study about antimicrobial of hydroponically grown pegaga extracts showed that gram positive microorganisms are typically more susceptible to antimicrobial agents than gram negative bacteria [9]. This might be because Gram negative bacteria possess an outer membrane which is not present in Gram positive bacteria. This membrane acts as a permeability barrier which limits access of the antimicrobial agents to their targets in the bacterial cells [4].

The antibacterial activity of these extracts might be due to the presence of flavonoid compounds. It has been reported that phenolic compounds and flavonoids from the extracts of many medicinal plants reveal antimicrobial activity. Flavonoids such as robinetin, myricetin and (-)-epigallocatechin inhibited the growth of *Proteus vulgaris* and *S.aureus* [10]. Another study indicated that kaempferol, myricetin, naringin, quercetin and rutin contributed to the inhibitory effect against microorganisms of *P. macrocarpa* [4]. According to Cushnie *et al* (2005), the antimicrobial activity of flavonoids compounds against human pathogenic microorganisms can be classified into three mechanisms [11]. The first mechanism is that flavonoids can inhibit the synthesis of nucleic acid since the B ring of the flavonoids relates to the intercalation or hydrogen bonding with the stacking of nucleic acid bases. The flavonoids also influence the synthesis of protein and lipid but to a lesser degree. Another antimicrobial mechanism of flavonoids is the inhibition of cytoplasmic membrane function by reducing membrane fluidity of bacterial cells, changing the permeability of the cellular membrane and damaging membrane function. Moreover, flavonoids show the inhibitory effect on bacteria by the inhibition of energy metabolism which is necessary for active uptake of various metabolites and for biosynthesis [11]. This explanation is in agreement with result of this research. Since the total flavonoids content in ethanol extract is higher than in water extract, the ethanol extract possess stronger antibacterial activity than the water extract.

IV. CONCLUSIONS

This study showed that the selected *M.pudica* extracts have antibacterial activity against *E.coli*, *S.aureus*, *B.cereus* and *S.typhi*. The inhibitory effect is stronger in Gram positive bacteria than the Gram negative bacterial. The higher antibacterial activity of ethanol 90% (v/v) extract compared to water extract probably relates to the flavonoid content in these two extracts. Further investigation should be done such as toxicity of the active compounds, their side effects, antibacterial activity of purified active compounds in order to deeply understand about bioactive components in *M.pudica*

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