Antioxidant and Antimicrobial Activities of the Extracts of *Sophora flavescens*

Cheng-Hong Yang, Chi-Chun Wu, and Li-Yech Chuang*

Abstract—The main goal of this study was to determine the antibacterial and antioxidant activities of various extracts, including 95% ethanol crude extracts, hexane fraction, ethyl ether fraction, ethyl acetate fraction, and aqueous fraction, from *Sophora flavescens*, an indigenous herb, widely used as a traditional medicine in Taiwan. Antioxidant activity was determined by the methods of DPPH radical scavenging test, trolox equivalent antioxidant capacity, and reduction capacity. In three methods, the extracts of ethyl acetate exhibited a higher antioxidant activity than other extracts. Antibacterial activity was performed by disk diffusion method, the minimum inhibitory concentration, time-killing curve and synergy effect. The clinical antibiotic isolates including gram-positive and gram-negative pathogens were used for antimicrobial activity assay. Experimental results showed that the extracts of ethyl acetate had a significant antibacterial activity against *Staphylococcus aureus*. A higher content of both total phenolics and flavonoids were found in the ethyl acetate extract which correlated with better biological activities compared with other extracts. These results reveal that the extracts of ethyl acetate from *Sophora flavescens* could be developed as a potential natural antioxidant and antibacterial agent.

Keywords—*Sophora flavescens*, antioxidant activity, antibacterial activity

I. INTRODUCTION

Several recent studies indicate that Chinese medicinal plants contain a wide variety of natural antimicrobial substances, such as terpenes, flavonoids and phenolic compounds [1]. Researchers have been interested in biologically active compounds isolated from Chinese medicines to inhibit the spread of pathogenic microorganisms. Multiple resistances in human pathogenic microorganisms have globally developed, however, and caused serious nosocomial infections. Among the nosocomial drug-resistant strains, Acinetobacter baumannii, *Pseudomonas aerugiosa* and *Staphylococcus aureus*, were the most prevalent isolates in Taiwan [2]. The recent emergence of drug-resistant strains is deeply worrisome and highlights the urgent need for novel antibacterial agents. From this point of view, it is important to identify new sources of safe and inexpensive antimicrobial substances that occur naturally.

As age or living increase in pressure, in vivo oxidation-reduction reactions of balance, anti-oxidation system and the free radical mechanism of the relationship between the dynamics of the balancing act is no longer. Due to excessive reactive oxygen species triggered by a number of age-related diseases, such as atherosclerosis, hypertension, diabetes, accelerated aging of the human body cause cancer or genetic mutations; these are the targets of concern to the medical profession [3].

*Sophora flavescens* is a species of evergreen shrub ranging throughout the temperate regions of Asia. This plant has a slow rate of growth, with adult species reaching about 1.5 meters in height. It keeps its leaves all year, and blooms yellow flowers during July and August. The roots and bark of *S. flavescens* are harvested in autumn and dried prior to use in topical and internal preparations. Asian traditional medicine systems consider roots of this species to possess the following properties: antibacterial, antihelmintic, astringent, diuretic, and tonic. In traditional Chinese medicine this herb is specifically directed towards addressing situations of “damp heat” and “wind qi-stagnation” [4].

II. METHODS

A. Disc diffusion method

The petri dish was prepared with a base layer of Muller Hinton (MH) agar (10 mL) and a top layer of 0.75% MH agar (5 mL), then inoculated with 50 μL of each bacterial suspension (10^6 cfu/mL). Paper discs (8 mm in diameter) were impregnated with 30 μL of crude extract (3 mg/disc), and placed on the inoculated plates, then incubated at 37 °C for 14 hr. The diameters of the inhibition zones (DIZ) were measured [5].

B. Minimum Inhibition Concentration (MIC), Minimum Bactericidal Concentration (MBC):

Taking different volumes of natural herbal extracts (0.2 g/mL) added to LB Broth medium, and in the same volume of DMSO as a negative control group. Wew ill have been cultured for 14 to 16 hours broth serial dilutions into 10^7 CFU/mL, 50 μL take added to the bacteria containing natural herbal LB Broth (the time the concentration of bacteria Broth 10^7 CFU/mL), at 37 °C incubator for 12 hours. Take 100 μL culture medium droplets in LA and sterile glass beads evenly on the surface of the medium, after inversion at 37 °C incubator for 12 hours, and
visual observation of the number of colonies of different concentrations change. Can 10^5 CFU / mL reduced the number of colonies 10^5 CFU/mL (99%) of the minimum inhibitory concentration (MIC), it is able to 10^2 CFU/mL reduced the number of colonies 10^5 CFU /mL (99.9%) as a minimum bactericidal concentration (MBC).

C. Synergy effect

The synergy effect is determined by the double disc diffusion method, in which 1 g of natural herbal extracts was dissolved in 1 mL of DMSO (1 g/mL), and various concentrations of different antibiotics (Ampicillin (Amp), Cephalosporin (CEP), Clindamycin (CC), Erytromyicine (Em 30 μL of each), Gentamycin (Gm), Kanamycin (KM), Pipericillin (PIP), Streptomycin (Sm), Trimethoprime / sulfamethoxazole (SXT), Tetracycline (Tc)) were used. For the synergy effect test, each of the disc intervals was approximately 2.5 cm, according to the shape of inhibition zone between antibiotics and extracts to determine the combined effect within the antibiotics and extracts.

D. Time-killing curve

Taking single colon was inoculated in 5 mL containing 5 μL Ampicillin (50 mg/mL) in LB Broth in incubator at 37 °C for 14 to 16 hours in LB Broth cultured broth was diluted to 10^7 CFU/mL, take 50 μL volume of broth and 2 times the MIC of natural herbal extracts, add 5 mL containing Ampicillin (50 mg/mL) LB Broth medium at 37 °C, 200 rpm shaking incubator, respectively, 0, 1, 2, 3 ,4, 6, 8, 10, 12, and 24-hour time point bacteria, the bacteria serial diluted in sterile glass beads evenly on the surface of the medium, after 37 °C incubator inverted 14-16 hours, calculate the number of colonies.

E. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The crude extract was diluted with methanol and then reacted with the DPPH solution. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. The absorbance at 517 nm of the reaction solution was measured by spectrophotometer. Inhibition of the DPPH radicals was calculated using the following equation:

\[ \% = \left( 1 - \frac{X_1 - X_2}{X_3} \right) \times 100\% \]

Where X1 is the absorbance of the tested sample (in a DPPH solution), X2 is the absorbance of the tested samples without DPPH and X3 is the absorbance of the control sample (which contains all reagents except for the test samples). The IC_{50} value represents the concentration of the tested sample that caused 50% inhibition [6].

F. Determination of total phenolics concentration

An aliquot of 0.2 mL sample and gallic acid in ddH2O were added to test tubes containing 1 mL Folin reagent and 1 mL sodium carbonate. After 2 hr, the absorbance was determined at 760 nm. Gallic acid (0-250 ppm) was used for calibration of the standard curve was Y = 0.0095·X + 0.0009 (where X = concentration of gallic acid equivalents expressed as milligrams of gallic acid per 100 g of dry weight of the plant material; Y = measured absorbance) and the correlation coefficient was R^2 = 0.9999.

G. Determination of total flavonoids concentration

An aliquot of 1.0 mL sample and quercetin in methanol were added to test tubes containing 0.1 mL potassium acetate, aluminium nitrate and 3.8 mL methanol. After 40 min, the absorbance was determined at 734 nm. The flavonoids were assessed by plotting the quercetin calibration curve (50-250 ppm) was Y = 0.0089·X + 0.0036 (where X = concentration of quercetin equivalents expressed as milligrams of quercetin per 100 g of dry weight of the plant material; Y = measured absorbance). The correlation coefficient was R^2 = 0.9976.

H. Trolox equivalent antioxidant capacity (TEAC) assay

An aliquot of 20 µL sample and different concentrations of Trolox were added to 1 mL 0.175 mM ABTS solution and then dark stand for 10 minutes. The absorbance was determined at 734 nm. Antioxidant capacity of the natural herbal extracts was determined based on the calibration curve of the different concentrations of Trolox. A trolox solution (final concentration 0-250 ppm) was used for calibration of the standard curve was Y = 0.0008·X - 0.6381 (where X = concentration of Trolox equivalents expressed as milligrams of gallic acid per 100 g of dry weight of the plant material; Y = measured absorbance) and the correlation coefficient was R^2 = 0.9979.

I. Reducing power test

An aliquot of 75 µL sample were added to test tubes containing 75 µL 1% K_3Fe(CN)_6 and Sodium phosphate buffer mixture, then incubated at 50 °C water bath. After cooling on ice, 75 µL of 10% TCA, 0.1% FeCl_3 and ddH2O were added to 300 µL and completely mixed for 14 minutes, then determined the absorbance at 700 nm. The slope of the plot represents the reduction capacity of the test herbal extracts.

III. RESULTS AND DISSUSION

A. Disc diffusion method

A total of 15 strains, including 5 of the standard strains and 10 of the clinical antibiotic resistant isolates, were test for the antibacterial activity. The results of disc diffusion method were shown in Table I. The extracts of ethyl acetate revealed slightly antibacterial activity against some of the test strains. Compared to the antibiotic, tetracycline, all of the extracts obtained from Sophora flavescens did not show significant antibacterial activity.

B. Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Two of the test strains, S. aureus 985 and oxacillin-resistant S. aurens 287, were selected for the determination of minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). As shown in Table II, the ethyl acetate extracts presented a significant antibacterial activity against
oxacillin-resistant *S. aurens* 287 with MIC value of 0.025 mg/mL and MBC of 0.04 mg/mL.

C. Synergy effect

Drug combination effects were investigated by the disc diffusion method. As shown in Fig. 1, the active ethyl acetate fractions presented different synergistic interactions for different bacteria strains. For *S. aureus* 985, the ethyl acetate extracts did not show any synergistic effect for the eleven tested antibiotics. For the oxacillin-resistant *S. aurens* 287, the ethyl acetate extracts presented synergistic effects with Clindamycin, Cephalosporin and Tetracycline.

![Oxacillin-Resistant S. aurens 287](image1)

![S. aureus 985](image2)

Fig.1 Double disc diffusion synergy test for the ethyl acetate fraction of *Sophora flavescens* extracts and antibiotics.

D. Time-killing curve

Due to their susceptibility, the time-killing curves of the clinical isolate (oxacillin-resistant *S. aurens* 287) were further analyzed. The results showed that the ethyl acetate extracts strongly inhibited the growth of the tested bacteria. Within 3 hrs, the extracts showed potential effects of antibacterial activity as a steep decline in the CFU number against the tested strain (Fig. 2). The antibacterial effects lasted for 24 hours.

![Time-killing curve](image3)

Fig. 2 Effect of the extracts from *Sophora flavescens* on growth of the test strain oxacillin-resistant *S. aurens* 287. The curves represent viable cell counts of the test microorganism.

E. Antioxidant activities of the extracts from *Sophora flavescens*

The various extracts of *Sophora flavescens* were assayed for antioxidant activity using DPPH radical scavenging capacity, Trolox equivalent antioxidant capacity, and reducing power test. In addition, the contents of total phenolics and flavonoids were also determined. As shown in Fig. 3, the free radical scavenging capacity was increased with the concentration of extracts increased. Compared to the IC_{50} values of various extracts from *Sophora flavescens*, the extracts of ethyl acetate (178 ppm) showed the highest free radical scavenging ability than the crude extracts (484 ppm) and water extracts (927 ppm) (Table III).

![Reducing power determination](image4)

Polyphenolic and flavonoid compounds commonly found in plants, mainly of plant secondary metabolites are often related to plant antioxidant. Comparing the results shown in Table III, the highest concentration of phenolic content was found in the extract of ethyl acetate (36.49 g Gallic acid / 100 g DW), followed by the crude extracts (28.62 g Gallic acid / 100 g DW), and then the water extracts (20.72 g Gallic acid / 100 g DW). Same results were found in the total flavonoid content determination; the highest concentration of total flavonoids was found in the extract of ethyl acetate (4.68 g Quercetin / 100 g DW), followed by the crude extracts (1.89 g Quercetin / 100 g DW), and then the water extracts (0.2 g Quercetin / 100 g DW). The phenolic and flavonoid contents showed a positive correlation with their antioxidant activities.
### TABLE I

**DISC INHIBITION ZOON (DIZ) OF THE EXTRACTS FROM SOPHORA FLAVESCENS**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Crude (1000 mg / mL)</th>
<th>Ethyl acetate (100 mg / mL)</th>
<th>Water (100 mg / mL)</th>
<th>Tetracycline (7.5 mg / mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. baumannii</em> ATCC 19606</td>
<td>11.70 ± 0.40</td>
<td>9.50 ± 0.30</td>
<td>9.40 ± 0.70</td>
<td>28.60 ± 0.00</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 6538P</td>
<td>19.30 ± 0.80</td>
<td>18.40 ± 0.50</td>
<td>10.80 ± 0.00</td>
<td>37.00 ± 0.00</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> ATCC 29260</td>
<td>12.20 ± 0.30</td>
<td>—</td>
<td>—</td>
<td>23.00 ± 0.00</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> ATCC 27853</td>
<td>21.50 ± 0.00</td>
<td>—</td>
<td>—</td>
<td>20.80 ± 0.00</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25257</td>
<td>19.40 ± 0.50</td>
<td>—</td>
<td>—</td>
<td>27.90 ± 0.00</td>
</tr>
<tr>
<td><em>A. baumannii</em> 814</td>
<td>11.80 ± 0.80</td>
<td>9.30 ± 0.40</td>
<td>9.00 ± 0.00</td>
<td>17.10 ± 0.00</td>
</tr>
<tr>
<td><em>A. baumannii</em> 817</td>
<td>12.50 ± 2.20</td>
<td>9.80 ± 0.30</td>
<td>9.30 ± 0.30</td>
<td>28.80 ± 0.00</td>
</tr>
<tr>
<td><em>S. aureus</em> 908</td>
<td>17.60 ± 0.60</td>
<td>17.20 ± 1.10</td>
<td>11.10 ± 0.20</td>
<td>38.40 ± 0.00</td>
</tr>
<tr>
<td><em>S. aureus</em> 985</td>
<td>17.80 ± 1.20</td>
<td>18.00 ± 0.30</td>
<td>10.80 ± 0.10</td>
<td>20.50 ± 0.00</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> 717</td>
<td>13.80 ± 1.80</td>
<td>10.20 ± 0.60</td>
<td>—</td>
<td>34.90 ± 0.00</td>
</tr>
<tr>
<td><em>E. coli</em> 9005UTI</td>
<td>12.10 ± 0.40</td>
<td>—</td>
<td>—</td>
<td>29.50 ± 0.00</td>
</tr>
<tr>
<td>Oxacillin-Resistant <em>S. aurens</em> 220</td>
<td>9.50 ± 0.00</td>
<td>9.30 ± 0.30</td>
<td>9.30 ± 0.30</td>
<td>27.30 ± 0.00</td>
</tr>
<tr>
<td>Oxacillin-Resistant <em>S. aurens</em> 287</td>
<td>17.40 ± 0.10</td>
<td>16.70 ± 0.30</td>
<td>11.30 ± 0.60</td>
<td>34.50 ± 0.10</td>
</tr>
<tr>
<td>Methicillin-Resistant <em>S. aurens</em> 2118</td>
<td>10.90 ± 0.40</td>
<td>10.30 ± 0.70</td>
<td>10.10 ± 0.40</td>
<td>31.40 ± 0.00</td>
</tr>
<tr>
<td>Methicillin-Resistant <em>S. aurens</em> 331</td>
<td>12.00 ± 0.50</td>
<td>9.70 ± 0.10</td>
<td>9.30 ± 0.30</td>
<td>31.60 ± 0.60</td>
</tr>
</tbody>
</table>

-: no active

### TABLE II

**MINIMUM INHIBITION CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF THE EXTRACTS FROM SOPHORA FLAVESCENS**

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Crude MIC (mg / mL)</th>
<th>Crude MBC (mg / mL)</th>
<th>Ethyl acetate MIC (mg / mL)</th>
<th>Ethyl acetate MBC (mg / mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> 985</td>
<td>&gt; 8.0</td>
<td>&gt; 8.0</td>
<td>&gt; 8.0</td>
<td>&gt; 8.0</td>
</tr>
<tr>
<td>Oxacillin-Resistant <em>S. aurens</em> 287</td>
<td>0.08 ± 0.000</td>
<td>0.09 ± 0.014</td>
<td>0.025 ± 0.007</td>
<td>0.04 ± 0.000</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Extracts</th>
<th>DPPH IC$_{50}$ (ppm)</th>
<th>TPC (g Gallic acid /100 g DW)</th>
<th>Flavonoid (g Quercetin /100 g DW)</th>
<th>TEAC (mmol Trolox /100 g DW)</th>
<th>Reducing Power (abs /10$^{-3}$ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>484.84 ± 16.54</td>
<td>28.62 ± 0.07</td>
<td>1.89 ± 0.20</td>
<td>78.05 ± 0.04</td>
<td>0.2</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>178.50 ± 30.70</td>
<td>36.49 ± 0.09</td>
<td>4.68 ± 0.50</td>
<td>78.22 ± 0.00</td>
<td>0.7</td>
</tr>
<tr>
<td>Water</td>
<td>927.16 ± 38.76</td>
<td>20.72 ± 0.10</td>
<td>0.2 ± 0.03</td>
<td>42.72 ± 0.04</td>
<td>0.1</td>
</tr>
<tr>
<td>BHT</td>
<td>33.87 ± 1.89</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.9</td>
</tr>
<tr>
<td>Vit C</td>
<td>6.48 ± 2.54</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18.3</td>
</tr>
</tbody>
</table>

- no detected

IV. CONCLUSION

The use of herbal extracts as antimicrobial and antioxidant agents has two distinct advantages: the natural origin and the associated low risk. This means that they are safer for people and the environment and that resistant pathogenic microorganism is less frequently developed. The present work shows the extracts of ethyl acetate from *Sophora flavescens* provide antimicrobial activities against oxacillin-resistant *S. aurens* strain. The antioxidant activity analyses have shown that the extracts of ethyl acetate have a good radical scavenging capacity. The experiment results revealed the ethyl acetate extracts of *Sophora flavescens* with a higher content of phenolic and flavonoid compounds, and have a better total antioxidant capacity and restore capability. Comprehensive experimental results show that the extracts of *Sophora flavescens* present good anti-oxidation and anti-bacterial role, do have the opportunity to become a new generation of pharmaceutical antioxidants and antibiotics. In conclusion, the results of this study suggest the possibility of using the extracts of *Sophora flavescens* as natural antioxidants and antimicrobials for the treatment of antibiotic resistant pathogens.

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