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Antibacterial and Antifungal Properties of Flavonoid Compounds from *Osmunda japonica thumb*

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Osmunda japonica thumb, widely used for human consumption, is also Chinese traditional medicine. In the study, we showed that the flavonoid compounds of *Osmunda japonica thumb* exert a potent antibacterial and antifungal activity. Results showed that the flavonoid compounds can remarkably inhibit the proliferation of *P. vulgaris*, *P. aeruginosa*, *E. coli*, *B. subtilis*, *A. niger* and *A. flavus* and its minimum inhibitory concentrations (MICs) was 50 µg/mL (*E. coli* and *B. subtilis*) and 100 µg/mL (*P. vulgaris*, *P. aeruginosa*, *A. niger* and *A. flavus*), respectively. The results provide promising baseline information for the potential use of the extract and flavonoids from this plant as antimicrobial agents to help control some microbial diseases.

Key Words: *Osmunda japonica thumb*, Flavonoid, Antimicrobial activity, Chinese medicine.

INTRODUCTION

Osmunda japonica thumb is a fern in the genus Osmunda native to eastern Asia, including Japan, China, Korea, Taiwan and the far east of Russia on Sakhalin. It is a deciduous herbaceous plant which produces separate fertile and sterile fronds. The sterile fronds are spreading, up to 80-100 cm tall, bipinnate, with pinnae 20-30 cm long and pinnules 4-6 cm long and 1.5-2.0 cm broad; the fertile fronds are erect and shorter, 20-50 cm tall. It grows in moist woodlands and can tolerate open sunlight only if in very wet soil. Like other ferns, it has no flowers, but rather elaborate sporangia, that very superficially might suggest a flower, from which the alternative name derives.

Osmunda japonica Thumb., also known as Ziqi or Japanese flowering fern, the rhizome with petiole of which can be dried and used as a medicine. Its Chinese medicinal name is Ziqiguanzhong. The rhizome of *Osmunda japonica Thumb*. contains lactones: osmundalin[1], dihydroisoosmundalin[2], (4R,5S)-osmundalactone, (4R,5S)-5-hydroxy-2-hexen-4-olide, (4R,5S)-5-hydroxyhexan-4-olide, (3S,5S)-3-hydroxyhexan-5-olide[3]; steroids: ponasterone A, ecdysone, ecdysterone[4]; it also contains polypeptides[5], proteoglycan[6], parasorboside[2], succinic acid[3]. The spores contains flavonoids: isoginkgetin, tris-O-methylamentoflavone, sciadopitysin, 4',4",7',7"-tetramethylamentoflavone, astragalin[7]. Flavonoids are an abundant class of natural product compounds and are widely distributed in the plant kingdom and in dietary foods[8]. They have been reported to possess a variety of biological activities including antiallergic, antidiabetic, antiinflammatory, antiviral, antiproliferative and anticarcinogenic, hepatoprotective and antioxidant activities. Since these secondary metabolites are synthesized by plants in response to microbial infection, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms[9]. Their antimicrobial activities against some Gram-negative and Gram-positive bacteria have been also reported in many papers[10]. Therefore, the flavonoids may be promising new class compounds in antimicrobial therapy.

In this work, we report the antimicrobial activities of flavonoids isolated from *Osmunda japonica thumb*. Then antibacterial and antifungal experiments were done by zone of Inhibition and MIC (minimum inhibitory concentration). The results showed that flavonoid compounds were effective in inhibiting proliferation of *P. vulgaris*, *P. aeruginosa*, *E. coli*, *B. subtilis*, *A. niger* and *A. flavus*.

EXPERIMENTAL

Osmunda japonica thumb was obtained from Anhui Science and Technology University. Rutin was purchased from Tianjin Chemical Co. (Tianjin, China). All chemicals used were analytical grade reagents. Deionized water was purified by a Milli-Q Water Purification system (Millipore, MA, USA).

Extraction of flavonoid compounds: The air-dried and powdered *Osmunda japonica thumb* was extracted with ethanol (95 %) under reflux (3 L × 30 L for 3 h). The combined

ethanol solution was concentrated in vacuum at 50 ºC to obtain the crude extract. The extract was further suspended in hot water and extracted in turn with petroleum ether and ethyl acetate. Based on the result of a TLC-bioautographic antibacterial assay[11], the ethyl acetate extract was subjected to ZORBAX SB-C_{18} chromatography column eluting with 0.04 % phosphoric acid-methanol (6:4, v/v) to obtain five fractions. Of them, fraction-4 (peak-4, 9.824 min), showing the strongest antimicrobial activity and similar peak to Rutin. This fraction was further identified by coupling the HCl-Mg reaction[12]. The flavonoid compounds concentration was quantified with a standard Rutin according to aluminum chloride method[13].

Determination of antibacterial activity of flavonoid compounds: The antibacterial study was performed for the determination of following parameters like, zone of inhibition and MIC (minimum inhibitory concentration). Different concentration of flavonoid compounds were tested for antibacterial activity by disc diffusion method[14]. Nutrient agar medium was inoculated with different microorganisms and once the media was solidified, it was punched with a 6 mm diameter well. The wells were then filled with different concentration of flavonoid compounds and control (DMSO) (concentration of flavonoid compounds was 50, 100 and 250 µg/mL). Agar plates containing bacteria and flavonoid compounds were incubated at 37 ºC for 24 h. Antimicrobial activity was evaluated by measuring the inhibition zone. Inhibition zones were recorded as the diameter of growth free zone, including the diameter of the well, in millimeters at the end of the incubation period. The tested drug was classified as active when the diameter of the inhibition zone was equal to or larger than 6 mm. Simultaneously standard antibiotic Penicillin for *P. vulgaris*, *P. aeruginosa*, *E. coli*, *B. subtilis* were used for comparison at a concentration 50 µg/mL each. The sample was tested in triplicate. Similar procedure was adopted for fungi, except PDA was used as a selective media for the effective growth of fungi. Nystatin was used as standard antibiotic for the comparison of zone of inhibition with different concentrations of flavonoid compounds. At the end of incubation period the zone of inhibition for the flavonoid compounds was measured for each bacteria and fungi and the results were tabulated.

Flavonoid compounds in different concentrations (50, 100 and 250 µg/mL) control (DMSO) and standard (nystatin 5 µg/mL), were transferred to the cups of each agar plate, incubated at room temperature (27 ºC) and examined for inhibition zones after 36 h of incubation to screen for antifungal activity. The flavonoid compounds were assayed for antifungal activity against the fungal strains, *A. niger*, *A. flavus*, *Heliminthosporium* spp., *Alternaria* spp.

The results for MIC of flavonoid compounds were studied by following ways: first, flavonoid compounds were diluted into different concentration with DMSO as thinner; then these flavonoid compounds were spread on nutrient agar solid medium; finally, bacteria and fungi were also spread on solid medium. Bacteria and fungi were incubated at 37 and 27 ºC, respectively. After 24 h (bacteria) and 36 h (fungi), their colony numbers were recorded. Inhibition ratio (proliferation ratio) was calculated by formula: proliferation ratio (%) = (A/B) × 100 %, where A is the colony numbers on flavonoid compounds medium, B is the colony numbers on control (DMSO) medium.

RESULTS AND DISCUSSION

Preparation of flavonoid compounds: By analysis of the chromatogram, the ethyl acetate extract contain five fractions. According to the peak time of standard sample Rutin, fraction-4 was collected from chromatography column (Fig. 1). Then the HCl-Mg reaction applied to the identification of sample, the result confirmed fraction-4 was flavonoid compounds. The flavonoid compounds concentration was quantified with a standard Rutin according to aluminum chloride method.

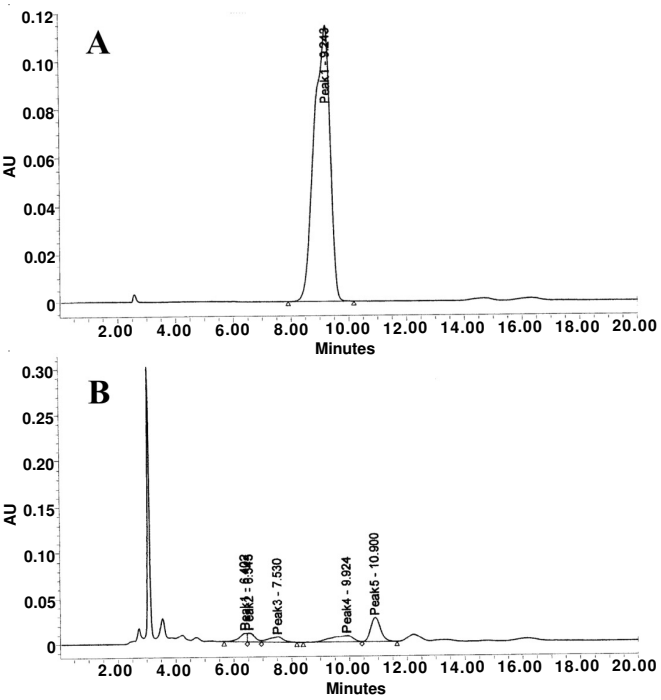

Fig. 1. HPLC chromatography. Panels A: the HPLC chromatography of the standard Rutin; Panels B: the HPLC chromatography of extract from *Osmunda japonica thumb*.

Antibacterial activity of flavonoid compounds: It was observed that all the four bacteria such as *E. coli*, *Pseudomonas aerogeinosa*, *Proteus vulgaris* and *Bacillus subtilis* show antibacterial activity against flavonoid compounds with zone of inhibition. When pathogenic bacterial strains were tested against flavonoid compounds such as C1, C2 and C3 at different concentration (50, 100 and 250 µg/mL), flavonoid compounds showed good inhibition area at one specific concentration (250 µg/mL); but at varied concentration, the above bacteria did not show or less area of zone of inhibition (Table-1).

TABLE-1
BACTERIA-RESTRAINING EFFECT OF FLAVONOID COMPOUNDS FROM *Osmunda japonica thumb*

Bacteria	Penicillin	50 µg/mL	100 µg/mL	250 µg/mL
P. vulgaris	3 mm	–	2 mm	5 mm
P. aeruginosa	4 mm	–	2 mm	6 mm
E. coli	5 mm	1 mm	3 mm	10 mm
B. subtilis	3 mm	1 mm	3 mm	7 mm

Dashed curve indicated that the zone of inhibition is zero.

Antifungal activity of flavonoid compounds: The antifungal activity of flavonoid compounds was tested against the four fungal species. *A. niger*, *A. flavus*, *Alternaria* spp. and *Helminthosporium* spp. At 50 µg/mL, the four fungal species did not show zone of inhibition to flavonoid compounds; At 100 µg/mL, *A. niger* and *A. flavus* showed zone of inhibition to flavonoid compounds, but *Alternaria* spp. and *Helminthosporium* spp. did not show any zone of inhibition; At 250 µg/mL, *A. niger* and *A. flavus* showed good zone of inhibition to flavonoid compounds, but Alternaria spp. and *Helminthosporium* spp. did not all the same show any zone of inhibition (Table-2).

TABLE-2
FUNGI-RESTRAINING EFFECT OF FLAVONOID COMPOUNDS FROM *Osmunda japonica thumb*

Fungi	Nystatin	50 µg/mL	100 µg/mL	250 µg/mL
A. niger	3 mm	–	2 mm	6 mm
A. flavus	4 mm	–	2 mm	7 mm
Alternaria spp.	6 mm	–	–	–
Helminthosporium spp.	6 mm	–	–	–

Dashed curve indicated that the zone of inhibition is zero.

MIC of flavonoid compounds to bacteria and fungi: Different organisms have different sensitivity to flavonoid. It is clear from Fig. 2 that the *E. coli* and *B. subtilis* were most sensitive to flavonoid compounds and 50 µg/mL flavonoid compounds can inhibit their proliferation. Whereas the effect of flavonoid compounds to *P. aerogeinosa*, *A. niger* and *A. flavus* were lowest; the sensitivity of *P. vulgaris* was middle.

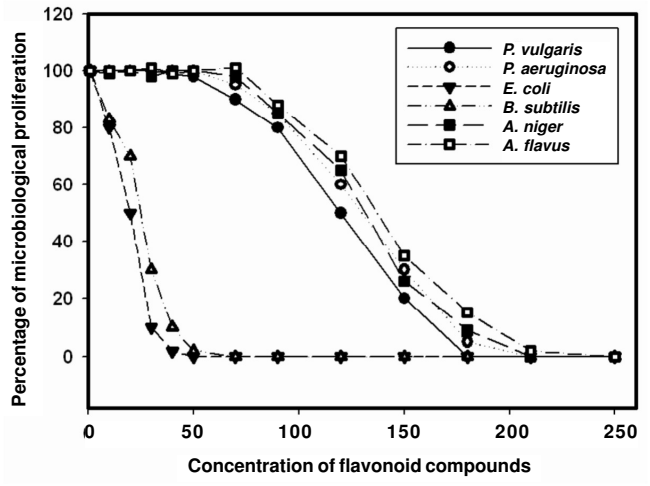

Fig. 2. Minimal inhibitory concentration of various organism

Osmunda japonica thumb belongs to traditional Chinese medicine, which aqueous and methanol extract possess the ability of antiviral *in vitro*[15]. Its polysaccharides can inhibit the growth of some bacteria *in vitro*[16]. Flavonoids are ubiquitous in photosynthesising cells and therefore occur widely in the plant kingdom[17]. They are found in fruit, vegetables, nuts, seeds, stems and flowers as well as tea, wine[18], propolis and honey[19] and represent a common constituent of the human diet[20]. Flavonoids are involved in providing colours for flowers, promote physiological survival of the plant, photosensitization, energy transfer, the actions of plant growth hormones and growth regulators, control of respiration and photosynthesis, morphogenesis and sex determination[18,20,21]. Increasingly, flavonoids are becoming the subject of medical research. They have been reported to possess many useful properties, including antiinflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity[17,20], antiallergic activity, antioxidant activity[18], vascular activity and cytotoxic antitumour activity[21].

In the study, flavonoid compounds were extracted from *Osmunda japonica thumb*. The experiments results showed that all the four bacterias such as *E. coli*, *Pseudomonas aerogeinosa*, *Proteus vulgaris* and *Bacillus subtilis* show antibacterial activity against flavonoid compounds; but in the antifungal experiments, flavonoid compounds only inhibited the growth of *A. niger* and *A. flavus* and it is invalid in the antifungal *Alternaria* spp. and *Helminthosporium* spp. aspects.

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