Antimicrobial Activity of *Oenanthe javanica* Seed Extract: An *In Vitro* Study

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Abstract

Water dropwort fruit (*Oenanthe javanica* (Blume) DC.), locally known as "Phak Chi Lom," is a renowned medicinal herb with a long history of traditional use, primarily for its gastrointestinal benefits. Its properties include relieving dizziness, soothing hiccups, suppressing nausea and vomiting, supporting digestion, and alleviating uncomfortable flatulence and bloating. Given the growing threat of antimicrobial resistance (AMR) and the high cost associated with treating recurring bacterial infections, investigating natural sources for new antimicrobial agents is critical.

This study aimed to evaluate the antimicrobial inhibitory efficacy of the *O. javanica* extract against four selected microbial strains using the Agar Disc Diffusion Assay. The extract consistently showed the most potent inhibitory effect against the Gram-positive bacterium *Bacillus subtilis*, yielding inhibition zones of 11.00±2.646 mm at 400 mg/ml and 12.00±0.577 mm at 200 mg/ml. The extract was effective against three of the four tested strains (*S. aureus*, *B. subtilis*, and *P. aeruginosa*) at high concentrations. However, the extract at all three tested concentrations failed to inhibit *Escherichia coli*, and at 100 mg/ml, its activity was strongly favored towards Gram-positive bacteria. The findings confirm the traditional usage and provide preliminary scientific justification for developing the extract into a natural antimicrobial agent to help address public health concerns.

Keywords: *Oenanthe javanica*, Water dropwort, Agar Disk Diffusion, Antimicrobial Activity, Herbal Extract

1. Introduction

Water dropwort fruit (Fennel Seed), scientifically known as *Oenanthe javanica* (Blume) DC., is a medicinal herb that has been used extensively in traditional medicine for a long time. Its prominent properties include relieving nausea, vomiting, flatulence, and bloating, as well as aiding digestion, which points to its significant biological potential.

Currently, the problem of infectious diseases caused by pathogenic microorganisms remains a major threat to public health. These pathogens are responsible for various illnesses, ranging

from food poisoning and hospital-acquired infections to urinary tract infections and septicemia (Department of Medical Sciences, 2023; Thomma & Sirithorn, 2012; Burakorn et al., 2011; Jaratpongpasut & Laappra, 2018). The treatment costs associated with these infections are high, making the search for natural antimicrobial agents a crucial strategy for mitigating these issues.

The researchers are therefore interested in studying the efficacy of the extract from *Oenanthe javanica* fruit in inhibiting the growth of four important pathogenic and potentially pathogenic microbial strains (namely, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli*). This research aims to validate the pharmacological activity based on traditional knowledge and to further develop the effective extract into health products, thereby increasing the economic value of this Thai herb.

1.1 Research Objective

To evaluate the antimicrobial efficacy of *Oenanthe javanica* extract.

2. Literature review

2.1 Herbal Information: Water Dropwort Fruit

Crude Drug Name: Water Dropwort Fruit (The part obtained from the dried seed/fruit)

Plant Source: Water Dropwort

Scientific Name: *Oenanthe javanica* (Blume) DC.

Synonym: *Oenanthe stolonifera* Wall. Ex DC.

Family: UMBELLIFERAE (Apiaceae)

Local Names: Phak chi Lom, Phak An, Phak An O, Phak Nong Chang (Northern Thailand), Jui Kheng Chai (Chinese)

Botanical Description

Oenanthe javanica is an annual or perennial herbaceous plant, growing approximately 10–100 cm in height. Its stem is hollow and succulent, and it often grows in damp or submerged areas. Its leaves are pinnately compound and bipinnate with serrated margins resembling saw teeth. The small, white flowers are arranged in a terminal umbel inflorescence. The fruit is a schizocarp (dry fruit that splits into two parts when ripe) and is characteristically round with ridges.

Medicinal Properties and Benefits

Fruit (Water Dropwort Fruit): The primary medicinal properties include helping to expel flatulence (carminative) from the intestines, relieving bloating, suppressing nausea and vomiting, aiding digestion, treating dizziness, and nourishing the lungs.

Whole Plant: Traditionally used for maintaining eyesight, nourishing the heart and blood, detoxifying the body, relieving phlegm and sore throat, treating edema (swelling), and treating beriberi.

Other Benefits: The plant is used as a pungent seasoning in cooking, helping to make dishes less greasy and easier to digest (Samunpri, 2017; Thaiherbal, 2015; Chansing, 2020).

2.2 Anti-inflammatory and Antioxidant Activity

Studies investigating the anti-inflammatory effects of isorhamnetin, hyperoside, and ethanolic extract from water dropwort (*Oenanthe javanica*) were conducted *in vitro* using human and mouse macrophage cells. The results showed that the ethanolic extract of *O. javanica* effectively reduced the secretion of the cytokine interleukin-1β (IL-1β) and inhibited the formation of the Asc pyroptosome complex, indicating that water dropwort possesses anti-inflammatory properties (Ahn., 2017).

Furthermore, *O. javanica* demonstrated anti-inflammatory and antioxidant effects in mice with Dextran Sulfate Sodium (DSS)-induced colitis. Treatment with the ethanolic extract suppressed the production of pro-inflammatory cytokines in macrophages and alleviated pathological damage to the colon. This treatment also mitigated symptoms such as weight gain and reduced colon length, inflammation, and epithelial tissue necrosis. It helped decrease the abundance of *Proteobacteria* (including *Escherichia*) and increase the abundance of *Muribaculum* genus bacteria. These findings suggest a beneficial impact on both inflammation and gut microbial composition in experimental colitis (Bae et al., 2022).

2.3 Antimicrobial Activity and Phytochemicals

Extracts from water dropwort have shown inhibitory activity against both Gram-positive and Gram-negative bacteria. Fractionation of the ethanolic extract revealed that certain fractions exhibited very high antibacterial activity against gastrointestinal pathogens, such as *Shigella flexneri*. The extract contains several groups of bioactive compounds believed to play a critical role in its antimicrobial action, including Phenols, Flavonoids, Alkaloids, Coumarins, and Saponins.

2.4 Antidiabetic Potential

Vadivelan et al. (2019) studied the potential of water dropwort root extracts to inhibit the activity of α-amylase and α-glucosidase (enzymes involved in carbohydrate breakdown). Extracts obtained using ethyl acetate and water layers showed lower inhibition of α-amylase and α-glucosidase compared to standard reference compounds. The root extract's major components include Flavonoids, Tannins, Phenolic compounds, Saponins, and Amino acids. Specifically, the total flavonoid content was 23.45 ± 1.33 mg (compared to rutin: 25.81 ± 0.82 mg), and the total triterpene content was 109.8 ± 5.6 mg (compared to ursolic acid: 95.6 ± 7.5 mg). The study concluded that water dropwort extract could be developed as a drug or food supplement with antidiabetic properties.

2.5 Acute Toxicity Study

An acute toxicity study of water dropwort was conducted by orally administering 15 g/kg of fresh water dropwort to mice for 14 days. The results showed that the extract did not cause mortality or induce changes in blood parameters such as blood glucose, total protein, albumin, urea, and creatinine. Furthermore, there were no signs of abnormal behavioral changes or toxicity to major organs, such as the liver and kidneys (Yu et al., 2017).

3. Methodology

3.1 Materials and Chemicals

Material: Water Dropwort Fruit (*Oenanthe javanica* (Blume) DC., dried seed/fruit part)
Solvent: 95% Ethanol

Pathogenic Microorganisms (4 Strains):

Gram-Positive Bacteria: *Staphylococcus aureus* ATCC6538P, *Bacillus subtilis* ATCC6633

Gram-Negative Bacteria: *Pseudomonas aeruginosa* TISTR 1287, *Escherichia coli* ATCC25922

Bacterial strains in this study obtained from stock culture from College of Allied Health Sciences, Suan Sunandha Rajabhat University, Thailand.

Culture Media: Mueller-Hinton Agar (MHA) and Mueller-Hinton Broth (MHB)

Control Agents: Ampicillin, Amikacin (Positive Controls), and Dimethyl Sulfoxide (DMSO) (Negative Control)

3.2 Microbial Culture Preparation

Each microbial strain was cultured on Mueller-Hinton Agar (MHA) prior to testing. The cultures were then adjusted in liquid medium to achieve a turbidity equivalent to the 0.5 McFarland Standard (CLSI, 2018), which was used as the initial Inoculum for the antimicrobial assays.

3.3 Extract Preparation by Maceration

The process began by finely grinding the dried water dropwort fruit and accurately weighing 200 grams of the material. This ground material was then placed in a jar and subjected to the maceration process by adding the solvent, 95% Ethanol, at a fixed ratio of 1 gram of raw material to 5 milliliters of solvent (1:5 w/v). The mixture was stirred and allowed to macerate at room temperature. The extraction was performed three times in total to ensure maximum component recovery. The liquid extract was filtered out and fresh solvent was added in each subsequent cycle: the first two cycles lasted 2 days each, and the final cycle lasted 3 days, resulting in a total extraction period of 7 days. All filtered liquid extracts were then pooled together. To obtain

the crude extract, the solvent (ethanol) was removed using a Rotary Evaporator at 50°*C*. Finally, the weight of the resulting crude extract was measured to calculate the product yield.

3.4 Testing for Antimicrobial Activity

3.4.1 Preparation of Stock Solution and Concentration Sets

The crude extract was dissolved in DMSO (Dimethyl Sulfoxide) to prepare a stock solution. This stock solution was then serially diluted to prepare various working concentrations for the antimicrobial assay.

3.4.2 Agar Disc Diffusion Assay

The prepared microbial inoculum (0.5 McFarland standard) was used to inoculate the entire surface of the MHA plates using the 3-way swab technique. This technique ensured uniform bacterial coverage. Subsequently, paper discs impregnated with the *Oenanthe javanica* extract at three different concentrations (400, 200, and 100 mg/ml) were placed onto the inoculated agar surface. Control discs, including the Positive Controls (Ampicillin, Amikacin) and the Negative Control (DMSO), were also placed on the plates. The plates were then incubated at the appropriate temperature and duration of 37°*C* for 24 hours. Following incubation, the diameter of the Zone of Inhibition (the clear area where microbial growth was suppressed) around each disc was carefully measured in millimeters (mm) to assess the antimicrobial effectiveness of the extract.

3.5 Statistical Analysis

All experiments were performed in at least three replicates (Triplicates). Descriptive statistics, including mean and standard deviation (SD), were used to analyze the data to test the effect of inhibiting the growth of microorganisms using the Disc Diffusion Assay.

4. Results and Discussion

For the preparation, it was prepared by maceration with 95% ethanol for this study. The percent yield was 7.087% as shown in Table 1. The characteristic of the *O. javanica* seed extract was sticky texture with dark brown.

Table 1: Thai name, scientific name, part used and percent yields.

Thai name	scientific name	part of used	% Yield (w/w)
			95% Ethanol
Phakchi lom	*Oenanthe javanica*	Seed	7.087%

Antimicrobial Activity of *O. javanica* Extract

The antimicrobial activity of the *O. javanica* extract was tested against four microbial strains using the Agar Disk Diffusion method.

- At 400 mg/ml Concentration: The extract showed the best inhibitory effect against *B. subtilis* (11.00±2.646 mm). This was followed by inhibition against *S. aureus* (10.00±0.000 mm) and *P. aeruginosa* (7.00±0.000 mm). No zone of inhibition was observed against *E. coli*, which was similar to the Negative Control (DMSO), as presented in Table 2. This finding is consistent with the research by Chansukh (2016), which tested the ethanolic extract of *Jasminum sambac* flower at 400 mg/ml and found inhibition zones against *S. aureus*, *B. subtilis*, and *E. coli* ranging from 7.00±0.0-7.33±0.58 mm, as shown in Table 2.
- At 200 mg/ml Concentration: The extract again demonstrated the best inhibitory effect against *B. subtilis* (12.00±0.577 mm). Inhibitory zones were also observed against *S. aureus* (10.00±0.000 mm) and *P. aeruginosa* 8.00±0.000 mm). Similar to the higher concentration, no zone of inhibition was found against *E. coli* or the Negative Control (DMSO), as shown in Table 2.
- At 100 mg/ml Concentration: The best inhibitory effect was against *B. subtilis* (12.00±1.155 mm), followed by *S. aureus* (11.00±0.000 mm). However, no zone of inhibition was detected against *E. coli* or *P. aeruginosa*, similar to the DMSO control, as shown in Table 2.

The results consistently show that the *O. javanica* extract is more effective against the Gram-positive bacteria (*B. subtilis* and *S. aureus*) than the Gram-negative bacteria, and it failed to inhibit *E. coli* at all tested concentrations.

When comparing the antimicrobial efficacy of the *O. javanica* extract with the findings of Thongkao et al. (2023), who tested a 95% ethanolic extract of Bang Chang Thai Cultivar Chili Pepper (*Capsicum annuum Var. acuminatum*) at a concentration of 100 mg/ml against various strains (including *S. aureus*, *S. epidermidis*, *E. coli*, *C. acnes*, and *C. albicans*), a significant difference was observed. Thongkao et al. (2023) reported that the antimicrobial activity of chili pepper was found only in the oil extract, which inhibited *C. albicans*, while the ethanolic extract could not inhibit any other pathogenic bacteria. Conversely, our results show that the *O. javanica* extract at 100 mg/ml demonstrated clear inhibitory activity against *S. aureus* (11.00±0.000 mm). This indicates that the *O. javanica* extract is significantly more effective at inhibiting bacteria than the ethanolic chili pepper extract in the comparative study. Therefore, the researchers propose that future studies should include additional testing of the *O. javanica* extract against fungal strains to gather comprehensive data and clearly ascertain the broad-spectrum antimicrobial potential of the extract.

Antimicrobial Activity of Antibiotics

The antimicrobial effectiveness of standard antibiotics was evaluated at a concentration of 1 mg/ml for comparison with the herbal extract.

The antibiotic Amikacin demonstrated its highest efficacy against the Gram-positive bacterium *B. subtilis*, exhibiting a zone of inhibition measuring 28.33±0.577 mm. Amikacin also showed strong activity against the two Gram-negative strains, *P. aeruginosa* (23.33±0.577 mm) and *E. coli* (22.33±0.577 mm), with the lowest inhibition zone recorded against *S. aureus* (20.67±0.577 mm).

Conversely, Ampicillin exhibited its most potent effect against the Gram-positive cocci, *S. aureus*, creating an impressively large zone of inhibition of 45.00±0.000 mm. Its effectiveness decreased against the Gram-negative *E. coli* (25.33±0.577 mm) and the Gram-positive rod *B. subtilis* (12.33±0.577 mm). Notably, Ampicillin showed no inhibitory activity whatsoever against *P. aeruginosa*, similar to the negative control (DMSO).

Table 2 Inhibitory Effect on Microbial Growth by Agar Disc Diffusion Assay

Extract / Antibiotic	**Extract Concentration**	**Diameter of Zone of Inhibition (mm ± SD) Against Microorganisms**			
		S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
O. javanica	400 mg/ml	10.00±0.000	11.00±2.646	N/A	7.00±0.000
	200 mg/ml	10.00±0.000	12.00±0.577	N/A	8.00±0.000
	100 mg/ml	11.00±0.000	12.00±1.155	N/A	N/A
	DMSO	N/A	N/A	N/A	N/A
Amikacin	1 mg/ml	20.67±0.577	28.33±0.577	22.33±0.577	23.33±0.577
Ampicillin	1 mg/ml	45.00±0.000	12.33±0.577	25.33±0.577	N/A

N/A = No Zone of Inhibition Detected

5. Conclusion

The preliminary study investigated the antimicrobial efficacy of the water dropwort (*Oenanthe javanica*) extract against four microbial strains: the Gram-positive bacteria (*S. aureus* and *B. subtilis*) and the Gram-negative bacteria (*E. coli* and *P. aeruginosa*), using the Agar Disk Diffusion method.

The results showed that the *O. javanica* extract successfully inhibited the growth of the tested strains, with the following findings:

- At the concentration of 400 mg/ml the extract inhibited microorganisms with zone diameters ranging from 7.00±0.000-11.00±2.646 mm, with the exception of *E. coli*.
- At the concentration of 200 mg/ml, the extract inhibited microorganisms with zone diameters ranging from 8.00±0.000-12.00±0.577 mm, with the exception of *E. coli*.
- At the concentration of 100 mg/ml, the extract inhibited microorganisms with zone diameters ranging from 11.00±0.000 - 12.00±1.155 mm, with the exception of both *E. coli* and *P. aeruginosa*.

The experiment clearly indicates that the *O. javanica* herbal extract at concentrations of 400 and 200 mg/ml is capable of inhibiting the growth of *S. aureus*, *B. subtilis*, and *P. aeruginosa*. At the 100 mg/ml concentration, the extract showed better inhibitory efficacy against Gram-positive bacteria than Gram-negative bacteria. Crucially, the herbal extract at all three tested concentrations failed to inhibit *E. coli*.

Therefore, the findings of this study may be beneficial and offer a valuable option for selecting effective concentrations to develop natural antimicrobial agents.

Acknowledgment

The author sincerely thanks Suan Sunandha Rajabhat University, Bangkok, Thailand, for their support and extends gratitude to the Science and Technology Services Center and the College of Allied Health Sciences at Suan Sunandha Rajabhat University for providing essential facilities throughout this work.

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