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ORIGINAL ARTICLE

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In-vitro Antibacterial activity, Extractive values and Phytochemical Profiling of *Oenanthe javanica* (Blume) DC Extracts

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Background: Phytochemicals are the physiologically active substances found in plants. These phytochemicals which can be found in a variety of plant parts including leaves, flowers, seeds, bark, roots, and pulps, have been employed as direct therapeutic agents. The perennial herb *Oenanthe javanica*, sometimes called Java water dropwort, water celery, water dropwort, or Indian pennywort, is endemic to East Asia and is specifically utilized in traditional medicine and culinary arts.

Purpose: The goal of this plant study is to determine *Oenanthe javanica* phytochemical composition, extractive values, and antibacterial activity.

Results: The extracts contained alkaloids, terpenoids, phenols, flavonoids, coumarins, and saponins, according to the results of the initial phytochemical analyses. Petroleum ether has a lower percentage yield extract than ethanol solvent. The agar-well diffusion technique has been utilized to ascertain *Oenanthe javanica* antibacterial activity. Both gram-positive and gram-negative bacteria were inhibited by the extracts. In comparison to petroleum ether, ethanol extracts demonstrated the highest level of susceptibility to the pathogens under study.

Conclusion: According to these results, *Oenanthe javanica* is a useful source of phytochemicals that appear to have promising antibacterial properties. *Oenanthe javanica* could be further investigated for toxicity and to obtain some novel antibacterial compounds in light of this bioactivity.

Key words: Oenanthe javanica, Phytochemical, Secondary metabolites, Antibacterial, Bio-active compounds

For the production of pharmacological lead compounds, medicinal plants have always been essential. As a result, the history of medicinal plants is as old as human history (Singh 2018). Early humans used plants to heal their illnesses because they were motivated by instinct, taste, and experience. Although modern medicines and drug development have advanced, medicinal plants continue to be a primary source of medicine for a significant section of the world population. They are invaluable sources of bioactive chemicals (Ali *et al.,* 2014). For thousands of years, extensive conventional healthcare systems have been based on plants, and these systems continue to yield innovative treatments for humanity. Medicinal plant therapy is founded on actual research spanning hundreds of thousands of years, even though some of the therapeutic qualities once attributed to plants have turned out to be false (Gurib-Fakim 2006).

Naturally occurring, physiologically active substances are called phytochemicals, produced from phytonutrients, or plant-based diets. Antibiotics and infections are warded off by phytochemicals. Although they are not required for a cell to survive, substances known as secondary metabolites are important for how a cell interacts with its surroundings. Plant defense against biotic and abiotic stressors is frequently mediated by these chemicals (Khatun *et al.*, 2023). Phytochemicals are categorized as either major or secondary ingredients based on how they function within the metabolism of plants. Common sugars, proteins, amino acids, pyrimidines and purines found in nucleic acids, chlorophyll, etc. are examples of primary components. Alkaloids, terpenes, flavonoids, lignans, plant steroids, saponins, phenolics, flavonoids, and glucosides are examples of the remaining plant compounds that are considered secondary components (Saxena *et al.*, 2013).

Oenanthe javanica (Blume) DC, is an aromatic perennial herb belonging to the Apiaceae family. It has been grown for millennia in both tropical and temperate regions of Asia, and its roots are in the ground. It has been utilized for centuries as a traditional medicine to treat an extensive range of ailments (Chan *et al.*, 2016). The leaves of *Oenanthe javanica*, also known as selum, have a distinct celery flavor, and they are eaten raw as ulam in Southeast Asia. *Oenanthe javanica* is eaten raw or seasoned in salads in Korea, and its soup is drink to cure hangovers caused by alcohol consumption (Kim *et al.*, 2009). The herb is used to treat jaundice, hypertension, polydipsia, fever, cold, abscesses, swellings, abdominal pain, leucorrhea, mumps, and difficulties urinating in traditional Chinese and Korean medicine (Park *et al.,* 1993). They have a distinct celery flavor. *Oenanthe javanica* is grown in early spring, and its aerial portions are eaten for their unique flavor and scent. It is also known as shui qin in China, minari in Korea, and seri in Japan. Young stems and leaves of *Oenanthe javanica* are used as a culinary ingredient in Manipur, North East, India, where they are known as "komprek" (Devi *et al.*, 2014).

Numerous biological actions of *Oenanthe javanica* have been documented, such as immune boosting, hepatoprotective, anti-inflammatory, antioxidant, and antiviral properties. Furthermore, toxicity tests have shown that *Oenanthe javanica* does not show any signs of acute or genetic toxicity. On the other hand, at high doses, oral administration of dry *Oenanthe javanica* power may cause a significant drop in weight and food consumption in mice and increase the rate of sperm malformation. Additionally, a high dose of *Oenanthe javanica* total phenolic acid extract demonstrated a reversible sub-chronic toxicity (Lu and Li 2019).

MATERIALS AND METHODS

Collection of plant material:

The complete plant material that was used in this study was collected in the month of February in Laphupat Tera, Bishnupur, Manipur, North-East, India. The University of Annamalai, Department of Botany has recognized the plant material.

Processing of plant materials:

The plant stem and leaves were cleaned using tap water, then distilled water, and then allowed to dry in the shade until all of the water had been removed. After that, the dried plant material that had shed was removed and ground into a rough powder. For analysis, the ground samples were carefully kept in an airtight glass

jar with the proper labeling.

Preparation of Plant Extracts:

The Soxhlet method was employed to extract the crude from the plant using one polar (ethanol) and one non-polar (petroleum ether) solvent. For the polar and non-polar solvent extraction, 100g of the dried and powdered plant material and 500ml of each solvent were utilized. The extractor siphon tube solvent is left in place until it turns colorless, which can take up to 24 hours. All of the extracts were dried and concentrated using a revolving vacuum evaporator. The dried extract was kept for later usage in a refrigerator at 4°C.

Percentage Yield of Extract (%)

The weight of the extracted crude extract (%) was divided by the weight of the plant powder (weighed before extraction) and multiplied by 100 to determine the percentage of extraction yield (%). After weighing the final extract, the extractive values of each solvent were calculated as follows:

$$\text{Percentage of yield (\%)} = \frac{\text{weight of extract}}{\text{weight of dry plant powder}} \times 100$$

Screening of phytochemicals

To identify alkaloids, saponins, terpenoids, flavonoids, phenol, coumarin, and cardiac glycosides, phytochemical analysis was performed in compliance with recognized protocols. The extracts were analyzed for the presence of bioactive components using the standard procedures (Kokate and Purohit 2005; Harbone 1973).

Alkaloids:

Mayer's test: Add 1 ml of extract to 1 ml of concentrated HCl, then add a few drops of Mayer's reagent. If a white or green precipitate forms, alkaloids are present..

Test for phenols

Ferric chloride test: Add 1 ml of 5% ferric chloride solution to 1 ml of extract; the formation of a reddish-brown precipitate suggests the presence of phenols.

Test for coumarins

When 1.5 ml of 10% NaOH is added to 1 ml of extract, coumarins are present because a yellow color forms.

Test for flavonoids

Lead acetate test: The presence of flavonoids is indicated by the formation of a yellow precipitate when 1 ml of the extract is mixed with 1 ml of 10% lead acetate solution.

Test for saponins

Foam test: When 1 ml of extract is combined with 1 ml of distilled water and shaken vigorously, foam forms, signifying the presence of saponins.

Test for terpenoids

Ferric chloride test: Add 2 ml of water to 1 ml of extract, then add 1 ml of a 10% ferric chloride solution. The creation of a strong color indicates the presence of terpenoids.

Test for cardiac glycosides

Keller- Kiliani test: To 1 ml of sample add 2 ml of glacial acetic acid followed by 2ml of glacial acetic acid, add 1 ml of 5% ferric chloride solution along with 1 ml of dilute HCl, formation of brown ring at the interface indicated the presence of cardiac glycosides.

Antibacterial Assay

Test organism

Two gram-negative strains of *Escherichia coli* and *Klebsiella pneumoniae* as well as two gram-positive strains of *Bacillus subtilis* and *Staphylococcus aureus* were provided by the Department of Microbiology at Annamalai University. On nutrient agar at 4°C, all test bacteria were kept alive. Every sample was extracted and then divided into three concentrations: 25, 75, and 100 mg/ml, each with three replications.

Determination of antibacterial activity

To obtain an extract concentration of 200 mg/ml, one gram of each crude extract was reconstituted in 20% dimethyl sulfoxide (DMSO). The following lower extract concentrations were achieved by serially diluting this two times: 100, 75, and 25 mg/ml. Using agar well diffusion techniques, the plant extracts activities were ascertained (Perez *et al.*, 1990; Alade *et al.*, 1993). A sterile cotton-tipped swab was used to streak a dried Mueller-Hinton agar surface with an 18-hour-old standardized inoculum of each test bacterial isolate in order to produce a confluent growth. After allowing the inoculated plates to dry, sterile standard 6 mm cork-borer holes were punched into the agar at equal intervals. Then, distinct

extract concentrations were added one at a time to the various wells that had been given the appropriate labels. As a control, a well that was bored into the center of the plate was filled with an equal volume of 20% DMSO. All test organisms underwent this process in triplicate, were left on the bench for thirty minutes, and then were incubated for twenty-four hours at 37°C. The observed zone of inhibition was measured and recorded to the closest millimeter at the conclusion of the incubation period.

Statistical analysis

The mean values were analyzed in one way and expressed as Mean ± Standard deviation (SD). The ANOVA test was employed to ascertain whether there exists a statistically significant variation in the susceptibilities of distinct test organisms to each extract. The Duncan multiple range test was employed to separate the variant means. P≤0.05 was considered significant.

RESULTS AND DISCUSSION

Percentage yield of extraction

Table 1 and Figure 1 show the percentage of the yield extract of *Oenanthe javanica* in each extract. According to the findings, ethanol extract has a higher extract yield (15.051%) than petroleum ether extract (4.713%), indicating that petroleum ether is not a particularly useful extractant for *Oenanthe javanica*. The amount of active ingredients in a specific amount of plant material after it is extracted with a solvent is determined by estimating the extractive value (Pawar and Jadhav 2015).

Phytochemical Analysis:

Table 2 lists the phytochemical components found in the ethanol and petroleum ether extracts of *Oenanthe javanica*. The majority of phytochemicals, with the exception of cardiac glycosides, are present in both the ethanol and petroleum ether extracts of *Oenanthe javanica,* according to preliminary phytochemical screening. In comparison to solvent extract from petroleum ether and ethanol extract, ethanol extract reveals more phytochemical compounds.

Alkaloids are a class of drugs that have diuretic properties, affect the central nervous system, and decrease appetite. It has been demonstrated by numerous studies that saponins have the unusual ability to precipitate and coagulate red blood cells. Additionally, terpenoids have been demonstrated shown to have antimicrobial, antifungal, antiviral, antiparasitic, antiallergenic, antispasmodic, antihyperglycemic, anti-inflammatory, and immunomodulatory qualities. Flavonoids also help to manage oxidative stress brought on by diabetes (Donipati and Sreeramulu 2015). To find abundant phytochemicals that could be the source of plant extracts antioxidant and antibacterial properties, preliminary phytochemical screening is typically carried out (Sagbo *et al.*, 2017).

Antibacterial Assay:

Four bacterial strains - two gram-positive and two gram-negative were tested for the antibacterial activity of the crude ethanol and petroleum ether extract of *Oenanthe javanica*. The results are shown in Table 3 and figure 2A and 2B. When tested against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, and *Klebsiella pneumoniae*, *Oenanthe javanica* ethanol and petroleum ether extracts demonstrated strong antibacterial activity. Significant antibacterial activity was demonstrated by the ethanol extracts against *Escherichia coli* and *Bacillus subtilis*, with respective zones of inhibition of 19.49±1.19 and 19.51±0.91. The zone of inhibition in petroleum ether extract, on the other hand, is smaller and ranges from 6.34±1.25 to 13.39±1.03. Based on the agar-well diffusion method, the study results indicate that *Oenanthe javanica* pure ethanol and petroleum ether extracts had the greatest antibacterial effects on the tested bacteria. Flavonoids ability to form complexes with soluble and extracellular proteins as well as the cell walls of bacteria has been used to exert their antimicrobial activities (Akiyama *et al.*, 2001). The antimicrobial activity is also attributed to plant steroids, alkaloids, and saponins (Hossain *et al.*, 2018). Antimicrobial properties of terpenoids are well established (Scortichini and Rossi 1991). Oils were extracted from the extract by the petroleum ether, which served as a defatting agent. Given that the majority of plant-based oils have antimicrobial activity, this could be a factor in the microbial susceptibility seen (Akgul and Saglikoglu 2005). The study of antimicrobial activities

could be attributed to the activity of these phytochemical constituents. However, more study on this plant is necessary to identify and isolate the active ingredients and determine each unique mechanism of action. Both gram-positive and gram-negative bacteria were inhibited by the ethanol, however greater inhibition zones were seen in the gram-positive microbes, most likely as a result of differences in the composition and structure of their cell walls. This outcome agrees with Mills-Robertson *et al.*, 2012 research.

Table 1. Percentage yield of two different solvent extracts

Sl. No.	Solvent used	Percentage yield
1.	Ethanol	15.051 %
2.	Petroleum ether	4.713%

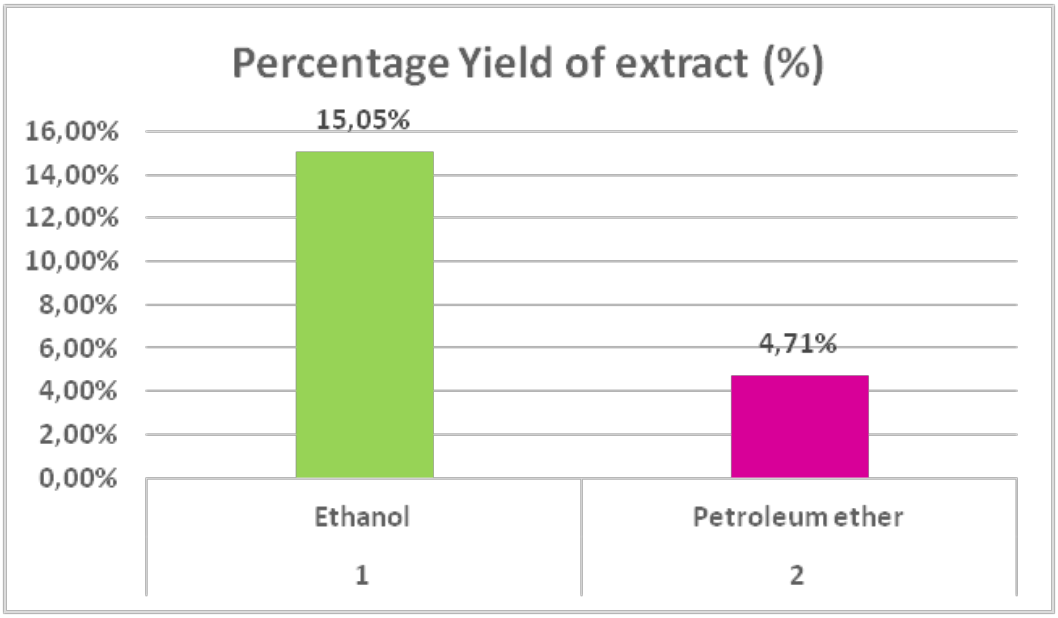

Figure 1: Percentage yield of two different extracts of *Oenanthe javanica*

Table 2. Qualitative phytochemical analysis of *Oenanthe javanica*

Sl. No.	Phytochemical compounds	Solvent extract of *Oenanthe javanica*	
		Ethanol	**Petroleum ether**
1.	Alkaloids (Mayer's test)	**++**	**+**
2.	Saponins (Foam test)	**++**	**+**
3.	Terpenoids (Ferric chloride test)	**+**	**-**
4.	Coumarins (Alkaline test)	**++**	**+**
5.	Cardiac glycosides (Keller-Kiliani test)	**-**	**-**
6.	Phenols (Ferric chloride test)	**++**	**+**
7.	Flavonoids (Lead acetate test)	**+++**	**++**

(+) low, (++) moderately present, (+++) highly present, (-) Absent

Table 3. Zone of inhibition (mm)against gram-positive and gram-negative bacteria

Sl. No.	Pathogenic Bacteria	Experimental units	Inhibition zone (mm) of *O. javanica*	
			Ethanol	Petroleum ether
Gram-Positive Bacteria				
1.	*Bacillus subtilis*	O_1	15.99±1.66	9.28±1.19
		O_2	17.34±1.32	11.52±0.73
		O_3	19.51±0.91	13.39±1.03
		PC	20.58±1.27	15.87±0.85
		NC	0	0
2.	*Staphylococcus aureus*	O_1	12.12±0.98	8.30±1.19
		O_2	14.26±0.99	10.28±1.02
		O_3	16.35±1.06	12.04±0.38
		PC	20.42±1.27	15.87±1.53
		NC	0	0
Gram-Negative Bacteria				
3.	*Escherichia coli*	O_1	13.64±1.23	7.34±0.77
		O_2	15.64±1.05	9.63±1.12
		O_3	18.49±1.19	11.29±1.20
		PC	20.58±0.86	15.01±0.56
		NC	0	0
4	*Klebsiella pneumoniae*	O_1	11.39±1.11	6.34±1.25
		O_2	13.54±1.34	8.30±1.12
		O_3	15.51±0.83	10.29±1.10
		PC	20.13±0.59	15.26±0.62
		NC	0	0

O_1=25%, O_2=75%, O_3=100%, PC=Positive control, NC= Negative control, *O. javanica=Oenanthe javanica*

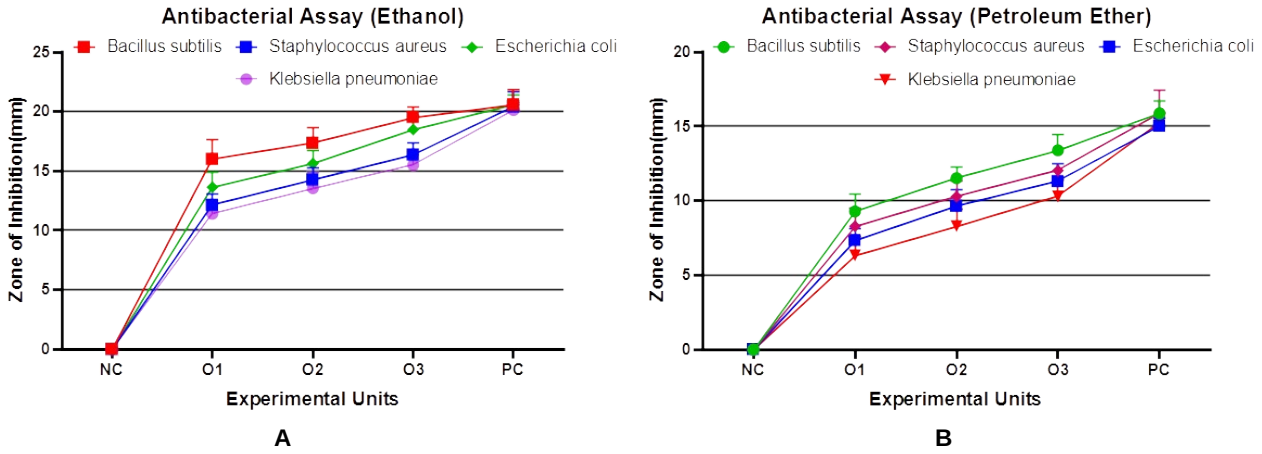

Figure 2: Antibacterial activity of *Oenanthe javanica* ethanol extract (**A**) and Petroleum ether extract (**B**) against gram-positive and gram-negative bacteria

CONCLUSION

In this study, *Oenanthe javanica* phytochemical components extractive values and antibacterial activities were examined. According to phytochemical screening, the antibacterial qualities of the crude extracts of *Oenanthe javanica* depend on the presence of phytochemicals such as alkaloids, saponins, phenols, terpenoids, flavonoids, and coumarins. Additionally, the plant exhibits potent antibacterial activity against a variety of gram-positive and gram-negative bacteria.

Thus, it is plausible to propose that *Oenanthe javanica* possesses a noteworthy therapeutic component. To isolate and identify the active principles present in the extracts that may have potential therapeutic uses, more research is needed.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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