



Research Article

ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT OF *OENANTHE JAVANICA* BLUME (DC) COLLECTED FROM IMPHAL WEST DISTRICT

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ABSTRACT

Oenanthe javanica Blume (DC) is an aromatic medicinal herb having pharmacological properties such as antioxidant, anti-quorum sensing, anti-coagulant, antitoxic, hepatoprotective, anti-hepatitis B virus and memory improvement. It is one of the most consumed vegetable throughout the year in Manipur. The present study was carried out to analyze the phytochemical constituents, antioxidant activity and total phenolic and total flavonoid content of *Oenanthe javanica*. Preliminary phytochemical analysis was done using standard procedure. Total phenolic, total flavonoid content and antioxidant activities were determined spectrophotometrically in crude aqueous and methanolic extracts. Preliminary phytochemical analysis indicates the presence of amino acids, carbohydrates, proteins, flavonoids, phenolic compounds, steroids and terpenoids, saponins, tannins, cardiac glycosides except alkaloids and phlobatannins. The present study demonstrates the antioxidant activity of *Oenanthe javanica* Blume (DC) due to the various phytochemicals present in the extract which confer their traditional uses as food and medicine.

Keywords: *Oenanthe javanica*, phytochemicals, phenolic, flavonoids & antioxidant

INTRODUCTION

Oenanthe javanica (Blume) DC is a perennial herb belongs to the family Apiaceae commonly known as water dropwort. The plant grows wild in freshwater, marshes and swampy fields and along ditches, canals and streams in many Asian countries. The tops are eaten raw in salads or as garnish similar to parsley. The young stems and leaves are also steamed with rice or boiled and chopped as greens¹. The plant is harvested from the wild for local use as food and medicine. It is occasionally cultivated in many regions of the tropics as a food. The plant contains vitamin E. Fruits yield an essential oil containing phellandrene. Seeds and fruits also yield fatty oil. Leaves are appetizer and used in digestive problems. Fresh shoot crushed and boiled with little water and diluted in distilled water. The filtrate is used as an ear-drop in ear-ache. The shoot part is taken as salad for digestion. The whole plant is depurative, febrifuge and styptic². Decoction is used in the treatment of epidemic influenza, fever and discomfort, jaundice, haematuria and metrorrhagia. The leaves are chewed with wild ginger and traditional ash salt as an antidote to poisoning. The leaves are rubbed onto the forehead in order to ease a headache. The stem is chewed and swallowed to ease a cough. The seed contains 3.5% essential oil. This is effective at large dilutions against pathogenic fungi³. Phytochemicals, persicarin and isorhamnetin isolated from *O. javanica* are reported to have anticoagulant activity⁴. *O. javanica* extract is also effective in overcoming alcohol intoxication by accelerating ethanol metabolism⁵. Caffeic acid isolated from *O. javanica* is reported to have hepatoprotective activity^{6,7}. Phenolic obtained from the plant showed anti-hepatitis B virus activity⁸. *O. javanica* is commonly consumed

in the North-eastern State of India, Manipur. It is known as kompek in Manipuri (Meiteilon). Water dropwort is one of the main ingredients in Manipuri Eromba and Singju, which has been the all-time favourite side dish for meals and as evening snacks too. Young stem and leaves are used as ingredient in chagempomba, a traditional dish of Manipur. Noticing the various medicinal uses of *O. javanica* and frequent consumption by local people, the present study was carried out to analyze the total phenolic content, total flavonoid content and antioxidant activity of crude aqueous and methanolic extracts of *O. javanica* collected from the Imphal- West district.

MATERIALS & METHODS

Plant sample

O. javanica were collected from Imphal-West district of Manipur, Northeast India. Identification of the sample was done by L. Somarjit Singh, Associate Professor, Department of Botany, Imphal College, Imphal. Stem and leaves of the plant were washed with tap water and then rinsed with distilled water, shade dried and ground into fine powder.

Soxhlet extraction

40g of powdered *O. javanica* was extracted separately using 400ml of methanol and double distilled water by soxhlation until the solvent become colourless in main chamber of the soxhlet extractor. The extract was evaporated to dryness and crude extract was obtained. The crude extracts were screened for the phytochemical constituents.

Phytochemical screening was carried out for aqueous and methanolic extracts of *O. javanica* using standard protocol⁹⁻¹³.

Determination of total phenolic content

The amount of total phenolic content in aqueous and methanolic extracts of *O. javanica* was determined with Folin-Ciocalteu reagent^{14, 15}. 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na₂CO₃ (2% w/v) were added to 0.5 ml of (3 replicates) extract solution (1mg/ml). The resulting mixture was incubated at 45°C for 15 mins. The absorbance of sample was measured at 760 nm using UV Visible Spectrophotometer (UV-2700). Gallic acid (10-50 µg/ml) was used as a standard compound.

Total phenolic contents in the plant extracts in gallic acid equivalent (GAE) were calculated by the following formula¹⁶:

$$T = (C \times V) / M$$

Where, T = total content of phenolic compounds, mg/g plant extract in GAE; C = concentration of gallic acid established from the calibration curve, µg/ml; V = volume of extract, ml; M = weight of the plant extracts, g.

Estimation of total flavonoid content

Total flavonoid content in the sample extracts were estimated by Aluminium chloride colorimetric method. The principle involved in Aluminium chloride (AlCl₃) colorimetric method is that AlCl₃ forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids (fig.1). Studies have reported Quercetin to be suitable reference for determination of total flavonoid content in plant sample extract. Therefore standard Quercetin solutions of various concentrations were used to make the calibration curve.

10 mg of quercetin was dissolved in 100ml methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 µg/ml using methanol. Stock solution of extracts was prepared by dissolving 100 mg of each extract in 5ml methanol and transferred to 10 ml volumetric flask and made up the volume with methanol. 10% aluminium chloride and 1M potassium acetate were prepared using distilled water.

The assay was determined using 0.5ml of each extract stock solution and each dilution of standard quercetin taken separately in test tubes. To each test tube 1.5ml methanol, 0.1ml aluminium chloride solution, 0.1ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank for all the dilution of standard quercetin and both aqueous and methanolic extracts were prepared in similar manner by replacing aluminium chloride solution with distilled water. All the prepared solutions were filtered through Whatmann filter paper if necessary before measuring their absorbance. Absorbance was taken at 415 nm against the suitable blank^{17,18}.

Determination of free radical scavenging assay

The free radical scavenging capacities of aqueous and methanolic extracts of *O. javanica* were determined using DPPH assay¹⁹. DPPH solution (0.004% w/v) was prepared in methanol. Stock solution (1mg/ml) of each of the extract and standard ascorbic acid (1mg/ml) were prepared using methanol. Various concentrations (30-150µg/ml) of the extracts and ascorbic acid were taken in test tubes and 2ml of freshly prepared DPPH

solution were added. The test tubes were protected from light by covering with aluminum foil. The final volume in each test tube was made to 4ml with methanol and incubated in dark for 30mins at room temperature. After incubation, the absorbance was read at 517nm using a spectrophotometer (UV-2700). Control sample was prepared containing the same volume of methanol and DPPH without any extract and reference ascorbic acid. Methanol was served as blank. The percent DPPH scavenging activity was calculated by using the following equation:

$$\% \text{ Scavenging activity} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

Estimation of reducing power

Various concentrations (30-150µg/ml) of the aqueous and methanolic *O. javanica* extracts from the stock solution of (1mg/ml) in methanol were prepared in different test tubes. Ascorbic acid (1mg/ml) at various concentrations was used as standard. To each tube, 2.5ml of phosphate buffer and 2.5ml of 1% potassium ferricyanide were added. This mixture was kept at 50°C in water bath for 20 minutes. After cooling, 2.5ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10mins (whenever necessary). The upper layers of solution (2.5ml) were transferred into fresh tubes and to each tube, 2.5ml of distilled water and 0.5ml of freshly prepared 0.1% ferric chloride solution were added. The absorbance was measured at 700 nm. Blank was prepared in similar manner excluding samples. Increased absorbance of the reaction mixture indicates increase in reducing power²⁰.

Determination of total antioxidant activity

The phosphomolybdenum method was used to evaluate the total antioxidant activity of the extract²¹. Antioxidants can reduce Mo (VI) to Mo (V) and the green phosphate / Mo (V) compounds at acidic pH, which have an absorption peak at 695 nm, were generated subsequently. 0.3 ml of the sample aqueous and methanolic extracts (1mg/ml) as well as ascorbic acid (1mg/ml) was mixed with 3.0 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) separately. Reaction mixture was incubated at 95°C for 90 min under water bath. Absorbance of all the mixtures was measured at 695 nm after cooling. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in micrograms (AAE). Total antioxidant activity was calculated by using the formula:

$$\text{Total antioxidant} = \frac{\text{O.D. of test sample} \times \text{concentration of standard in } \mu\text{g}}{\text{made up volume of sample}}$$

RESULTS

Phytochemical constituents present in aqueous and methanolic extracts of *O. javanica* are listed in table 1. Preliminary phytochemical screening shows the presence of most of the phytochemicals in both aqueous and methanolic extracts of *O. javanica* except alkaloids and phlobatannins. Presence of oil was noted in methanolic extract and not in aqueous extract.

Total phenolic content was estimated in crude aqueous and methanolic extracts of *O. javanica* by Folin-Ciocalteu method. Standard curve of gallic acid is illustrated in fig. 2. Total phenolic content in the sample extracts were calculated using the above mentioned formula and data obtained from the gallic acid calibration curve. Total phenolic content in aqueous and

methanolic extracts were found to be 48.81 mg/g (GAE) and 41.86 mg/g (GAE) respectively indicating a higher total phenolic content in aqueous extract than methanol extract.

Total flavonoid content in crude aqueous and methanolic extracts of the plant sample was quantified by aluminum chloride method. The quercetin standard calibration curve is shown in fig. 3. From the standard curve of quercetin ($y=0.018x$, $R^2=0.997$), concentration values of both extracts were obtained and total flavonoid content (TFC) was calculated by using the following formula²²

$$TFC = R * D.F * V * 100 / W$$

Where R - Result obtained from the standard curve, D.F - Dilution factor, V - Volume of stock solution, 100 - For 100 g dried sample and W - Weight of plant sample used in the experiment.

Total flavonoid content was found to be higher in methanolic extract than aqueous extract of the plant. Table 2 indicates the total phenolic and total flavonoid content in crude aqueous and methanolic extracts of *O. javanica*.

DPPH assay showed an increase in concentration increases the free radical scavenging activity for the reference standard, ascorbic acid and crude aqueous and methanolic extracts of *O. javanica*. The assay indicates a dose dependent manner for standard and both the extracts of the plant sample as illustrated

in fig.4. Percentage DPPH scavenging activity of aqueous and methanolic extracts was comparable with standard ascorbic acid and methanolic extract showed higher scavenging activity than aqueous extract. For aqueous extract 73.2µg/ml is required to scavenge 50% of DPPH radical while it require less than 30µg/ml methanolic extract to scavenge 50% of DPPH radical. Similar DPPH scavenging activity was noted for the standard and methanolic extract of the plant from 60µg/ml to 150 µg/ml concentration. At the highest concentration (150µg/ml) used for the study, the DPPH scavenging activity of the standard, aqueous and methanolic extracts of *O. javanica* were 99.79%, 83.94% and 97.09% respectively.

Reducing power assay indicates an increasing order for standard as well as for both the extracts of plant sample and is shown in fig. 5. Reducing power noted in aqueous and methanolic extract is almost equal with methanolic extract showing a slight higher in reducing power than aqueous extract of the plant.

Similar trend was noted in total antioxidant activity where methanolic extract showed higher activity than aqueous extract. At highest concentration used for the study, total antioxidant activity shown by methanolic extract was 224.5µg AAE/mg of extract while that of aqueous extract was 58.21µg AAE /mg of extract. Total antioxidant activity shown by various concentrations (30-150 µg/ml) of crude aqueous and methanolic extracts of *O. javanica* is shown in table 3.

Table 1: Phytochemical constituents present in the whole plant extract of *O. javanica*

Phytochemical constituents	Tests	<i>Oenanthe javanica</i> whole plant	
		Aqueous extract	Methanolic extract
Amino acids	Ninhydrin test	+	+
Alkaloids	Hager's test	-	-
Carbohydrates (reducing sugar)	Benedict's test	+	+
	Fehling's test	+	+
Proteins	Xanthoproteic test	+	+
Flavonoids	Alkaline reagent test	+	+
	Lead acetate test	+	+
Phenolic compounds	Lead acetate test	+	+
	Ferric Chloride test	+	+
Steroids and Terpenoids	Salkowski's test	+	+
	Froth test	+	+
Saponins	Froth test	+	+
Tannins	Lead acetate test	+	+
	Ferric chloride test	+	+
Cardiac glycosides	Keller-killiani test	+	+
Oil		-	+
Phlobatannins		-	-

Table 2: Total phenolic and total flavonoid content in crude extracts of *O. javanica*

<i>O. javanica</i> whole plant extract			
Total phenolic content in mg GAE /g of extract		Total flavonoid content in µg QE /100g of dried extract	
Aqueous	Methanol	Aqueous	Methanol
48.81 ± 0.02	41.86 ± 0.06	4.52 ± 0.06	19.80 ± 0.07

Assays were performed in triplicates. Values are expressed as means ± SD

Table 3: Total antioxidant activity of *O. javanica*

Concentration (µg/ml)	Total antioxidant activity in µg AAE /mg of extract	
	Aqueous	Methanol
30	8 ± 0.05	55.51 ± 0.04
60	22.01 ± 0.04	100.32 ± 0.03
90	30.09 ± 0.05	149.16 ± 0.04
120	38.17 ± 0.03	169.38 ± 0.09
150	58.21 ± 0.14	224.5 ± 0.03

Assays were performed in triplicates. Values are expressed as means ± SD

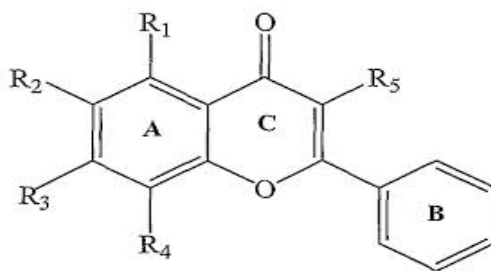


Figure 1: Basic structure of flavonoid

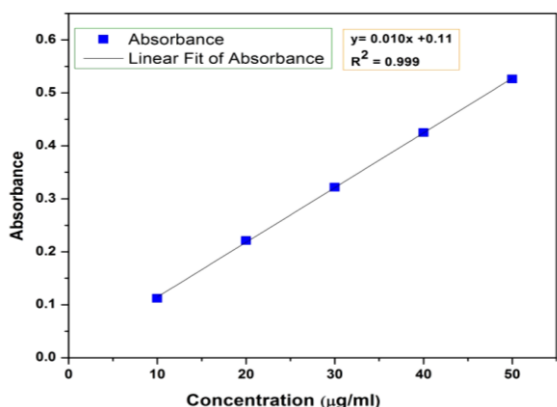


Figure 2: Standard curve of gallic acid

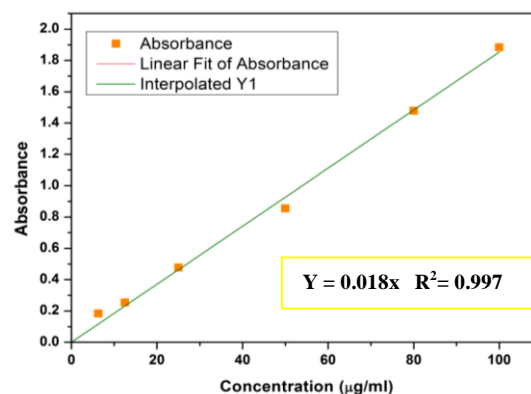
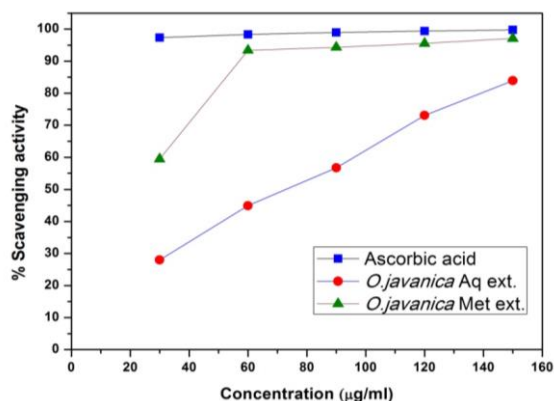


Figure 3: Standard curve of Quercetin



Aqueous =Aq, methanolic =Met & extract = ext

Figure 4: DPPH scavenging activity shown by standard and crude extracts of *O. javanica*

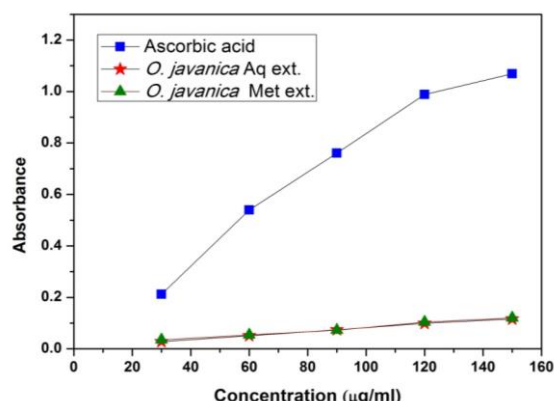


Figure 5: Reducing power shown by standard and crude extracts of *O. javanica*

DISCUSSION

Phytochemical screening indicates the presence of most of the phytoconstituents in the crude aqueous and methanolic extracts of *O. javanica*. The plant has been reported to have constituents of phenylpropanoids, flavonoids and phenolic acids, notably, persicarin and isorhamnetin²³. Preliminary phytochemical screening is commonly performed for the identification of substantial phytochemicals that may be involved in the antioxidant activity of plant extracts²⁴⁻²⁶. In the present study, total phenolic content was analyzed in the crude extracts as they are the primary compounds responsible for antioxidant activity. Total phenolic content in aqueous extract as per our study is 48.81mg GAE/g whereas according to Reihani and Azhar is 31-33mg GAE/g²⁷. The difference in total phenolic content may be due to difference in geographical region and climatic condition. Phenolic compounds are the most abundant phytochemical in

plants and considered as important natural antioxidants. Phenolic compounds exhibit their antioxidant activity by various mechanisms such as donation of hydrogen atoms to free radicals and through connection to transition metal ions resulting in more stable forms²⁸. Various physiological actions performed by polyphenols were related to the prevention of neurodegenerative and cardiovascular diseases, cancer, among others, mainly because of their high antioxidant capacity²⁹. Many studies illustrates that water is the best solvent for extraction of phenol from most plant species. Our result also indicates water, a better solvent to extract phenol from *O. javanica* than methanol. In contrast to total phenolic content, total flavonoid content was found to be higher in methanolic extract of *O. javanica*. Among the polyphenol compounds, the most studied subclass is the flavonoids which in plants are commonly found conjugated to sugars³⁰. Flavonoid extracted from *O. javanica* has neuroprotective activities. Neuroprotection of *O. javanica* is

related with increased or maintained intracellular antioxidant enzymes by *O. javanica*^{31,32}. DPPH assay of the present study showed a dose dependent manner. The results of the DPPH free radical scavenging assay suggest that whole plant of *O. javanica* have potent antioxidant property which may be due to presence of various phytochemicals in the extracts mainly phenols and flavonoids. Isorhamnetin isolated from the aerial part of *O. javanica* was reported to have strong radical scavenging activity³³. As per Hwang et al., phenolic and flavonoid content was higher in ethanol extract than aqueous extract and also showed higher DPPH scavenging activity and reducing power³⁴. DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples³⁵. It is a stable free radical with a characteristic absorption at 517 nm that was used to study the radical-scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. Antioxidants, on interaction with DPPH, transfer either an electron or hydrogen atom to DPPH, thus neutralizing its free radical character³⁶. The color changed from purple to yellow and the absorbance at wavelength 517 nm decreased. Similar trends were noted in reducing power assay and total antioxidant activity of crude aqueous and methanolic extracts of *O. javanica*, with increased in concentration reducing power and antioxidant activity increases. Reducing power of the extracts may be contributed by bioactive compounds in the extract which possess electron donating abilities. Presence of reducers causes the conversion of the Fe³⁺ complex to the ferrous (Fe²⁺) form which serves as a significant indicator of its antioxidant capacity³⁷.

The present study showed methanolic extract of *O. javanica* has higher antioxidant activity than aqueous extract which may be due to higher flavonoid content in the methanolic extract, which would justify its traditional use. Water is found to be a better solvent for extracting phenols while methanol is a better solvent for extracting flavonoid from *O. javanica*. Whole plant of *O. javanica* can be one of the potential sources of natural antioxidants. Further investigation can be done to find out the possible causes and their mechanisms responsible for the antioxidant property of *O. javanica*.

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