**Antimicrobial, Cytotoxic, and DPPH Scavenging Activities of Oenanthe Javanica**

**Abstract**

Medicinal plants are an integral part of life in many communities. They are source of vital therapeutic aid for assuaging human ailments and that is chiefly due to existence of biologically active phytochemicals. Oenanthe javanica, aquatic perennial herb, known for its medicinal values. The present research was conducted to investigate the chemical composition of essential oil extracted from O. javanica through GC-MS analysis; to evaluate the phytochemical constituents, cytotoxic capability and antioxidant activity of different extracts; and finally, to evaluate the antimicrobial potential of essential oil and different extracts of O. javanica. GS-MS analysis shown that seven constituents are present in the essential oil. Phytochemical investigation has shown that O. javanica contains following components alkaloids, flavonoids, tannins, phenol, and saponins in all its extracts except n-hexane extract which lacks saponins. Among all extracts only chloroform extract is found to be cytotoxic and best values for DPPH scavenging potential was exhibited by ethanolic extract. Some of the extracts of O. javanica possess antimicrobial potential whereas essential oil was only active against tested fungal strains.

**Keyword**: Oenanthe javanica, essential oils extraction, phytochemical screening, cytotoxic, antimicrobial and scavenging properties.

**1. Introduction**

 Phytochemicals are non-nutritive components of plants which provides protection against certain diseases [1]. Medicinal plants are gift to living world and contributing important role in health despite the modernization in medicinal field [2] [3]. Presently, World Health Organization explores that, world’s 80% population depend on plants for their health [4]. Plants are the largest source of traditional medicines having several functions such as antimicrobial, anticancer, antioxidant, cytotoxic, antiviral and much more [5]. 20 % of plants of world have been investigated to explore their pharmacological or biological properties [6]. Antioxidants of the plants scavenges the destructive effect of oxidants during oxidative stress both as raw extract and as pure chemical constituent [3]. The isolated phytochemicals are then examined via different biological assays for their activities [7]. Oenanthe javanica (aquatic perennial herb), family Umbelliferae member, commonly known as water dropwort is mostly grows in ditches, swampy places, canals and streams of Asia and Australia. It has distinctive taste and fragrance [8-11]. Koreans uses O. javanica for seasoning of soaps and stews and as food. As a traditional medicine Chinese uses it for the cure of fever, jaundice, leucorrhea, abdominal pain, hypertension, urinary infection, mumps, and hepatitis [11] [12]. Researchers reported volatile compounds and number of phytochemicals of this plants like pulegone, β-pinene, limonene, germacrene, and coumarins, pthalic acid ester, flavonoids, and phenolics [13] [14]. Flavonoids of O. javanica have antimutagenic properties against aflatoxin B1 induces mutations and have ability to scavenge heavy metals (Cd and Pb) from polluted water [15] [11]. Protective effect against hepatitis B virus (HBV) has been shown by the total phenolics of O. javanica in animal models [16]. Anticancer, neuroprotective, hepatoprotective, antidiabetic, and antithrombotic properties of O. javanica has been reported by researchers [17].

 Essentials oils can be applied to following fields: food preservations, phytopathology, medicine, clinical microbiology, and pharmaceutical botany. Essentials oils’ antimicrobial capacity of Lamiaceae family members has been evaluated by many researchers, whereas few of them studied the essential oils chemical composition. A hyphenated technique Gas chromatography-Mass spectrophotometry (GC-MS) is vital for qualitative and quantitative analysis of essential oils along with their biological activities evaluation [18].

 Crude extract can be first examined for phytonutrients and active fractions [7]. Brine shrimp lethality assay (BSLT) is a front-line screening assay for detecting a broad spectrum biological activity of crude plant extract. This predicts the pesticide, cytotoxic and anticancer ability of crude extract [19] [20]. For phytochemist several bioassays are available, but DPPH radical scavenging assay is getting more attention due to its high sensitivity and simplicity [6] [21]. Emerging of various diseases makes the finding of new antimicrobial drugs necessary with diverse and active chemical compounds having ability to neutralize fatal microorganisms [22] [23]. Medicinal plants can have the new potential antimicrobials compounds due to outstanding chemical diversity. Active compounds have been reported in plant extracts in previous literature with antimicrobial capacity through bioassays such as antifungal and antibacterial assays [24].

 In current research our objectives were, first, evaluate the chemical composition of essential oils from O. javanica, collected from Pakistan, through GC-MS analysis, second, to determine phytochemical constituents and cytotoxic capability of different extracts (methanol, ethanol, n-hexane, chloroform, and ethyl acetate) of O. javanica, third, to evaluate the antimicrobial potential of different extracts and essential oils of O. javanica, and, finally to check the DPPH radical scavenging potential of extracts.

**2. Materials and methodology**

2.1. Plant collection

 The O. javanica was collected from the Sufaid dheray (a local area of district Peshawar, Khyber Pakhtunkhwa, Pakistan) and identified by Prof. Dr. Saleem of Botany Department, Islamia College Peshawar, Pakistan.

2.2. Essential oil extraction

 The essential oil was extracted by conventional steam distillation technique from the aerial parts of the O. javanica. In distilled water (2000 mL) fresh plant (100 mg) was added and then placed in 2000 mL round bottom flask. Water was boiled under control conditions to extract oil from plant for 3 hrs. After extraction, anhydrous sodium sulphates was used for the removal of water from the oil and they were stored at 4 ℃ until further use in vials [25].

2.3. GC-MS spectrometry and identification of compounds of essential oils

 Gas chromatography-Mass spectrophotometry instrument equipped with fused silica column (60 m x 0.25 mm i.d., 0.25 µm film thickness) was used for determining essential oils composition. The temperature of the instrument’s oven was set from 600C to 250 ℃ at 5 ℃ min-1 rate and then held for 10 min at 250 ℃ (transfer line temperature was 250 ℃). Helium gas, as carrier gas, was used at rate of 1.1 mL/min. Finally, by comparison of retention times (Rt) and mas mass spectra with those given in literature the essential oil components were identified.

2.4. Extract preparation

 The fresh collected plant (O. javanica) was shade dried and then dry plant was powdered through Willy Mill to 60 mesh size. Extracts was prepared by soaking 500 g of powdered dried plant in 2000 mL of methanol, ethanol, n-hexane, chloroform and ethyl acetate separately at room temperature for 48 hrs. Extracts were then filtered via Whatman No. 1 and concentrated using rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan). The extracts were then stored at 4 ℃ for further bioassays. The MeJ, EtJ, nHJ, ChJ, and EaJ abbreviations were used for representing methanolic, ethanolic, n-hexane, chloroform, and ethyl acetate respectively to avoid unnecessary repetitions.

2.5. Phytochemicals screening

 Phytochemicals screening of MeJ, EtJ, nHJ, ChJ, and EaJ for the evaluation of alkaloids, flavonoids, tannins, saponins, and phenol was carried out:

2.5.1. Test for alkaloids

 For alkaloids detection we followed the protocol as with one modification i.e. instead of 1 mL of sample 0.4 gm of extract was used. 0.4 g of extract (MeJ, EtJ, nHJ, ChJ, and EaJ) was mixed with 3 mL of Hager’s reagent (saturated solution of picric acid) separately. Formation of yellow colored precipitate was taken as indication for presence of alkaloids [26].

2.5.2. Test for flavonoids

 50 mg of extract (MeJ, EtJ, nHJ, ChJ, and EaJ) was mixed with distilled water (100 mL) and then filtered via whatman filter paper. To 10 mL of filtrate 5 mL of dilute ammonia solution was added followed by concentrated sulfuric acid few drops. Yellow coloration will confirm the presence of flavonoids [27].

2.5.3. Test for tannins

 For detection of tannins we followed the protocol as 50 mg of MeJ, EtJ, nHJ, ChJ, and EaJ extract was boiled in distilled water (20 mL) separately and then filtered to get filtrate. To filtrate few drops of FeCl3 (0.1%) was added. Change in color to brownish green or blue black was an indication for the presence of tannins [27].

2.5.4. Test for saponins

 Screening test for saponins was done by following the procedure as MeJ, EtJ, nHJ, ChJ, and EaJ (20 mg of each) was mixed with distilled water (20 mL) and boiled for 5 minutes in water bath and then filtered. Distilled water (5 mL) was then mixed with filtrate (10 mL) and vigorously shaken for formation of froth. Later to forth form few drops of olive oil were mixed and vigorously shaken to observe emulsion development [28].

2.5.5. Test for phenol

 2 mg of extract (MeJ, EtJ, nHJ, ChJ, and EaJ) were properly mixed with 2 mL of ethanol separately. Few drops of ferric chloride solution were added and observed for change in coloration [28] [29].

2.6. Brine shrimp lethality assay

 O. javanica cytotoxic potential was determined using Brine Shrimp Lethality Assay (BSLA) [30]. Different concentration (1000 µg/mL, 100 µg/mL, and 10 µg/mL) of each extract (MeJ, EtJ, nHJ, ChJ, and EaJ) were prepared in methanol and transferred into vials, then allowed for evaporation of methanol.

Brine Shrimp eggs were taken from the Department of Biochemistry (Quaid-i-Azam University, Islamabad). Artificial and filtered sea water was prepared by dissolving sea salt (38 gm) in distilled water (1 L) for hatching of eggs. The sea water was added in plastic container having two partitions separated by porous wall; one partition is for light and other one is dark (covered) area. After incubation at room temperature (23 to 28 ℃) for 24 to 72 hrs, the hatched larvae moved from dark partition to lighter one. 10 shrimps were added with pipette in each vial and sea water final volume is adjusted to 5 mL in each vial. Each vial was left naked under lamp for 24 hrs. The living shrimps were calculated in each vial and this was used for the determining the LD50 value, the concentration at which it could kill 50% larvae, for each extract. Each concentration was tested in triplicate.

2.7. Antimicrobial activity

 Antibacterial and antifungal potential of O. javanica’s extracts and essential oil against three bacterial strains of gram-negative class (Escherichia coli ATCC 15224, Klebsiella pneumonia MTCC 0618, and Xanthomonas) and three fungal strains (Aspergillus nigar ATCC 6275, Candida albicans 90028, and Acremonium) that were collected from Department of Microbiology (Quaid-i-Azam University, Islamabad), via agar well diffusion assay [31]. Different concentrations (2 mg/mL and 3 mg/mL) of each extract were prepared in DMSO (Dimethyl sulfoxide) whereas oil was given as pure. On dried agar plates using a sterile swab inoculum of each bacterial strain and spores of fungal strains were swabbed. 100 µL of each extract and pure essential oil were administered in each well. Petri plates were then set for incubation at 37 ℃ for 24 hrs. After that zone of inhibition was measured and average value was calculated. Streptomycin and Flumetazole was used as standard drug for antibacterial and antifungal potential evaluation, respectively [32] [33].

2.8. DPPH radical scavenging potential assay

 In vitro 2, 2’- diphenyl-1-picrylhydrazyl (DPPH) assay was used to determine the O. javanica extract’s free radical scavenging potential [34]. 0.24 g of DPPH was dissolved in an analytical methanol (100 mL) and stored at 20 ℃ for further use. DPPH solution optical density was adjusted at 0.98 (±0.02) at 517 nm via spectrophotometer at the time of use. Crude extracts’ stock solution was prepared by mixing it in analytical methanol (95%) to make a concentration of 1 mg/mL. Further series of concentration (100 µL, 200 µL, 300 µL, 400 µL, and 500 µL) were prepared by diluting the stock solution. DPPH aliquot (900 µL) was mixed with test sample (100 µL) in the Eppendorf. Followed by well shaking, the reaction mixture was incubated for 15 minutes under dark at 27 ℃. Ascorbic Acid was used as standard whereas control has only methanol (100 µL in 900 µL DPPH solution). Using spectrophotometer (SP-200) the absorbance of the working dilutions, standard and control was measured at 517 nm. Free radical scavenging potential was estimated by the following formula:

$$Scavenging Potential=\left[\frac{Control absorbance-Sample absorbance}{Control absorbance}\right]x100$$

**3. Results**

3.1. Essential oils extraction and composition

 The steam distillation of aerial parts of O. javanica for 3 hrs yielded 0.35% (w/w). The n-hexane fraction of essential oils was investigated by GC-MS and total of seven chemical constituents were eluted between 1.367 and 25.890 min based on comparison between their relative retention times and mass spectra with those obtained from authentic samples, Wiley libraries and NITS/NBS libraries spectra shown in Table 1. Whereas GC-MS chromatogram of O. javanica‘s essential oil is shown in Figure 1.

****

Figure 1: GC-MS chromatogram of O. javanica‘s essential oil.

Table 1: Chemical constituents present in essential oil of Oenanthe javanica.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Chemical Constituent** | **Molecular Formula** | **Molecular Weight** | **Retention Time** | **Percent area (%)** |
| Ethanol | C2H6O | 46 | 1.36 | 24.07 |
| Trichloromethane | CHCl3 | 118 | 1.63 | 11.54 |
| Dichloromethanesulphonyl chloride | CHCl3O2S | 182 | 2.42 | 2.28 |
| Apiol 1,3-benzodioxole, 4,7-dimethoxy-5-(2-prppenyl) benzene | C12H14O4 | 222 | 17.29 | 3.04 |
| Pithalic acid, di(6-methylhept-2-yl) ester | C24H38O4 | 390 | 23.84 | 2.58 |
| Pithalic acid, di(2-propylpentyl) ester | C24H38O4 | 390 | 24.00 | 2.88 |
| 4-methylthiomethylnitrostyrene 4-methylthiophenyl-beta-nitropropene | C10H11N2 | 209 | 25.89 | 0.45 |

3.2. Phytochemical screening

 Phytochemical screening tests of MeJ, EtJ, ChJ, and EaJ confirmed the existence of alkaloids, flavonoids, tannins, phenol and saponins as shown in Table 2. Whereas nHJ extract contains before mention constituents except saponins.

Table 2: Qualitative analysis of Oenanthe javanica.

|  |  |
| --- | --- |
| **Phytochemicals** | **Extracts** |
| MeJ | EtJ | nHJ | ChJ | EaJ |
| Alkaloids | + | + | + | + | + |
| Flavonoids | + | + | + | + | + |
| Tannins | + | + | + | + | + |
| Phenol | + | + | + | + | + |
| Saponins | + | + | – | + | + |

+ = Presence, - = Absence, MeJ; O. javanica methanolic extract, EtJ; O. javanica ethanolic extract, nHJ; O. javanica n-Hexane extract, ChJ; O. javanica chloroform extract, EaJ; O. javanica ethyl acetate extract.

3.3. Antimicrobial potential of extracts and essential oil

 Agar well-diffusion method was used to evaluate the antimicrobial potential of extracts (MeJ, EtJ, nHJ, ChJ, and EaJ) and essential oil of O. javanica. Extracts and essential oil of O. javanica were tested against three bacterial strains (Escherichia coli ATCC 15224, Klebsiella pneumonia MTCC 0618, and Staphylococcus aureus ATCC 6538) and three antifungal strains (Aspergillus nigar ATCC 6275, Candida albicans 90028, Acremonium). The antibacterial and antifungal activity was compared against standard antibiotic drug (Streptomycin) and antifungal drug (Flumetazole), respectively.

 Escherichia coli was inhibited by extract EaJ (3 mg/mL) and nHJ (3 mg/mL) while remaining of the extracts and essential oil failed to inhibit the growth of Escherichia coli. On the other hand, Klebsiella pneumonia’s growth was restricted by extract EaJ (3 mg/mL) and ChJ (3 mg/mL) while remaining extract were unable to inhibit the growth of the Klebsiella pneumonia. None of extract and essential oil were able to inhibit the growth of bacterial strain Staphylococcus aureus where as the standard drug streptomycin (1 mg/mL) has inhibited all three bacterial strains under study.

 The invitro antifungal activity of extracts and essential oil of O. javanica is shown in Table 3 in the form of zone of inhibition. In case of antifungal activity, only essential oil (100 µl/ml) shown the antifungal potential against Aspergillus nigar while against the fungal strain Candida albicans essential oil and other extracts have shown potential to restrict the fungal growth. Essential oil (100 µl/ml) and extract EtJ (3 mg/mL) failed to show any antifungal activity against the fungal strain Acremonium while other extracts have shown some antifungal potential. Flumetazole was active against all three fungal strains. The zone of inhibitions of extracts and oil of O. javanica against antibacterial and antifungal strains are shown in Table 3.

Table 3: Antimicrobial potential of extract and essential oil of Oenanthe javanica.

|  |  |  |
| --- | --- | --- |
| **Extract / Antibiotic** | **Conc.** | **Zone of inhibition** |
| **Antibacterial** | **Antifungal** |
| *E. coli*(ATCC 15224) | *K. pneumonia*(MTCC 0618) | *S. aureus*(ATCC 6538) | *A. nigar*(ATCC 6275) | *C*. *albicans* (90028) | A*cremonium* |
| MeJ | 3 mg/mL | - | - | - | - | 11.66±1.53 | 7.9±0.36 |
| EtJ | - | - | - | - | 9.00 ± 1.73 | - |
| nHJ | 3.06±0.40 | - | - |  | 10.33±1.51 | 5.96±0.95 |
| ChJ | - | 2.00±0.20 | - | - | 9.66 ± 1.52 | 8.03±0.57 |
| EaJ | 3.03±0.35 | 2.13±0.32 | - | - | 26.16±1.25 | 8.36±0.55 |
| Essential oil | 100µl/ml | - | - | - | 20.33±1.52 | 25.33±1.54 | - |
| Streptomycin | 1 mg/mL | 7.00±1.73 | 09.20±0.15 | 19.00±3.01 |  |  |  |
| Flumetazole |  |  |  | 10.66±2.08 | 10.00±1.01 | * 1. ±2.51
 |
| Negative control  | 100% DMSO | - | - | - | - | - | - |

“-” sign shows that extract or essential oil shown no activity against respective microbial strain. Values are expressed as mean±SD (n=3). MeJ; O. javanica methanolic extract, EtJ; O. javanica ethanolic extract, nHJ; O. javanica n-Hexane extract, ChJ; O. javanica chloroform extract, EaJ; O. javanica ethyl acetate extract, E. coli; Escherichia coli, S. aureus; Staphylococcus aureus, A. nigar; Aspergillus nigar; C. albicans; Candida albicans,

3.4. Cytotoxic potential of extracts

 Brine shrimp lethality assay for different extracts (MeJ, EtJ, nHJ, ChJ, and EaJ) was performed for cytotoxic screening which has shown that ChJ extract has lowest LD50 value (47.07) while the other extracts (EtJ, nHJ, and EaJ) exhibited higher values of LD50 (>1000). Table 4 shows the LD50 values for understudy extracts of O. javanica.

Table 4: Cytotoxic activity (% mortality) of extracts of Oenanthe javanica.

|  |  |  |  |
| --- | --- | --- | --- |
| **Extracts** | **Different concentrations (µg/mL)** | **LD50** | **Result** |
| 1000 | 100 | 10 |
| MeJ | 42±1.31 | 21±1.44 | 16±1.29 | >1000 | Non-Toxic |
| EtJ | 40±1.33 | 20±1.28 | 15±1.96 | >1000 | Non-Toxic |
| nHJ | 45±1.92 | 20±1.30 | 15±0.32 | >1000 | Non-Toxic |
| ChJ | 100 | 55±1.32 | 30±1.44 | 47.04 | Highly Toxic |
| EaJ | 30±0.56 | 25±0.34 | 15±0.57 | >1000 | Non-Toxic |

Values are expressed as mean±SD (n=6), MeJ; *O*. *javanica* methanolic extract, EtJ; *O*. *javanica* ethanolic extract, nHJ; *O*. *javanica* n-Hexane extract, ChJ; *O*. *javanica* chloroform extract, EaJ; *O*. *javanica* ethyl acetate extract

*3.5. DPPH Scavenging potential of extracts*

 The scavenging potential of the extracts of *O*. *javanica* on DPPH radical is shown in Table 5. The LC50 values were in following order: ChJ>nHJ>EtJ>MeJ>EaJ. The LC50 value of EtJ (45.63±5.88) is closer to the LC50 value of the ascorbic acid (31.59±6.01). All other fractions showed highest LC50 values than ascorbic acid.

**Table 5:** LC50 values of various DPPH inhibiting activities of *Oenanthe javanica*

|  |  |
| --- | --- |
| **Extract/Standard** | **DPPH scavenging Potential** |
| LC50 (µg/ml) | R2 |
| MeJ | 95.06±2.30c | 0.985 |
| EtJ | 45.71±5.88d | 0.938 |
| nHJ | 139.5±4.60ab | 0.990 |
| ChJ | 154.51±12.10a | 0.979 |
| EaJ | 127.20±1.1b | 0.984 |
| Ascorbic Acid | 31.65±6.01d | 0.868 |

Values are expressed as mean±SD (n=6), a-d (Mean with different letters) specify significance at *p* <0.01, MeJ; *O*. *javanica* methanolic extract, EtJ; *O*. *javanica* ethanolic extract, nHJ; *O*. *javanica* n-Hexane extract, ChJ; *O*. *javanica* chloroform extract, EaJ; *O*. *javanica* ethyl acetate extract.

**4. Discussion**

 Therapeutic plants are vital source for curing various diseases around the world [35]. With the passage of time importance of medicinal plants is increasing due to useful phytochemicals they contain to cure such diseases [36]. In order to analyze important bioactive compounds in therapeutic plants phytochemical screening is imperative step. Plant have a large of number of compounds having different polarities. When these compounds are dissolved in different solvents (having different polarities), they distribute themselves under “like dissolves like” rule [37] [38]. In our study we obtained maximum yield in following order ethanol>n-hexane>chloroform>ethyl acetate>methanol, when we used them for extraction purposes. Phytochemical analysis gives important clue about medicinal importance of medicinal plants [39]. Number of phytochemicals are known to posses’ therapeutic properties such as antibacterial, antifungal [40] and anti-oxidant [41] activities etc. In this work we conducted phytochemical screening test to confirm the existence of tannins, phenols, terpenoids, saponins, alkaloids and flavonoids in the MeJ, ChJ, EtJ, nHJ, and EaJ extracts of O. javanica. All extracts possessed above tested phytochemicals except n-hexane which lack the presence of saponins. Phytochemicals gives medicinal features to plant. Tannins have good activities against diarrhea, hemorrhage, virus, bacteria, fungi, parasites, and have cytotoxic potential [39] [42] [43]. Phenols and flavonoids have anti-oxidant and anti-inflammatory activity [44]. Alkaloids have a good effect on neurological disorders like Alzheimer disease [45]. Saponins present in plants have vital role in defense against pathogenic microbes [46] [47]. Our plant extract has shown antimicrobial activities, cytotoxic and antioxidant potential which confirms the presences of tannins, saponins, phenol, flavonoids, and terpenoids. The presence of these phytochemicals puts light on medicinal importance of O. javanica.

 In the present research the antimicrobial activity of extracts (MeJ, EtJ, nHJ, ChJ, & EaJ) and essential oil of O. javanica was determined against three bacterial strains (E. coli, K. pneumonia and S. aureus) and three fungal strains (A. nigar, Candida albicans, Acremonium). Researchers suggests that plants rich in flavonoids have antibacterial potential. Antibacterial potential was shown by flavonoids rich extract of species of Capsella [48] and Hypericum [49]. Tannins are reported to have antibacterial activity [50] [51]. Antibacterial activity of all extracts and essential oil was checked in the form of zone of inhibition and no MIC was observed because plant shown the lower activity against under test bacterial strains. Only ChJ and nHJ extract were active against E. coli and K. pneumonia whereas other extracts and essential oil failed to show any activity against tested bacterial strains. Antifungal activities shown by Terminaliz sericea is due to presence of saponins and tannins [52]. Whereas flavonoids were also being reported for having antimicrobial potentials [53]. Only essential oil was active against A. nigar. All extracts and essential oil have shown antifungal potential against Candida albicans. Essential oil and EaJ extract have shown significant activity against Candida albicans. Against Acremonium MeJ, nHJ, ChJ, and EaJ have shown very low antifungal activity. The work was also done on Fagonia oliveria and investigations revealed the antifungal potential of extracts of F. oliveria with different solvents against five fungal strains; ethyl acetate extract was most active against A. flavus. In. our study also ethyl acetate has shown maximum inhibition, but it was against Candida albicans [54].

 Cytotoxic activity of plant was checked by using the brine shrimp lethality assay to kill the laboratory cultures brine shrimps (Artemia nauplii) as described [30]. MeJ, EtJ, ChJ, nHJ and EaJ extracts of O. javanica were tested for cytotoxic potential. LD50 is minimum concentration at which 50% of shrimps can be killed. In our study, chloroform extract has shown lowest LD50 value that is 47.04. The results stated that LD50 values above 1000 µg/mL were considered as non-toxic; 500-100 µg/mL as light toxic; 250-400 µg/mL as medium toxic; and below 249 µg/mL as highly toxic [55]. All other extracts of O. javanica are non-toxic.

 Antioxidants present in medicinal plants play vital role in scavenging the free radicals. DPPH is very popular, sensitive and short time assay to evaluate the potential of natural antioxidants of plant extracts and compounds to scavenge free radicals [56] [57]. Reports shows that flavonoids have strong antioxidative capacity than nonflavonoids [58] and research shows that tannins have more potential to quench free radical [59]. We investigated the DPPH scavenging potential of all extracts of O. javanica. Chloroform extract showed good scavenging ability against DPPH. All the extract showed LC50 higher than ascorbic acid used as standard. It is reported that leaves of Indigofera Aspalathoides showed significant antioxidant potential. Among all chloroform fraction was most active with lowest IC50 value in DPPH assay regardless of having less polyphenolics [60].

**5. Conclusion**

 GC-MS analysis of essential oil of O. javanica confirmed the presence of 7 chemical constituents in essentials oil. Our study shows that the extracts of O. javanica contains bioactive constituents namely alkaloids, flavonoids, tannins, phenols, saponins and biologically active against tested microbes. Cytotoxic activity, and DPPH scavenging potential performed for different extracts verified medicinal importance of O. javanica.

**References**

[1] Chede PS. Phytochemical analysis of Citrus sinensis pulp. International Journal of Pharmacognosy and Phytochemical Research. 2012; 4(4):221-3.

[2] Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. Journal of medicinal plants research. 2009; 3(2):67-72.

[3] Zengin G, Aktumsek A, Guler GO, Cakmak YS, Yildiztugay E. Antioxidant Properties of Methanolic Extract and Fatty Acid Composition of Centaurea urvillei DC. subsp. hayekiana Wagenitz. Records of Natural Products. 2011 Apr 1; 5(2).

[4] Ramesh P, Okigbo RN. Effects of plants and medicinal plant combinations as anti-infectives. Afr. J. Pharm. Pharmacol. 2008 Sep; 2(7):130-5.

[5] Islam MR, Reza AA, Chawdhury KA, Uddin J, Farhana K. Evaluation of in vitro antioxidant activity and cytotoxicity of methanolic extract of Sida cordata leaves. Int J Biol Pharm Res. 2014; 5(2):196-200.

[6] McLaughlin JL, Hostettmann K. Methods in plant biochemistry. Assays for Bioactivity. 1991; 6:1-33.

[7] Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Springer science & business media; 1998 Apr 30.

[8] Huang ZM, Yang XB, Cao WB. Textual study on Oenanthe javanica documented in ancient Chinese medicinal literatures. Chinese Traditional and Herbal Drugs. 2001; 32(1):59-62.

[9] Huopalahti R, Linko RR. Composition and content of aroma compounds in dill, Anethum graveolens L., at three different growth stages. Journal of agricultural and food chemistry. 1983 Mar; 31(2):331-3.

[10] Kasting R, Andersson J, von Sydow E. Volatile constituents in leaves of parsley. Phytochemistry. 1972 Jul 1; 11(7):2277-82.

[11] Park JC, Ha JO, Park KY. Antimutagenic effect of flavonoids isolated from Oenanthe javanica. Journal of the Korean Society of Food Science and Nutrition (Korea Republic). 1996, 25: 588–592.

[12] Ai G, Huang ZM, Liu QC, Han YQ, Chen X. The protective effect of total phenolics from Oenanthe Javanica on acute liver failure induced by D-galactosamine. Journal of Ethnopharmacology. 2016 Jun 20; 186:53-60.

[13] Song GS, Kwon YJ. Analysis of the volatile constituents of Oenanthe stolonifera DC. J Korean Soc Food Nutr. 1990;19(4):311-4.

[14] Zhang J, Li SH, Gu RH. Chemical constituents in oenanthe javanica. Chinese Traditional and Herbal Drugs. 2012;43(7):1289-92.

[15] Lee BS, Chung MH, Tu OJ. A study on removal of cadmium and lead from water by Oenanthe Stolonifera DC. Kor. J. Env. Hlth. Soc. 1995;21(1):47-55.

[16] Han YQ, Huang ZM, Yang XB, Liu HZ, Wu GX. In vivo and in vitro anti-hepatitis B virus activity of total phenolics from Oenanthe javanica. Journal of Ethnopharmacology. 2008 Jun 19; 118(1):148-53.

[17] Yang XB, Huang ZM, Cao WB, Zheng M, Chen HY, Zhang J. Antidiabetic effect of Oenanthe javanica flavone. Acta Pharmacologica Sinica. 2000 Mar 1; 21(3):239-42.

[18] Daferera DJ, Ziogas BN, Polissiou MG. GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on Penicillium digitatum. Journal of agricultural and food chemistry. 2000 Jun 19; 48(6):2576-81.

[19] Colegate SM, Molyneux RJ, editors. Bioactive natural products: detection, isolation, and structural determination. CRC press; 2007 Dec 14.

[20] He L, Orr GA, Horwitz SB. Novel molecules that interact with microtubules and have functional activity similar to Taxol™. Drug discovery today. 2001 Nov 15; 6(22):1153-64.

[21] Peteros NP, Uy MM. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. Journal of Medicinal Plants Research. 2010 Mar 4; 4(5):407-14.

[22] Khan RA, Khan MR, Sahreen S, Ahmed M. Assessment of flavonoids contents and in vitro antioxidant activity of Launaea procumbens. Chemistry Central Journal. 2012 Dec; 6(1):1-1.

[23] Khan RA, Khan MR, Sahreen S, Ahmed M. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of Sonchus asper (L.) Hill. Chemistry Central Journal. 2012 Dec; 6(1):1-7.

[24] Khan AM, Qureshi RA, Gillani SA, Ullah F. Antimicrobial activity of selected medicinal plants of Margalla hills, Islamabad, Pakistan. Journal of Medicinal Plant Research. 2011 Sep 16; 5(18):4665-70.

[25] Abdellatif F, Hassani A. Chemical composition of the essential oils from leaves of Melissa officinalis extracted by hydrodistillation, steam distillation, organic solvent and microwave hydrodistillation. J. Mater. Environ. Sci. 2015; 6(1):207-13.

[26] Ali S, Salman SM, Jan MT, Afridi M, Malik MS. Comparative studies of various phyto nutrients in citrus fruits. Pak. J. Chem. 2014; 4(2):72-6.

[27] Sofowora A. Research on medicinal plants and traditional medicine in Africa. The Journal of Alternative and Complementary Medicine. 1996 Sep 1; 2(3):365-72.

[28] Biu AA, Yusufu SD, Rabo JS. Phytochemical screening of Azadirachta indica (neem) (Meliaceae) in Maiduguri, Nigeria. Bioscience research communications. 2009; 21(6):281-3.

[29] Awang DV. Tyler's herbs of choice: the therapeutic use of phytomedicinals. CRC Press; 2009 May 4.

[30] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta medica. 1982 May; 45(05):31-4.

[31] Dhingra OD, Sinclair JB. Basic plant pathology methods. CRC press; 2017 Nov 22.

[32] Sirajuddin M, Ali S, Haider A, Shah NA, Shah A, Khan MR. Synthesis, characterization, biological screenings and interaction with calf thymus DNA as well as electrochemical studies of adducts formed by azomethine [2-((3, 5-dimethylphenylimino) methyl) phenol] and organotin (IV) chlorides. Polyhedron. 2012 Jun 19; 40(1):19-31.

[33] Magaldi S, Mata-Essayag S, De Capriles CH, Pérez C, Colella MT, Olaizola C, Ontiveros Y. Well diffusion for antifungal susceptibility testing. International journal of infectious diseases. 2004 Jan 1; 8(1):39-45.

[34] Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology. 1995 Jan 1; 28(1):25-30.

[35] Bhatia H, Sharma YP, Manhas RK, Kumar K. Ethnomedicinal plants used by the villagers of district Udhampur, J&K, India. Journal of ethnopharmacology. 2014 Feb 3; 151(2):1005-18.

[36] Petrovska BB. Historical review of medicinal plants’ usage. Pharmacognosy reviews. 2012 Jan; 6(11):1.

[37] Jones WP, Kinghorn AD. Extraction of plant secondary metabolites. Natural products isolation. 2005:323-51.

[38] Starmans, D.A. and Nijhuis, H.H., 1996. Extraction of secondary metabolites from plant material: a review. Trends in Food Science & Technology, 7(6), pp.191-197.

[39] Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against Staphylococcus aureus. Journal of antimicrobial chemotherapy. 2001 Oct 1; 48(4):487-91.

[40] Lemos TL, Matos FD, Alencar JW, Craveiro AA, Clark AM, McChesney JD. Antimicrobial activity of essential oils of Brazilian plants. Phytotherapy Research. 1990 Apr; 4(2):82-4.

[41] Vardar-unlu G, Cadan F. Deferera, Polissiou, M. Sokmen, M. Donmez, E and tap bektas. J. Agric. Food. Chem. 2003:51-63.

[42] Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. Critical reviews in food science and nutrition. 1998 Aug 1; 38(6):421-64.

[43] Talmale SA, Bhujade AM, Patil MB. Phytochemical analysis of stem bark and root bark of Zizyphus mauritiana. Int. J. Innov. Sci. Eng. Technol. 2014; 1:526-35.

[44] Wu JH, Tung YT, Chien SC, Wang SY, Kuo YH, Shyur LF, Chang ST. Effect of phytocompounds from the heartwood of Acacia confusa on inflammatory mediator production. Journal of agricultural and food chemistry. 2008 Mar 12; 56(5):1567-73.

[45] Maelicke A, Samochocki M, Jostock R, Fehrenbacher A, Ludwig J, Albuquerque EX, Zerlin M. Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer’s disease. Biological psychiatry. 2001 Feb 1; 49(3):279-88.

[46] Iturbe-Ormaetxe I, Haralampidis K, Papadopoulou K, Osbourn AE. Molecular cloning and characterization of triterpene synthases from Medicago truncatula and Lotus japonicus. Plant molecular biology. 2003 Mar; 51:731-43.

[47] Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzysta M. Antimicrobial activity of saponins from Medicago sp.: structure‐activity relationship. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2006 Jun; 20(6):454-7.

[48] El-Abyad MS, Morsi NM, Zaki DA, Shaaban MT. Preliminary screening of some Egyptian weeds for antimicrobial activity. Microbios. 1990 Jan 1; 62(250):47-57.

[49] Dall'Agnol R, Ferraz A, Bernardi AP, Albring D, Nör C, Sarmento L, Lamb L, Hass M, Von Poser G, Schapoval EE. Antimicrobial activity of some Hypericum species. Phytomedicine. 2003 Jan 1; 10(6-7):511-6.

[50] Kapu SD, Ngwai YB, Kayode O, Akah PA, Wambebe C, Gamaniel K. Anti-inflammatory, analgesic and anti-lymphocytic activities of the aqueous extract of Crinum giganteum. Journal of Ethnopharmacology. 2001 Nov 1; 78(1):7-13.

[51] Schulz V, Hansel R, Tyler VE. Fitoterapia racional: um guia de fitoterapia para as ciências da saúde. Manole; 2002.

[52] Fyhrquist P, Mwasumbi L, Hæggström CA, Vuorela HI, Hiltunen R, Vuorela P. Ethnobotanical and antimicrobial investigation on some species of Terminalia and Combretum (Combretaceae) growing in Tanzania. Journal of Ethnopharmacology. 2002 Feb 1; 79(2):169-77.

[53] Al-Fatimi M, Wurster M, Schröder G, Lindequist U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. Journal of Ethnopharmacology. 2007 May 22; 111(3):657-66.

[54] Sam, T. W. "Toxicity testing using the brine shrimp: Artemia salina In: Colgate SM; Molyneux RJ (eds) Bioactive Natural Products: Detection, Isolatiion and Structural Determination CRC Press, USA." (1993): 442-456.

[55] McLaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. Drug information journal. 1998 Apr; 32(2):513-24.

[56] Khan MA, Rahman AA, Islam S, Khandokhar P, Parvin S, Islam MB, Hossain M, Rashid M, Sadik G, Nasrin S, Mollah MN. A comparative study on the antioxidant activity of methanolic extracts from different parts of Morus Alba L. (Moraceae). BMC Research Notes. 2013 Dec; 6:1-9.

[57] Villaño D, Fernández-Pachón MS, Moyá ML, Troncoso AM, García-Parrilla MC. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta. 2007 Jan 15; 71(1):230-5.

[58] Ademiluyi AO, Oboh G. Antioxidant properties of condiment produced from fermented bambara groundnut (Vigna subterranea L. Verdc). Journal of Food Biochemistry. 2011 Aug; 35(4):1145-60.

[59] Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. High molecular weight plant polyphenolics (tannins) as biological antioxidants. Journal of agricultural and food chemistry. 1998 May 18; 46(5):1887-92.

[60] Philips A, Philip S, Arul V, Padmakeerthiga B, Renju V, Santha S, Sethupathy S. Free radical scavenging activity of leaf extracts of Indigofera aspalathoides-An in vitro analysis. J Pharm Sci Res. 2010 Jun 1; 2(6):322-8.