



Received on 27 October 2018; received in revised form, 30 January 2019; accepted, 31 January 2019; published 01 July 2019

## EVALUATION OF *IN-VITRO* ANTIOXIDANT AND ANTICANCER ACTIVITY OF *MONOCHORIA VAGINALIS* LEAVES ON Hep2 AND HeLa CELL LINES

M. Rama Prabha\* and G. K. Nivethitha

Post Graduate and Research Department of Botany, Thiagarajar College (Autonomous), Affiliated to Madurai Kamaraj University, Madurai - 625009, Tamil Nadu, India.

### Keywords:

*Monochoria vaginalis*,  
Anti-oxidant, Anticancer, Hep2,  
HeLa, Methanol extract, DPPH

### Correspondence to Author:

**M. Rama Prabha**

Post Graduate and Research  
Department of Botany, Thiagarajar  
College (Autonomous), Affiliated to  
Madurai Kamaraj University, Madurai  
- 625009, Tamil Nadu, India.

**E-mail:** me.ramaprabha@gmail.com

**ABSTRACT:** The entire plant (*Monochoria vaginalis* (Burm. f.) C. Presl.) except root is being used as a vegetable and has been used in traditional Indian system of medication for treating various diseases. But there are not many scientific data on the antioxidant and anticancer activity of *M. vaginalis*. Therefore, we aimed to determine the *in-vitro* antioxidant and anticancer activity of methanolic extract of *M. vaginalis* leaves. Standard biochemical methods evaluated the phytochemical investigation. *In-vitro* antioxidant activities of the methanolic extract of leaves were determined against DPPH, hydroxyl radical, reducing power assay, metal chelating activity, and hydrogen peroxide radicals. Also, we evaluated the anticancer activity of methanolic extract on Hep2 and HeLa lines by MTT calorimetric assay. Phytochemical analysis established the presence of rich secondary metabolites of total phenol (52.66 GAE mg/g), and flavonoids (305.88 QE mg/g). The methanolic extract of *M. vaginalis* exhibited strong antioxidant property (with IC<sub>50</sub> value) against DPPH (52.24 mg/ml), hydroxyl radical (76.08 mg/ml), reducing power (58.15 mg/ml), metal chelating activity (35.70 mg/ml), and hydrogen peroxide scavenging activity (59.87 mg/ml). Also, the methanolic extract of *M. vaginalis* was found to be a potent anticancer activity (with IC<sub>50</sub> value) on Hep2 (31.20 ± 0.02 µg/ml) and HeLa (44.58 ± 0.42 µg/ml) cell lines. In conclusion, the methanolic extract of *M. vaginalis* leaves have strong antioxidant potential and cytotoxicity activity. So, *M. vaginalis* can be used as a good source of natural antioxidants for health benefits and can be an anticancer agent. However, further study is needed to isolation and characterization of active molecules, which may provide potential benefits source of natural antioxidants and anticancer properties.

**INTRODUCTION:** Oxidative stress defined as the imbalance between the oxidants and antioxidants, and it leads to some chronic illness including diabetes mellitus, cancer, cardiovascular disease, arthritis and aging<sup>1</sup>. Reactive oxygen species (ROS) is the collective term given to a group of free radicals such as superoxide (O<sub>2</sub><sup>•-</sup>), hydroxyl (OH<sup>•</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and nitrogen oxide (NO) radicals.

Antioxidants are molecules, can prevent the chain reaction of oxidation by providing an electron to oxidants<sup>2</sup>.

Many researchers have reported and demonstrated that antioxidant neutralizes the free radical formation and regulate the intracellular balance of the body<sup>3</sup>. However, the emerging attention in healthcare is replacing synthetic molecules and discovering the new natural antioxidants, and these secondary metabolites of plants containing bioactive molecules including alkaloids, flavonoid, and polyphenols may supply to the antioxidant activity<sup>4</sup>. Natural antioxidant molecules are considered as more beneficial than synthetic antioxidant molecules to the human body due to the harms<sup>5,2</sup>.

	<b>QUICK RESPONSE CODE</b> <b>DOI:</b> 10.13040/IJPSR.0975-8232.10(7).3340-48
	The article can be accessed online on <a href="http://www.ijpsr.com">www.ijpsr.com</a>
<b>DOI link:</b> <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.10(7).3340-48">http://dx.doi.org/10.13040/IJPSR.0975-8232.10(7).3340-48</a>	

Several of different naturally present flavonoids were identified from plant sources<sup>6</sup>, but very few of important pharmacological research has established that major flavonoids<sup>7, 8</sup>. Numerous herbs are successfully used in commercial health products, and food sources like a plant, vegetable, and fruits now come to the vision. For centuries India has been using several native herbs for food and medicine rationale. The root of *M. vaginalis* is widely used in traditional system of medication, and the entire part of the plant except root has been used as a vegetable. *M. vaginalis* leaves have been used to treat a cough; root has been used for stomach, liver complaints, asthma and toothache<sup>9, 10</sup>. However, there are not many reports on the free radical scavenging and cytotoxic properties. Hence, the present experiment was to evaluate the antioxidant and anticancer activity of methanolic extract of *M. vaginalis*.

#### MATERIALS AND METHODS:

**Plant Material:** The fresh leaves of *M. vaginalis* were collected from Otthakadai, Madurai, Tamil Nadu, India. The specimen sample (TC/BOT/HERB/84) was authenticated by taxonomist, and the plant was deposited to the herbarium cabinet of Thiagarajar College, Madurai, Tamil Nadu, India.

**Preparation of *M. vaginalis* Leaves Extract:** The collected fresh leaves of *M. vaginalis* were cleaned and washed with normal water, gently dried in shade conditions to remove the water content and then pulverized in the mechanical grinder, passed through a 40 mesh sieve and stored in an airtight container. The powdered sample (10 g) was extracted with 100 ml of methanol by using Soxhlet apparatus until the discolorisation of the solvent, and filtered through Whatman no. 1 filter paper. The filtrate was concentrated under reduced pressure and controlled temperature. The collected extracts were stored in a small container at -20 °C, and used for the further experiment.

**Estimation of Total Phenolic Content and Total Flavonoids Content:** The total phenolic content of *M. vaginalis* was determined by using Folin-Ciocalteu reagent following the method of Ainsworth and Gillespie<sup>11</sup> with minor changes. Dried crude plant extracts stock solutions (1 mg/ml) were prepared and diluted with deionized water to prepare 100 µg/ml. A total of 0.5 ml (100

µg/ml) of *M. vaginalis* plant extract was mixed with 2 ml of freshly prepared Folin-Ciocalteu reagent (1:10 diluted with de-ionized water) and further neutralized with 4 ml of sodium carbonate solution (7.5% w/v). The reaction mixture was incubated at room temperature for 30 min and subjected to shaking intermittently. The absorbance was then measured at 765 nm using UV-visible spectrophotometer. The results were expressed as gallic acid equivalents (GAE) per gram of sample. Each experiment was conducted in triplicates.

The total flavonoid content was estimated by a colorimetric method as reported by Chang *et al.*,<sup>12</sup> with few changes. A total of 0.5 ml (100 µg/ml) of *M. vaginalis* plant extract was mixed with 1.5 ml of ethanol (95% v/v), 0.1 ml of aluminium chloride hexahydrate (10% w/v), 0.1 ml of potassium acetate (1 M), and 2.8 ml of deionized water. The reaction mixture was incubated for 40 min at 25 °C, and absorbance was measured at 415 nm against blank. The total flavonoid content was determined by using quercetin as standard. The results were expressed as quercetin equivalents (QE) per gram of sample. Each experiment was conducted in triplicates.

**DPPH Radical Scavenging Assay:** The hydrogen atom or electron donating abilities of the compounds were measured from the bleaching of the methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable free radical, DPPH as a reagent. 0.1 mM DPPH-methanol solution was mixed with 1ml of the sample with different concentrations (10-100 mg/ml). After the solution was incubated for 30 min at 25 °C in the dark, the decrease in the absorbance was measured at 517 nm in spectrometer<sup>13</sup>.

Methanol was used as a control. Ascorbic acid was used as a standard. The inhibition of DPPH radicals by the samples was calculated according to the following equation:

$$\text{Percentage of DPPH inhibition} = (A_C - A_S) / A_C \times 100$$

Where  $A_C$  = absorbance of control and  $A_S$  = absorbance of the sample. A percent inhibition versus concentration curve was plotted, and concentration of sample required for 50% inhibition was expressed an  $IC_{50}$  value.

**Reducing Power Assay:** 0.5 ml of sample with different concentrations (10-100 mg/ml) was mixed with 0.5 ml of a 0.2M phosphate buffer (pH 6.6) and 0.5 ml of a 1% potassium ferricyanide solution. The mixture was incubated in a water bath at 50 °C for 20 min. Subsequently, 0.5 ml of 10% (w/v) trichloroacetic acid solution was added, and the mixture was then centrifuged at 3000 rpm for 10 minutes. Finally, 0.5 ml of the supernatant layer solution was mixed with 0.5ml of distilled water and 0.1% ferric chloride, and the absorbance of the reaction mixture was measured at 700 nm<sup>14</sup>. Three replicates were made for each test sample. Increased absorbance of the reaction mixture indicated increased reducing the power of the sample. Ascorbic acid was used as the standard.

**Hydrogen Peroxide Radical Scavenging (H<sub>2</sub>O<sub>2</sub>) Assay:** A solution of H<sub>2</sub>O<sub>2</sub> (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (10-100 mg/ml) in methanol were added to a H<sub>2</sub>O<sub>2</sub> solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm<sup>15</sup>. The blank solution contained the phosphate buffer without H<sub>2</sub>O<sub>2</sub>. Ascorbic acid was used as a standard. The percentage of H<sub>2</sub>O<sub>2</sub> scavenging was calculated as:

$$\text{Percentage of scavenged (H}_2\text{O}_2) = (A_0 - A_1 / A_0) \times 100$$

Where A<sub>0</sub> is the absorbance of control, and A<sub>1</sub> is the absorbance of the test.

**Metal Chelating Activity Assay:** The chelation of ferrous ion by the extract was estimated. The chelation test initially includes the addition of 100 µl of ferrous chloride and 250 µl of ferrozine to different concentration of standard and sample extract (10-100 mg/ml). The mixture was finally quantified to 1.3 ml with methanol, shaken vigorously and left in room temperature for 10 min. The antioxidant present in the sample chelates the ferrous ions from the ferrous chloride. The remaining ferrous combine with ferrozine to form ferrous-ferrozine complex. The intensity of the ferrous-ferrozine was measured at 562 nm<sup>16</sup>. Ascorbic acid was used as a standard.

$$\text{Percentage of scavenging effect} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where A<sub>control</sub> is the absorbance of the ferrozine - Fe<sup>2+</sup> complex and A<sub>sample</sub> is the absorbance of the test compound. The percent of inhibition of absorbance at 562 nm was calculated.

**Hydroxyl Radical Scavenging Assay:** Stock solution of EDTA (1 mM), FeCl<sub>3</sub> (10 mM), ascorbic acid (1 mM), H<sub>2</sub>O<sub>2</sub> (10 mM) and deoxyribose (10 mM) was prepared in distilled water. The attempt was performed by adding up 0.1 ml EDTA, 0.01 ml of FeCl<sub>3</sub>, 0.1 ml H<sub>2</sub>O<sub>2</sub>, 0.36 ml deoxyribose, 1 ml of sample extract (10-100 mg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid added.

The mixture was incubated at 37 °C for 1 h. 1.0 ml of 10% trichloroacetic acid and 1.0 ml of 0.5% thiobarbituric acid to urbanized the pink color measured at 532 nm<sup>17</sup>. Ascorbic acid was used as a standard. The hydroxyl radical scavenging activity was determined by comparing the absorbance of the control with that of the samples.

$$\text{Percentage of scavenging effect} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where A<sub>control</sub> is the absorbance of the extract and A<sub>sample</sub> is the absorbance of the test compound. The percent of inhibition of absorbance at 532 nm was calculated.

**Cell line and Culture:** Vero (African green monkey-kidney) HeLa (human cervical carcinoma) and Hep 2 (human laryngeal carcinoma) cell lines were obtained from Veterinary College, Vepery, Chennai. The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO<sub>2</sub> at 37 °C.

**MTT Cell Viability Assay:** Cells were seeded in 24-well plates at density 1 × 10<sup>5</sup>, cells were incubated in 37 °C with 5% CO<sub>2</sub> condition. After 100% confluence of the cells, the sample was treated and incubated for 24 h. Followed by the incubation; the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. After that 100µl (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 h. After incubation period, 1ml of DMSO was added to all the wells. The absorbance at 570 nm was measured with UV-Spectrophotometer using DMSO as the blank<sup>18</sup>. Measurements were performed and the concentration required for a 50% inhibition (IC<sub>50</sub>)

was determined. The % cell viability was calculated using the following formula:

$$\% \text{ cell viability} = A_{570} \text{ of treated cells} / A_{570} \text{ of control cells} \times 100$$

**Statistical Analysis:** All statistical analyses of data were done in triplicate, and made with using the Microsoft Excel. Experimental results were expressed as a mean  $\pm$  standard error of the mean. One-way analysis of variance (ANOVA) and followed by Post hoc Dennett's test was used to determine statistical significance, and the significant level was considered  $p < 0.05$ .

**RESULTS AND DISCUSSION:** Free radicals are molecule that spontaneously produced as by-products under certain environmental conditions in the biological systems during the normal metabolic process which may cause extensive damage to tissues and biomolecules by lipid peroxidation, breakdown of DNA strands, denaturation of proteins and disrupting cellular functions leading to various diseases including cancer, diabetes mellitus, chronic inflammation, and neurodegenerative disorders<sup>19</sup>. Although plenty of synthetic (molecules) drugs are available in the market to protect oxidative damage to the cells, the major drawback is their side effects, so it has restricted to use. To overcome this problem, the consumption of natural antioxidants from food supplementation and traditional medicine is a choice.

Numerous of researchers seeking medication and management of diseases free from side effects caused by synthetic compounds. Therefore, the medicinal importance of Phytomolecules including alkaloids, phenols, flavonoids, tannins, saponins, terpenoids and steroids from plants have been briefly assessing their health benefits, because of their richness of antioxidant molecules<sup>20</sup>. Plants are one of the abundant natural sources which are used to synthesis new drugs in the modern medicine directly or indirectly, and these medicinal plants have prepared by refined traditional medicine practices which have been used for thousands of year by people in the world.

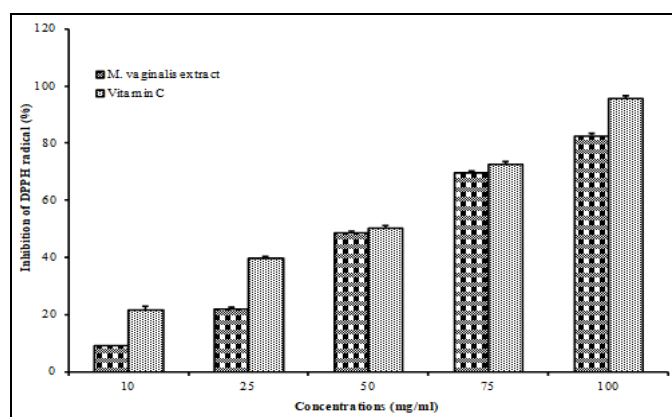
Since last few decades, researchers have more attention to medicinal plants screening for their pharmaceutical importance<sup>21</sup>. Therefore in this study, we determined the antioxidant property of

*M. vaginalis* leaves against DPPH, OH<sup>•</sup>, reducing power, metal chelating and H<sub>2</sub>O<sub>2</sub><sup>-</sup> free radicals.

**Total Phenolic and Flavonoid Contents:** The determined total phenolic content was expressed as mg of GAE/g of fresh weight. The total phenolic content in methanolic extract of *M. vaginalis* leaves was 52.66 GAE mg/g. The greater amount of phenolic content in leaves was supported its higher antioxidant potential. The total flavonoid content was determined by using quercetin as standard. The total flavonoid content of methanolic extract of *M. vaginalis* leaves was 305.88 QE mg/g. Numerous previous studies have demonstrated that the phenolic compounds are potent antioxidants and these are responsible for various biological activities including antiaging, anticancer, anti-diabetic and prevention of cardiac disorders<sup>22</sup>. Also, a strong relationship has been reported between total phenolic compounds and antioxidant activity, due to the redox property which is mainly involved in neutralizing and absorbing free radical<sup>23</sup>. Flavonoids play a significant role in plant physiology and are components of the diet of numerous herbivores and omnivores, including humans. This flavonoid compounds showed the strong biochemical, and pharmacological activities in the mammalian system, like anti-inflammatory, antioxidant, free radical-scavenging, immunomodulatory, hepatoprotective, antimicrobial activities and has been widely used in the pharmacological research and development of natural medicine<sup>24</sup>.

**DPPH Radical Scavenging Activity:** The DPPH radical scavenging activity results in various concentrations of methanol extract of *M. vaginalis* leaves and known antioxidant vitamin C is represented in **Fig. 1**. Results showed the enhanced DPPH radical scavenging property increased with increasing concentration of methanolic extract at the concentration of 10-100 mg/ml when compared with known antioxidant. Also, we determined the concentration which can scavenge 50% of the radical (IC<sub>50</sub>) by *M. vaginalis* extracts and was 52.24 mg/ml. The DPPH radical scavenging property results showed that the reaction of *M. vaginalis* leaves extracts with purple colored DPPH radical converted to pale yellow  $\alpha$ -diphenyl- $\beta$ -picryl hydrazine which increased with increasing concentrations.

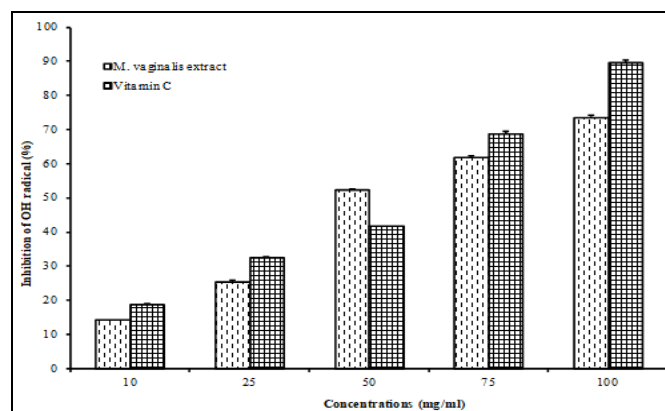
This radical scavenging activity occurs depends upon their antioxidant capacities could be due to the high phenolic and flavonoids contents present in the methanolic extract of *M. vaginalis* leaves, especially phenols that can donate hydrogen proton<sup>25</sup>. Phenols have been stated to own DPPH radical scavenging activity either one by giving a hydrogen to the nitrogen-centered free radicals of DPPH altering it to a stable diamagnetic molecule identified as diphenyl-picryl hydrazine or by single electron transfer to ABTS radical<sup>26</sup>. Our study results agreement with Sharachandra and Rajashekhar<sup>27</sup>, and Oyedemi *et al.*,<sup>28</sup> who reported that the antioxidant potential of *Cyanobacteria* and *Strychnos henningsii* to the higher concentration of phenolic compounds respectively.



**FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF *M. VAGINALIS* EXTRACT AND ASCORBIC ACID.** Data expressed as mean  $\pm$  Standard error of the mean. Values differ significantly at  $P < 0.05$ .

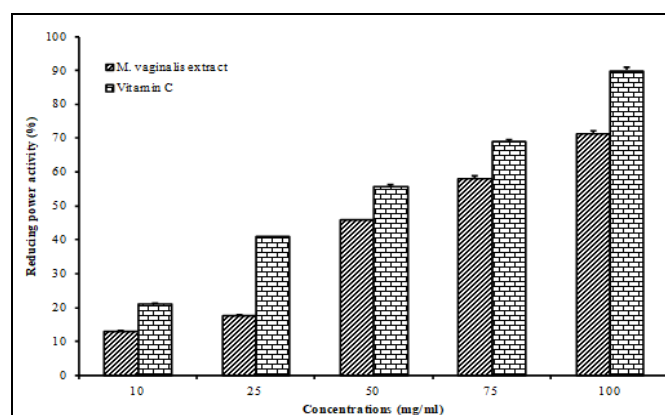
**Hydroxyl Radical Scavenging Activity:** The efficacy of the methanolic extract of *M. vaginalis* leaves to inhibit hydroxyl-radical-mediated damage was evaluated at a concentration of 10-100 mg/ml. It was observed that the *M. vaginalis* showed the minimum activity of 14.38% at 10 mg/ml and maximum activity of 73.66% at 100 mg/ml concentration, indicating that the hydroxyl radical scavenging activity of *M. vaginalis* occurred in a dose-dependent manner and  $IC_{50}$  value of extract was 76.08 mg/ml **Fig. 2**. The Fenton reaction produces hydroxyl radicals ( $OH^{\bullet}$ ) which degrade DNA deoxyribose, by using reduced transition metals and  $H_2O_2$ , which is known to be the most reactive species of all the reduced forms of dioxygen and may cause to DNA fragmentation and DNA strand breakage<sup>29</sup>. The scavenging of  $OH^{\bullet}$  radical is an important for the measurement of antioxidant activity because of very high reactivity

of the  $OH^{\bullet}$  radical, allowing it to counter with a wide range of biomolecules found in living cells, such as sugars, amino acids, lipids, and nucleotides. Thus removing  $OH^{\bullet}$  is very important for the protection of living systems. This  $OH^{\bullet}$  radical scavenging activity may be due to the presence of hydrogen donating capacity of phenolic compounds in the extract.



**FIG. 2: HYDROXYL RADICAL SCAVENGING ACTIVITY OF *M. VAGINALIS* EXTRACT AND ASCORBIC ACID.** Data expressed as mean  $\pm$  Standard error of the mean. Values differ significantly at  $P < 0.05$ .

**Reducing Power Activity:** The antioxidant efficacy of *M. vaginalis* leaves was calculated to assess their potential to reduce  $Fe^{3+}$ -TPTZ (ferric tripyridyltriazine) by electron donation, to ( $Fe^{2+}$ ) ferrous which has an intense blue color, which is associated with the presence of a redundant molecule or complex that serves as the electron donor and/or free radical scavengers<sup>30</sup>. Among the study results, *M. vaginalis* extract possessed better ferric reducing abilities ( $IC_{50}$  -58.15 mg/ml) at all the measured concentration in a dose-dependent manner than known compound **Fig. 3**.

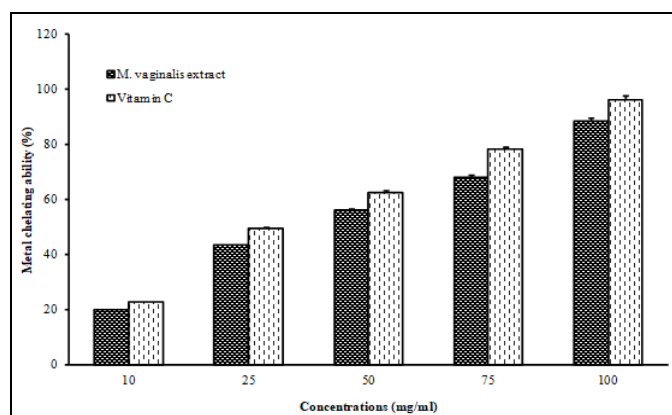


**FIG. 3: REDUCING POWER ABILITY OF *M. VAGINALIS* EXTRACT AND ASCORBIC ACID.** Data expressed as mean  $\pm$  Standard error of the mean. Values differ significantly at  $P < 0.05$ .

In this study, we noted a concentration-dependent increase in the absorbance of the reaction mixture for both plant extract and the standard drug (vitamin C), indicating that the extracts can reduce oxidative stress. Nevertheless, studies have also suggested that secondary plant metabolites such as phenol and flavanol are involved mainly in the antioxidant activities of the extracts<sup>31-33</sup>. The result of the current study supports the findings of El-Hashasa *et al.*,<sup>34</sup> who reported that the reducing power of a plant correlates with its phenolic content.

**Metal Chelating Ability:** In this assay system, the extract and known compound (ascorbic acid) interfered with the formation of ferrous and ferrozine complex, suggesting its chelating effect. The formation of the ferrozine-ferrous complex was not complete in the presence of a methanolic extract of the *M. vaginalis* leaves. The ability of chelating ferrous ions also increased with an increase in *M. vaginalis* concentrations. The absorbance of Fe<sup>2+</sup>-ferrozine complex linearly decreased dose-dependently. The values shown in **Fig. 4** demonstrated the potential of *M. vaginalis* as peroxidation protector.

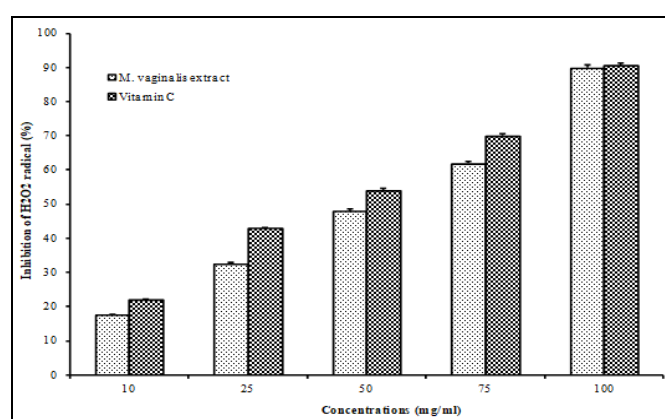
Metal chelating activity is based on the chelating effect of Fe<sup>2+</sup> ions by the reagent ferrozine, which is a quantitative formation of a complex with Fe<sup>2+</sup> ions. The formation of the complex is disrupted by another chelating agent, which results in the reduction of the formation of the red color in the complex<sup>16</sup>. Determining the rate of reduction of the color, therefore, allows estimation of the chelating activity of the co-existing chelator.



**FIG. 4: METAL CHELATING ACTIVITY OF *M. VAGINALIS* EXTRACT AND ASCORBIC ACID.** Data expressed as mean  $\pm$  Standard error of the mean. Values differ significantly at  $P < 0.05$ .

In this study, both extract (IC<sub>50</sub> - 35.70 mg/ml) and standard compound (ascorbic acid) interfered with the formation of a ferrous complex with the reagent ferrozine, suggesting that it has a chelating effect and captures the ferrous ion before ferrozine. The absorbance of Fe<sup>2+</sup>-ferrozine complex decreased linearly in a dose-dependent manner. It is reported that certain phenolic compounds have properly oriented functional groups, which can chelate metal ions<sup>35</sup>. The iron chelating activity of the extract may be attributed due to the presence of endogenous chelating agents mainly phenolics.

**Hydrogen Peroxide Radical Scavenging Activity:** Results showed that methanolic extract of *M. vaginalis* had a strong potential in eradicating hydrogen peroxide at all the tested concentrations, but significantly lower than the standard compound **Fig. 5**. The scavenging property of plant extract at the concentration of 100 mg/ml showed 89.89%, and this scavenging property is similar to that standard compound 90.56% dependently decreased in hydrogen peroxide activity. *M. vaginalis* displaying the most effective antioxidant activity, having the lowest IC<sub>50</sub> value of 59.87 mg/ml when compared with vitamin C. The results of the current study agreed with Brihi *et al.*,<sup>36</sup> who reported previously on antioxidant activity of alkaloids of *Fumaria capreolate*. H<sub>2</sub>O<sub>2</sub> is an important reactive oxygen species based on its ability to cause oxidative degradation of cell membrane lipids to give rise to the occurrence of mutagenesis and cytotoxicity. It is rapidly broken down into water and oxygen, thereby producing hydroxyl radical that can initiate lipid peroxidation and cause DNA damage in the body<sup>37</sup>.



**FIG. 5: HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF *M. VAGINALIS* EXTRACT AND ASCORBIC ACID.** Data expressed as mean  $\pm$  Standard error of the mean. Values differ significantly at  $P < 0.05$ .

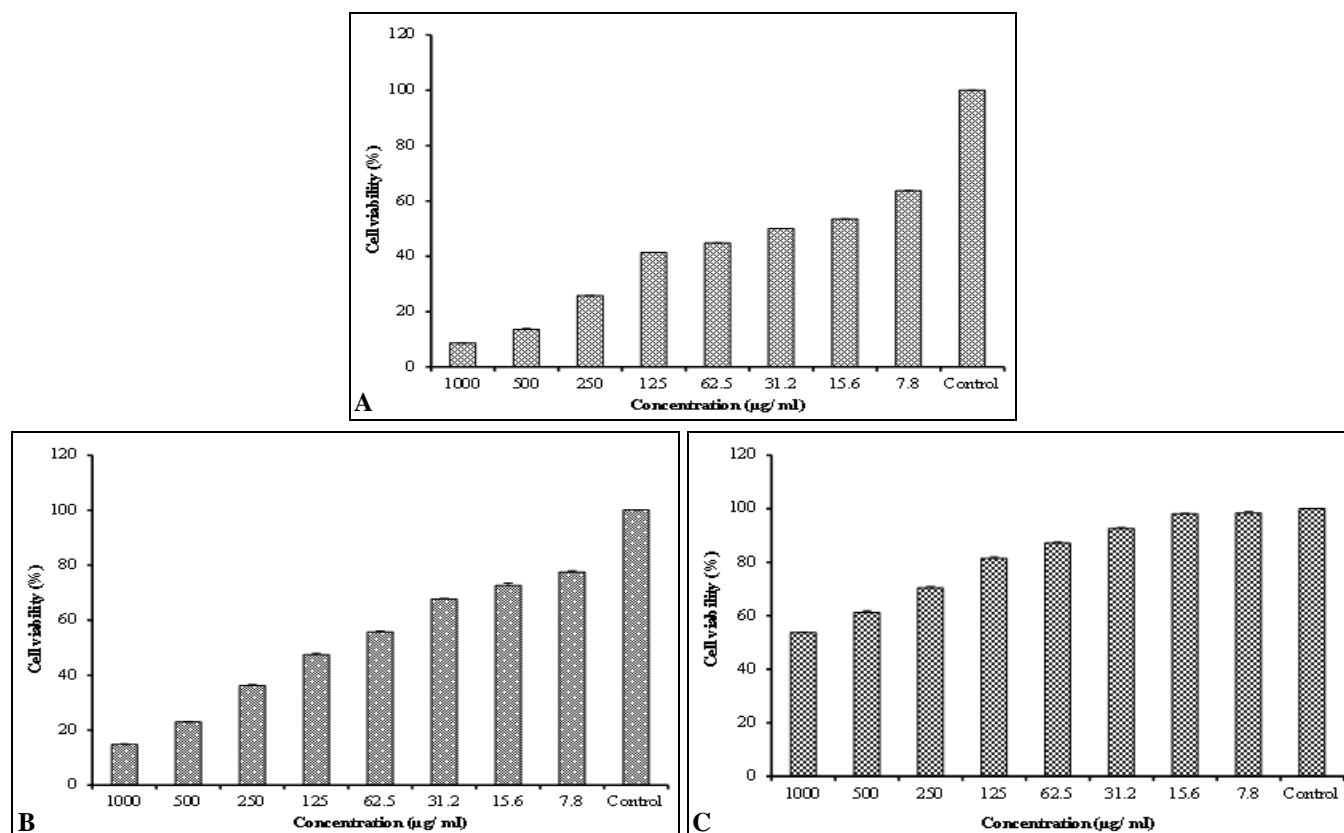
Therefore, eradication of hydrogen peroxide radical to prevent the body system from this radical is very important. The higher activity observed against hydrogen peroxide in this study may be attributed to the high polyphenolic content. Therefore, it can be deduced from this study that *M. vaginalis* extract converted the hydrogen peroxide to water and oxygen, causing a decrease in hydrogen peroxide concentration in assay mixtures as the concentration of the extract increases.

### Cytotoxicity Effect of *M. vaginalis* Leaf Methanol Extract on Hep2, HeLa and Vero Cells Lines:

The result of MTT assays confirmed that the methanolic extract of *M. vaginalis* leaves reduced cancer cells viability percentages. The methanol extract was found to induce more cytotoxicity towards cancer cell lines Hep2, HeLa increased with increasing concentration **Fig. 6A, 6B** and **6C**. The plant extract effect is similar to that of the standard drug, which is commonly used for cancer treatment. The results of the present study revealed morphological changes and cells shrinkage of the cells leading to the cell death induced by the plant extract.

The assessment of the anticancer action of the plant extracts is essential for safety. Therefore, MTT assay is generally used to the screening of the crude extracts as well as the isolated compounds for the evaluation of toxicity. It could also provide information on possible cytotoxic effects on the tested plant extracts or compounds. MTT assay depends on the reduction of MTT by mitochondrial dehydrogenase by producing a purple formazan product. Also, it is generally used as an *in vitro* experimental model to evaluate the cytotoxic effects of the variety of plant extracts or compounds against cancer cell lines.

*In-vitro* cytotoxicity test using Hep2, HeLa and Vero cancer cell lines were performed to screen potentially toxic compounds that affect basic cellular functions and morphology. The methanolic extract of *M. vaginalis* extracts showed *in-vitro* growth inhibition effects on the two cancer cell lines of Hep2, HeLa and there is no significant reduction on the cell viability of Vero cell lines. Such selective effects were concentration as well as, incubation period dependent.



**FIG. 6: CYTOTOXICITY EFFECT OF *M. VAGINALIS* LEAF METHANOL EXTRACT ON Hep2 (A), HeLa (B) AND VERO (C) CELLS LINES.** Data expressed as mean  $\pm$  Standard error of the mean. Values differ significantly at  $P < 0.05$ .

With respect to concentration (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.5 µg/ml, 15.6 µg/ml, and 7.8 µg/ml) were evaluated in triplicates. Therefore, the inhibition of cell growth by *M. vaginalis* extracts might be due to the power of the solvent in surpassing effect of several bioactive constituents, the presences of phenolic, flavonoids compounds and other antioxidant agents that are present in *M. vaginalis*.

**CONCLUSION:** To the best of our knowledge, the reactive oxygen species such as free radicals play an important role in metabolic disorders, particularly metabolic diseases including diabetes, liver diseases, hypertension, and cancer. Research on natural molecules from the medicinal plant has been increasing. The result of the current study indicates that the leaves of *M. vaginalis* possess antioxidant potential and could serve as free radical inhibitors or scavengers, or act as primary antioxidants and can be an anticancer agent. A significant relationship between the antioxidant capacity and total phenolic content was previously reported. By this kind of investigation, it would be easier to treat and prevent the human diseases occurring due to the free radical. The current study results showed that *M. vaginalis* extract exhibited potent antioxidant activity. Thus present data suggest that *M. vaginalis* can be used as a good source of natural antioxidants for health benefits and can be an anticancer agent. However, further research is needed for the isolation and identification of the active components in *M. vaginalis* extract.

**ACKNOWLEDGEMENT:** The authors are grateful to the University Grants Commission (Project title: Assessments of nutraceuticals, medicinal and bioremediation properties of an unexplored aquatic weed *Monochoria vaginalis*; Project No: MRP-5245/14 (SERO/UGC)), India for providing funding and their continuous support to carry out this research.

**CONFLICT OF INTEREST:** The authors declared that there are no conflicts of interest.

## REFERENCES:

- Halliwell B: Oxidative stress and cancer: have we moved forward? *Biochem J* 2007; 401(1): 1-11.
- Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ and Li HB: Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. *Int J Mol Sci* 2017; 18(1): E96.
- Baiano A and Del Nobile MA: Antioxidant compounds from vegetable matrices: biosynthesis, occurrence, and extraction systems. *Crit Rev Food Sci Nutr* 2016; 56(12): 2053-68.
- Li Y, Zhang JJ, Xu DP, Zhou T, Zhou Y, Li S and Li HB: Bioactivities and health benefits of wild fruits. *Int J Mol Sci* 2016; 17(8): pii1258.
- Wannes WA, Mhamdi B, Sriti J, Jemia MB, Ouchikh O, Hamdaoui G, Kchouk ME and Marzouk B: Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. *Food Chem Toxicol* 2010; 48(5): 1362-70.
- Middleton E, Kandaswami C and Harborne JB: *The Flavonoids: Advances Research Since*. Chapman and Hall, London, UK 1995; 72(3): A73.
- Kang TH, Cho H, Oh H, Sohn DH and Kim YC: Flavonol glycosides with free radical-scavenging activity of *Saururus chinensis*. *Fitoterapia* 2005; 76: 115-17.
- Sung SH, Kwon SH, Cho NJ and Kim YC: Hepatoprotective flavonol glycosides of *Saururus chinensis* herbs. *Phytother. Res* 1997; 11: 500-03.
- Longman O: *Indian Medicinal Plants: A Compendium of 500 sps*. Orient. Longman Pvt. Ltd., Chennai 2002: 52.
- Parrotta JA: *Healing Plants of Peninsular India*, CABI Publications, Wallingford, UK 2001: 597.
- Ainsworth EA and Gillespie KM: Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat Protoc* 2007; 2(4): 875-77.
- Chang CC, Yang MH, Wen HM and Chen JC: Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 2002; 10(3): 178-82.
- Duan XJ, Zhang WW, Li XM and Wang BG: Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chem* 2006; 95(1): 37-43.
- Aiyegoro OA and Okoh AI: Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium*. *BMC Complement Altern Med* 2010; 10: 21-28.
- Sarma R, Kumari S, Elancheran R, Deori M and Devi R: Polyphenol rich extract of *Garcinia pedunculata* fruit attenuates the hyperlipidemia induced by high fat diet. *Front Pharmacol* 2016; 7: 294.
- Dinis TC, Madeira VM and Almeida LM: Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Arch Biochem Biophys* 1994; 315(1): 161-69.
- Klein SM, Cohen G and Cederbaum AI: Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical generating system. *Biochemistry* 1981; 20(21): 6006-12.
- Mostafa AA, Al-Rahmah AN, Surendra Kumar R, Manilal A and Idhayadhulla A: Biological evaluation of some imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one derivatives as anticoagulant agents and inhibition of MCF-7 breast cancer cell line. *Int. J. Pharm* 2016; 12: 290-03.
- Chanda S, Dave R and Kaneria M: *In-vitro* Antioxidant Property of Some Indian Medicinal Plants. *Res J Med Plant* 2011; 5(2): 169-79.
- Swargiary A and Roy B: Phytochemical screening and antioxidant property of shoot extracts of *Alpinianigra* (Gaertn.) Burt (Family - Zingiberaceae). *Med Plants Int J*



- Phytomed Ind 2015; 7(1): 48-54.
21. Meher R, Rao DM and Reddy KA: Phytochemical screening, antioxidant activity and antimicrobial activity of *Spathodea campunata* Stem extracts. Int J Phytomed. 2015; 7(2): 209-218.
  22. Dixon RA, Xie DY and Sharma SB: Proanthocyanidins-a final frontier in flavonoid research? New phytol 2005; 165(1): 9-28.
  23. Itagaki S, Kurokawa T, Nakata C, Saito Y, Oikawa S, Kobayashi M and Iseki K: *In-vitro* and *in-vivo* antioxidant properties of ferulic acid: a comparative study with other natural oxidation inhibitors. Food Chem 2009; 114(2): 466-471.
  24. Rijke E de, Out P, Niessen WMA, Ariese F, Gooijer C and Brinkman UAT: Analytical separation and detection methods for flavonoids. J. Chromatogr. A 2006; 1112: 31-63.
  25. Lugas A, Hovari J, Sagi KV and Biro L: The role of antioxidant phytonutrients in the prevention of diseases. ActaBiol Szeged 2003; 47(1-4): 119-125.
  26. Kumarappan CT, Thilagam E and Mandal SC: Antioxidant activity of polyphenolic extracts of *Ichnocarpus frutescens*. Saudi J Bio Sci 2012; 19: 349-55.
  27. Sharathchandra K and Rajashekhar M: Antioxidant activity in the four species of *Cyanobacteria* isolated from a sulfur spring in the western ghats of Karnataka. Int J Pharm Bio Sci 2013; 4(1): 275-85
  28. Oyedemi SO and Afolayan AJ: *In-vitro* and *in-vivo* antioxidant activity of aqueous leaves extract of *Leonotis leonurus* (L.) R. Br. Int J Pharmacol 2011; 7: 248-56.
  29. Halliwell B and Gutteridge J: Formation of a thiobarbituric acid reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. FEBS Letters 1981; 128(2): 347-52.
  30. Wintola OA and Afolayan JA: Phytochemical constituents and antioxidant activities of the whole leaf extract of *Aloe ferox* Mill. Pharmacogn Mag 2011; 7(28): 325-33.
  31. Sudha A and Srinivasan P: Bioassay-guided isolation and antioxidant evaluation of flavonoid compound from aerial parts of *Lippianodiflora* L. Biomed Res Int 2014; 549836: 1-10.
  32. Venkataramanamma D, Aruna P and Singh RP: Standardization of the conditions for extraction of polyphenols from pomegranate peel. J Food Sci Technol 2016; 53(5): 2497-03.
  33. Perez-Gutierrez RM, Garcia-Campoy AH and MuñozRamirez A: Properties of flavonoids isolated from the bark of *Eysenhardtia polystachya* and their effect on oxidative stress in streptozotocin-induced diabetes mellitus in mice. Oxid Med Cell Longev 2016; 1-13.
  34. El-Hashash MM, Abdel-Gawad MM, El-Sayed MM, Sabry WA, Abdel-Hameed ES and Abdel-Lateef EE: Antioxidant properties of methanolic extracts of the leaves of seven Egyptian *Cassia* species. Acta Pharm Zagreb Croat 2010; 60(3): 361-67.
  35. Thompson M, Williams CR and Elliot GEP: Stability of flavonoid complexes of copper(II) and flavonoid antioxidant activity. AnalChimActa 1976; 85(2): 375-81.
  36. Bribi N, Bouguezza Y and Maiza BF: Evaluation of erythrocytes toxicity and antioxidant activity of alkaloids of *Fumaria capreolata*. Int J Pharm Bio Sci 2013; 4(2): 770-76.
  37. Rajat G and Panchali D: A study on antioxidant properties of different bioactive compounds. J Drug Deliv Therap 2014; 4(2): 105-15.

**How to cite this article:**

Prabha MR and Nivethitha GK: Evaluation of *in-vitro* antioxidant and anticancer activity of *Monochoria vaginalis* leaves on Hep2 and HeLa cell lines. Int J Pharm Sci & Res 2019; 10(7): 3340-48. doi: 10.13040/IJPSR.0975-8232.10(7).3340-48.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Play store)