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Evaluation of antioxidant activity of *Mimosa pudica* L. extracts

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Abstract

Antioxidant activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of leaf, stem and root of *Mimosa pudica* L. was observed through DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Five concentrations (12.5, 25.0, 50.0, 100.0 and 200.0µg/ml) were taken for each extract as well as the standard and the absorbances were measured at 517nm using a spectrophotometer against methanol blank. The activity was increased by the increment of concentrations of the extracts. In case of leaf, the highest scavenging percentage was found in chloroform extract (86.40%) at 200.0µg/ml concentration. But for stem and root, the highest scavenging percentages were found in ethyl acetate extracts (73.72% and 83.79% respectively) at same concentration. The ethyl acetate extracts showed the highest activity among all the extracts where the IC_{50} values were 65.152µg/ml, 76.036µg/ml and 65.000µg/ml and the lowest was found in petroleum ether extracts where the IC_{50} values were 130.129µg/ml, 147.891µg/ml and 186.449µg/ml for leaf, stem and root respectively and that was for ascorbic acid (standard) was 18.012µg/ml.

Keywords: Antioxidant; Phytochemicals; Reactive oxygen species; DPPH (2, 2-diphenyl-1-picrylhydrazyl); Diamagnetic molecule; Spectrophotometer

1. Introduction

Plants contain bioactive constituents that are used as traditional medicines as well as modern medicine and these natural compounds are used for phytotherapy and pharmaceutical drugs (Sahu *et al.* 2015). A large number of factors such as xenobiotics, radiation or exposure to heavy metals are responsible for inducing oxidative stress and enhancing the production of free radicals (Shinde *et al.* 2016). Antioxidants derived from natural sources have less adverse effects and very much promising for its better efficacy. The damage of cells are prevented by the antioxidants significantly by scavenging the free radicals and reactive oxygen species developed in different diseases as hepatic failure, diabetes mellitus, renal failure, atherosclerosis, inflammation, cancer, etc. (Bulkley 1983, Halliwell and Gutteridge 1993, Niki 1995 and Frei 1999). Herbal plants, vegetables and fruits possess the antioxidants such as Vitamin C, Vitamin E, flavonoids, polyphenol, phenolics, tannins and proanthocyanidins. So the consumption of antioxidant rich diet may prevent the oxidative stress induced degenerative diseases (Habib and Ibrahim 2011, Hendra *et al.* 2011 and Gulcin 2012). The present study serves as a basis for further research to isolate the bioactive compounds for discovery of new herbal drugs.

2. Material and methods

DPPH (2,2-diphenyl-1-picrylhydrazyl) was used to evaluate the free radical scavenging activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of leaf, stem and root of *Mimosa pudica* L.

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2.1. DPPH free radical scavenging principle

DPPH radical is widely used to evaluate the free radical scavenging capacity of antioxidants (Choi *et al.* 2000). This free radical is reduced to corresponding hydrazine when it reacts with hydrogen donors. It can make stable the free radicals in aqueous or methanol solution.

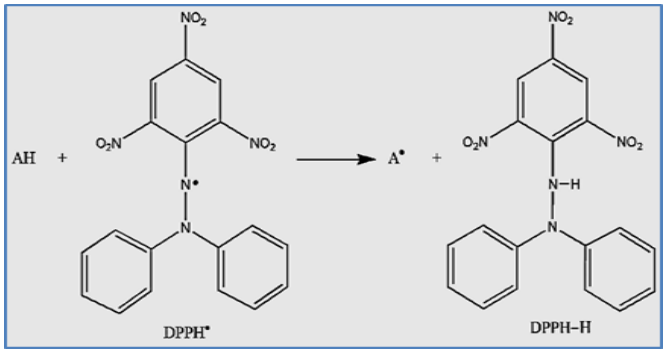

Figure 1 Principle of antioxidant molecule

Figure 2 Antioxidant activity of *M. pudica*

By following this method, it is possible to determine the antiradical power of an antioxidant by measuring the decrease in absorbance of DPPH at 517nm. Resulting from a color change from purple to yellow, the absorbance decreased when DPPH is scavenged by an antioxidant through donation of hydrogen to form a stable DPPH molecule. In the radical form, this molecule has an absorbance at 517nm which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Figure 1).

2.2. Experimental procedure

DPPH radical scavenging activity of the extracts was measured by the method developed by Manzorro *et al*. (1998). Stock solution was made by mixing 1mg extract in 1ml methanol. 3.94mg DPPH was added in 100ml methanol and vortex well. Extracts and standard of each concentration (12.5, 25.0, 50.0, 100.0 and 200.0µg/ml) were mixed with 1.5ml of DPPH solution (0.1mM). The reaction was carried out at room temperature in a dark place for 30 minutes and the absorbance was measured at 517nm. IC_{50} values (concentration of samples required to scavenge 50% of free radicals) were calculated from the regression equation, developed by plotting concentration of samples versus percentage inhibition of free radicals (Figure 3). Ascorbic acid was used as positive control.

2.3. Preparation for antioxidant activity test

At first, all equipment's were washed and sterilized carefully. Different concentrations *i.e.* 12.5, 25.0, 50.0, 100.0 and 200.0µg/ml of the extracts and a standard were taken in test tubes. 1.5ml of methanol solution of DPPH was added into each of the test tubes. The test tubes were then incubated at room temperature for 30 minutes in dark place to complete the reaction. Then the absorbances of solutions were measured at 517nm using a spectrophotometer against blank. Methanol was used as a blank. Control sample was prepared containing the same amount of methanol and DPPH without plant extracts and it was incubated under the same conditions as rest of the sample solutions (Figure 2).

2.4. Reading and analysis of data for antioxidant activity test

The percentage (%) inhibition activity of DPPH was calculated from the following equation:

$$\% I = \{(A_0 - A_1)/A_0\} X 100$$

Where, A_0 is absorbance of the control and A_1 is the absorbance of the extract/standard.

The percent inhibitions were plotted against concentration and IC_{50} was calculated from graph.

3. Results and discussion

The free radical scavenging capacity of different solvent extracts of leaf, stem and root of *M. pudica* were estimated using the stable DPPH (2-2-Diphenyl-1-picrylhydrazyl) radical with absorbance at 517nm. Ethyl acetate crude extracts showed well antioxidant activity in comparison to ascorbic acid as standard. Different solvent extracts of leaf, stem and root showed moderate to high antioxidant properties. Absorbance at 517nm was gradually decreased with higher concentrations of the samples in respect to control. It was quantitatively measured from the change in absorbance and percent of scavenging activity was calculated. The activity was increased by increment of concentration of the extracts. All the extracts of leaf, stem and root possessed significant DPPH free radical scavenging activity.

Table 1 DPPH free radical scavenging activity of different extracts of leaf of *M. pudica* and Ascorbic acid as standard at different concentrations

Concentrations (µg/ml)	Scavenging (%)				
	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Ascorbic acid
12.5	7.45	9.67	41.29	26.69	45.41
25.0	16.92	22.66	50.45	38.97	72.47
50.0	32.23	36.96	63.75	53.17	88.96
100.0	48.74	67.88	69.39	66.97	94.25
200.0	66.67	86.40	74.62	76.03	96.11

Table 2 DPPH free radical scavenging activity of different extracts of stem of *M. pudica* and Ascorbic acid as standard at different concentrations

Concentrations (µg/ml)	Scavenging (%)				
	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Ascorbic acid
12.5	3.02	16.82	36.15	10.07	45.41
25.0	9.06	27.09	47.73	16.31	72.47
50.0	21.95	37.76	56.29	33.53	88.96
100.0	43.00	63.24	67.27	50.55	94.25
200.0	62.24	72.61	73.72	62.24	96.11

Among the different extracts of leaf of *M. pudica*, the highest scavenging activity was found in chloroform extract at 200.0µg/ml concentration which was slightly lower than that of standard ascorbic acid; while petroleum ether, ethyl acetate and methanol extracts showed moderate scavenging activity (Table 1). Rajendran *et al.* (2010) denoted that the chloroform extract obtained from *M. pudica* have significant antioxidant activity and the antioxidant potential might be due to the phytoconstituents like alkaloids, glycosides, flavonoids, steroids and phenolic compounds. The reducing power of *M. pudica* leaf extracts was very potent and the activity increased with quantity of sample. The chloroform extract not only scavenged off free radicals but also inhibited the generation of free radicals. The present study showed more or less similar results. Das *et al.* (2014) depicted that the IC_{50} values of methanolic leaf extract of *M. pudica* through DPPH free radical scavenging assay was 126.71µg/ml and that of for ascorbic acid was 20.13µg/ml. They mentioned that the DPPH reading was very close to ascorbic acid as the leaf extract of the plant was in crude form. These findings were nearly close to the present result where the IC_{50} values of DPPH free radical scavenging activity of methanolic leaf

extract and ascorbic acid were 84.700µg/ml and 18.012µg/ml respectively. But Arokiyaraj *et al.* (2012) showed somewhat different result; where they mentioned that the methanolic extract of leaf of *M. pudica* had a significant free radical scavenging activity generated by DPPH where the IC_{50} was 9.0mg/ml. Almalki (2016) denoted that the hexane extract of leaves of *M. pudica* showed a significant scavenging effect on DPPH free radical (IC_{50} 20.83mM) and vitamin C (22.6mM) and the leaves of *M. pudica* were good antioxidant agent. But in the present study the petroleum ether leaf extract showed moderate DPPH scavenging effect where the IC_{50} was 130.129µg/ml.

Table 3 DPPH free radical scavenging activity of different extracts of root of *M. pudica* and Ascorbic acid as standard at different concentrations

Concentrations (µg/ml)	Scavenging (%)				
	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Ascorbic acid
12.5	10.57	9.06	27.09	35.95	45.41
25.0	15.11	17.02	48.74	43.50	72.47
50.0	24.17	26.38	65.56	50.76	88.96
100.0	34.84	38.17	74.22	64.35	94.25
200.0	49.35	57.40	83.79	78.95	96.11

Table 4 IC_{50} values of different extracts of leaf, stem and root of *M. pudica* and Ascorbic acid as standard

Extracts	IC_{50} values (µg/ml)				
	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Ascorbic acid
Leaf	130.129	94.293	65.152	84.700	18.012
Stem	147.891	105.414	76.036	135.030	
Root	186.449	159.276	65.000	79.213	

Among the different extracts of stem, the highest scavenging activity was found in ethyl acetate extract at all concentrations and all the extracts showed moderate scavenging activity in comparison to ascorbic acid (Table 2). DPPH free radical scavenging assay was carried out by Tunna *et al.* (2015) to determine the free radical scavenging potential of extracts of aerial parts of *M. pudica*. The initial ethanol extract (mother extract) and subsequent subfractions (hexane, ethyl acetate, acetone and methanol) were evaluated for their free radical scavenging activity and found that the methanol extract (initial or mother extract) possessed the lowest IC_{50} value (7.18±0.0005). The hexane fraction showed a higher IC_{50} value but a weaker antioxidant activity of 92.302±0.0077. In the present study, the petroleum ether extracts showed more or less similar results as hexane fraction where the IC_{50} values were 130.129µg/ml and 147.891µg/ml for leaf and stem respectively. The methanol crude extract of aerial part of *M. pudica* showed moderate antioxidant activity compared to ascorbic acid as standard where the IC_{50} values were 296.62µg/ml and 131.29µg/ml for methanol extract and ascorbic acid respectively against DPPH free radical scavenging assay (Chowdhury *et al.* 2008). The present study, also showed moderate antioxidant activity for methanol crude extracts of leaf and stem *i.e.* the aerial parts of the plant where the IC_{50} values were 84.700µg/ml and 135.030µg/ml for methanol extracts of leaf and stem respectively.

Among the different extracts of root, the highest scavenging activity was found in ethyl acetate extract at all concentrations which was slightly lower than that of standard ascorbic acid. On the other hand, petroleum ether and chloroform extracts showed lower scavenging activity and methanol extract showed moderate scavenging activity (Table 3).

The ethyl acetate extracts showed the highest antioxidant activity among all the extracts where the IC_{50} values were 65.152µg/ml, 76.036µg/ml and 65.000µg/ml and the petroleum ether extracts showed the lowest activity where the IC_{50} values were 130.129µg/ml, 147.891µg/ml and 186.449µg/ml for leaf, stem and root respectively. The IC_{50} value of the standard ascorbic acid was found 18.012µg/ml (Table 4).

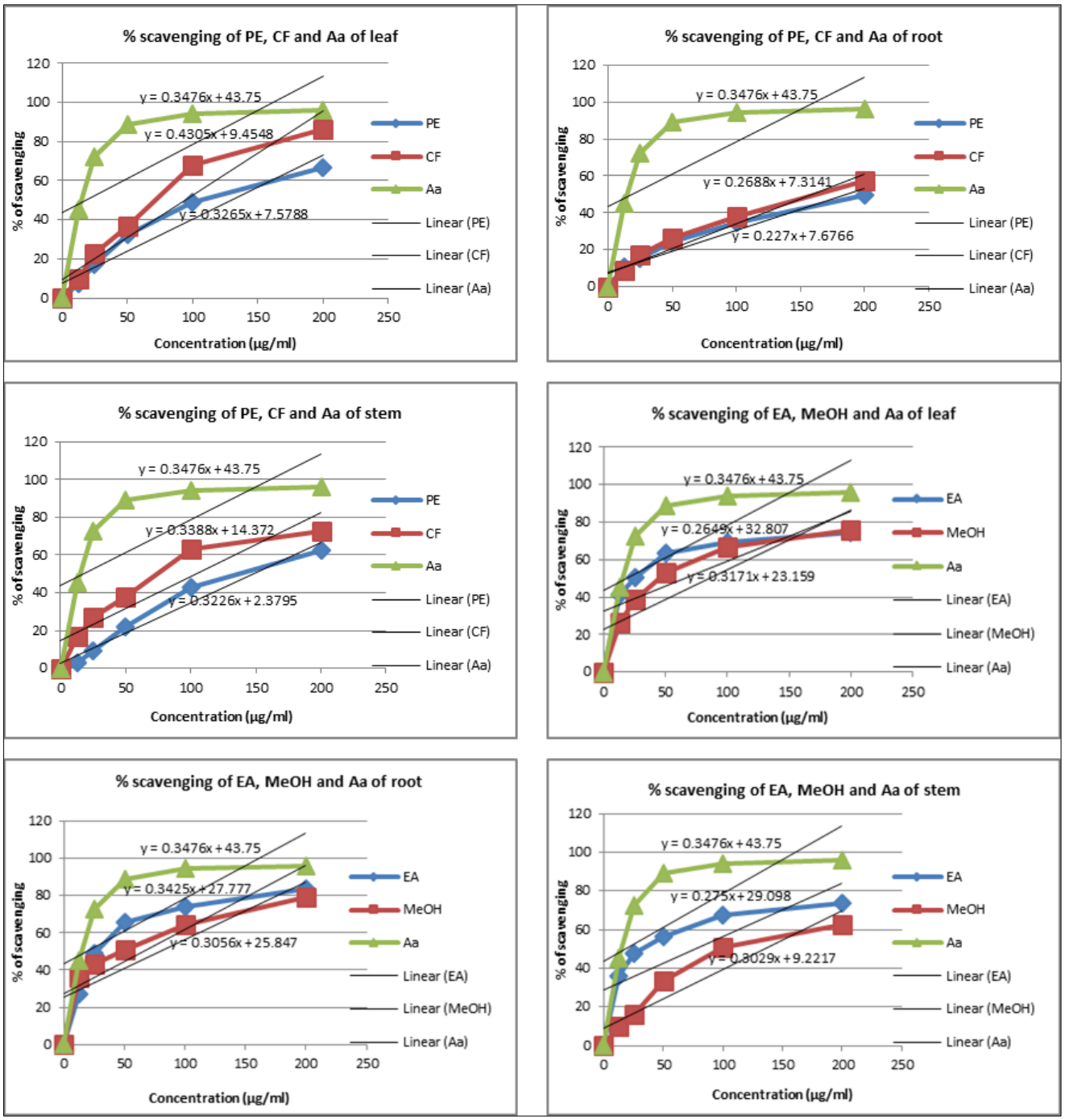

Figure 3 Regression lines between concentration and % of scavenging of different solvent extracts of *M. pudica* and the standard for DPPH free radical scavenging activity

4. Conclusion

In the present study the antioxidant activity of different solvent extracts of *M. pudica* was evaluated at different concentrations. Among the extracts, the highest activity or the lowest IC_{50} value was observed in ethyl acetate extracts and the lowest activity or the highest IC_{50} value was observed in petroleum ether extracts of leaf, stem and root. But in respect of scavenging activity, the highest percentage was found in chloroform extract of leaf (86.40%) at 200µg/ml concentration. The IC_{50} values of DPPH radical scavenging activity could be arranged as ethyl acetate > methanol > chloroform > petroleum ether for leaf and root, and as ethyl acetate > chloroform > methanol > petroleum ether for stem.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest exist.

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