Abstract

Objective: The antioxidants recovered from medicinal plants were widely used in the treatment of disease. Therefore, we aimed to investigate the antioxidant potentials of leaf extract of *Mimosa pudica* Linn. Methods: The plant extract was collected by using the hexane solvent and the crude extract was collected by vacuum distillation. *In vitro* antioxidant activities of the extract were exerted by evaluating 2,2-Diphenyl-Picrylhydrazyl (DPPH), hydroxyl, nitric oxide and superoxide radicals, in addition the total phenolic content also measured. Results: The hexane extracts from *Mimosa pudica* Linn. revealed significant scavenging effect on DPPH (IC$_{50}$ 20.83 mM), hydroxyl (IC$_{50}$ 19.37 mM), nitric oxide (IC$_{50}$ 21.62 mM) and superoxide (IC$_{50}$ 22.19 mM) radicals. Conclusion: The antioxidant activities exhibited by *M. pudica* could be used for the treatment of various disorders. Further studies are needed in this direction.

Keywords: Antioxidant Activity, Hexane Extract, Mimosa Pudica

1. Introduction

Biological systems are recurrently unprotected to oxidative stress, which is a contributing factor for numerous human health disorders\(^1\). The reactive molecules such as O$_2$•−, OH•, ROS, H$_2$O$_2$ and O$_2$ are mainly involved in the initiation of oxidative\(^2\). Among that, ROS are generated from the physiological actions of living system such peroxidation reaction of lipids, alterations in the as nucleic acids, amino acid structures and modifications in the chemical structure of the sugars. It is very important to neutralize the ROS moieties in the human system. Defective in the oxidation of the ROS in the system leads to various disorders to human\(^1\). ROS damage was protected by enzymatic and non-enzymatic antioxidant systems exists in the human body. However, the inherent defense could not be enough to control continuous oxidative stress. Therefore, the intake of metabolites with antioxidant function is very important to maintain the level of the redox level in the human body. At present varied number of chemically synthesized antioxidants are available in the market to slow down the ROS level in the living system whereas, most of them have severe demerits towards human health\(^1-8\). Since, natural antioxidant compounds are used in food and it might protect our biological systems against damages mediated by ROS and thus helping in prevention and curing of oxidative related disorders.

*Mimosa pudica* L. is a traditional medicinal plant, known as Lajjalu in Ayurveda. It is widely used in the treatment of antidepressant, aphrodisiac and antiasthmatic respectively\(^9-11\). Additionally, the metabolites such as phenolic compounds and flavanoids recovered from this plant posses anticancer, antidiabetic and antiarthritic properties\(^12\). Especially, the roots, fruits, flowers and stem parts of the plants were widely used in the treatment of multiple ailments. Hence, the numbers of reports related to the antioxidants from medicinal plants are less; it was the aim of this study to investigate the antioxidant and free radical scavenging effect of *M. pudica* hexane extract.

2. Materials and Methods

2.1 Chemicals and Reagents

DPPH (1, 1-Diphenyl, 2-Picryl Hydrazyl), TBA (Thiobarbituric Acid), NBT (Nitro Blue Tetrozolium), TCA (Trichloro Acetic Acid), EDTA (Ethylene Diamine Tetra...
Acetic Acid), Ferric Chloride, BHT (Butylated Hydroxy Toluene), curcumin, Vitamin C and other reagents and chemicals were procured from Merck chemical company.

2.2 Preparation of Extract
The collected plants were washed thoroughly with ice cold tap water twice to remove the dust particle and further washed with sterile distilled water. After that the plant materials were shaded dried before the organic solvent extraction. After that, the dried plants were powdered using blender and 50 gram of the powered samples was mixed with 300 ml of hexane in 1000 ml conical flask. The flask was tightly packed and kept in the shaking incubator for 48 h. After that the mixture was filtered using filter paper and further centrifuged at 8000 rpm for 15 min to remove the debris. The collected debris free solvent phase were vacuum evaporated and stored in the brown bottle and stored at 4°C for lab experiments.

2.3 Total Phenolic Content (TPC)
Standard Folin-Ciocalteu (FC) analytical methodology was used for the quantification of total phenolic components in the organic solvent extracts13. For the quantification, 1.0 ml of plant extract was mixed with 46 ml of sterile room temperature distilled water in a 100-ml volumetric flask. After that, 1.0 ml of freshly prepared FC reagent was transferred to the conical flask and mixed thoroughly for three minutes for proper mixing. To that, freshly prepared 2% Na₂CO₃ solutions was added and incubate at room temperature for 2 h with slight shaking. The change in the color was read at 760 nm using spectrophotometer. Gallic acid was used as a standard phenolic compound. The amount of total phenolic compound in the extract was determined as µg of Gallic Acid Equivalent (GAE) per mg dry weight.

2.4 In Vitro Antioxidant Assays
2.4.1 Reducing Ability Assay
For accessing the reducing ability of the extract, the protocol followed by Oyaizu (1986) was implemented14. Briefly, the extract was taken in different concentrations such as 5-25 mM were mixed with equal volume of sterile distilled water together with 2.5 ml of 1% K₃Fe(CN) and 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and further kept in the shaking incubator at 50°C for 20 min. After the incubation, 2.5 ml of freshly prepared ice cold 10% TCA was carefully added to the reaction mixtures and centrifuged at 5000 rpm for 5 min. Finally, the collected supernatant was suspended with 2.5 ml sterile room temperature distilled water and freshly prepared FeCl₃ (0.5 ml, 0.1%).

2.4.2 DPPH Radical Scavenging Assay
DPPH quenching ability of hexane extract of M. pudica was determined according to15. DPPH (0.15%) reagent solution was freshly prepared using methanol and stored in the ice cold condition for the routine experiments. For the experiments, different proportions of the extract was mixed with 1 ml of DPPH solution and incubated under dark condition for 10 min. After incubation, the change in the color intensity was measured at 515 nm using spectrophotometer. The antiradical activity was expressed as IC₅₀ (µg/ml). The ability to scavenge the DPPH radical was calculated using the following formula:

\[ \text{DPPH scavenging effect} \% = \frac{A_0 - A_1}{A_0} \times 100 \]  

Where A₀ is the absorbance of the control at 30 min, and A₁ is the absorbance of the sample at 30 min.

2.4.3 Hydroxyl Radical Scavenging Assay
In16 method was followed for the determination of this assay. For this assay, all the used reagents and chemicals were prepared freshly. Briefly, different proportions of the extracts were mixed with 0.1 ml of 28 mM 2-deoxy-2-ribose, 0.1 ml H₂O₂ (1 mM), 0.2 ml of 200 µM FeCl₃ and 1.04 mM EDTA (1:1 v/v). After that the reaction mixtures were incubated at 37°C for 1 h. The content of scavenged hydroxyl molecules were read at 532 nm. The antioxidant activities of the plant extracts were compared with BHT and vitamin C by using the Formula (1).

2.4.4 Nitric Oxide Radical Inhibition Assay
The scavenging of nitric oxide radical was evaluated by the methods of17. For this assay, all the reagents were prepared freshly using the sterile distilled water. The variation in the color change was determined by measuring the absorbance at 540 nm.

2.4.5 Superoxide Radical Scavenging Assay
In18 analytical method was used form the determination of the scavenging activity of the superoxide radicals. For this assay, all the reagents were prepared freshly using the sterile distilled water. The variation in the color change was determined by measuring the absorbance at 560 nm.
The antioxidant activities of the plant extracts were compared with BHT and vitamin C by using the Formula (1).

### 2.5 Statistical Analysis

The data of the study was subjected to one way ANOVA. Statistical analysis was done using SPSS ver. 11 software.

### 3. Results

The solvent hexane was used in this study. The average percentage yield of each extracting solvent was based on triplicate analysis of 500g samples. The hexane solvent yielded the highest amount of extract which means

**Figure 1.** Reductive ability of HEMP, butylated hydroxy toluene and vitamin C. Each value represents the mean ± SEM of triplicate experiments.

**Figure 2.** (a) DPPH radical scavenging effect of HEMP, butylated hydroxy toluene and vitamin C. (b) The OH scavenging effect of HEMP, butylated hydroxy toluene and vitamin C. (c) Nitric oxide radical scavenging effect of HEMP, butylated hydroxy toluene and curcumin. (d) Superoxide radical scavenging effect of HEMP, butylated hydroxy toluene and vitamin C. Each value represents the mean ± SEM of triplicate experiments.
that the leaves of M. pudica contain mostly of lipophilic compounds. The TPC in M. pudica was in the range of 7.35±0.1-48.03±2.24 GAE mg/g. The highest was observed in hexane fraction with a value of 48.03±2.24 GAE mg/g. The antioxidant and free radical scavenging activity of M. pudica hexane extract was determined. The reducing power of M. pudica hexane extract was showed in Figure 1 and 2. In nitric oxide radical inhibition assay at concentration of 21.62 mM of M. pudica hexane extract 50% of nitric oxide generated by incubation was scavenged. The results were depicted in Figure 2(c). The IC_{50} values for BHT and curcumin were 18.6 mM and 90.8-16.33-35 was in the range. The IC_{50} of other functional group containing chemical structures of 19–32 anthocyanins, glucosinolates and 36-38 phenolic compounds were responsible for the antioxidant properties. Other toxic molecules with the characteristics' of hydrogen peroxide and muscle cells which are mainly involved in the synthesis of enzymes for the slow-down of the oxidative stress and protect the life of human.

4. Discussion

Metabolites observed from the medicinal plants play an important role as antioxidants. In vitro antioxidant effects of several plant extracts, Marrubium peregrinum L., Turnera ulmifolia Linn., Stevia rebaudiana Bert reported in the recent studies. However, many studies concluded that the potential applications of plant metabolites belonged to alkaloids, falvonoids, saponins, glucosinolates, anthocyanins, phenolic compounds and other functional group containing chemical structures were responsible for the antioxidant properties and their activities were mainly varied with respect to the functional constituents. The leaves of M. pudica were reported to be good antioxidant agent. In this study we fractionated M. pudica hexane extract from M. pudica leaves investigated the anti radical activity. It is predicted that the hexane extract contains the phytochemicals which could be exhibiting the scavenging activity for maintain the redox level. Literature claimed that the secondary metabolites present in plant extracts interact with the oxygen molecules and minimize the synthesis of nitric oxide. Other toxic molecules with the characteristics' of hydrogen peroxide affect the human cells such as hepatic and muscle cells which are mainly involved in the synthesis of enzymes for the slow-down of the oxidative stress and protect the life of human.

5. Conclusion

Hexane extract of M. pudica revealed antioxidant and free radical scavenging. Therefore, future studies have to carry to identify the novel molecules which are responsible for the scavenging activities.

6. Conflict of Interest

The authors declare that there are no conflicts of interest.

7. References

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