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Research Article

EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF *MIMOSA PUDICA* THORNS

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ABSTRACT

Objective: *Mimosa pudica* is a traditional medicinal plant. The natural antioxidant compounds from plants mop up the free radicals causing cell damage and maintain the biological systems. The aim of the present study is to evaluate the free radical scavenging potential of the ethanolic and aqueous thorn extracts of *M. pudica*.

Methods: The ethanolic and aqueous thorn extracts of *M. pudica* were analyzed for the phytochemicals and for free radical scavenging activity by 1, 1 diphenyl 1-2-picric hydrazine (DPPH), 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and nitric oxide free radical scavenging method.

Results: Flavonoids, saponins, glycosides, alkaloids, terpenoids, and coumarins were the phytoconstituents revealed in ethanolic and aqueous thorn extracts of *M. pudica*. At 250 µg/mL concentrations, aqueous thorn extracts of *M. pudica* exhibited 73.41% radical scavenging activity by DPPH method and 26.10% inhibition by nitric oxide free radical scavenging method. However, the ethanolic extracts of *M. pudica* thorns exhibited 73.35% inhibition by ABTS free radical scavenging method at 250 µg/mL concentrations.

Conclusion: The results obtained suggest that the plant extracts from *M. pudica* could serve as a potential source of antioxidant in slowing down the process of aging and age-related or oxidative stress-related degenerative diseases. Moreover, the isolation of bioactive principle responsible for the antioxidant activity and formulation of novel therapeutic agents can be further studied.

Keywords: *Mimosa pudica*, Free radical scavenging, Phytoconstituents, Bioactive principle.

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INTRODUCTION

Plants are the largest reservoir of the bioactive principles which have a long history of use in modern medicine and in certain systems of traditional medicine. Plant-derived compounds form the basis for pharmaceutical drugs and phytotherapy [1]. Antioxidants react through free radical or molecular oxygen quenching, being capable of either delaying or inhibiting the oxidation processes which occur under the influence of molecular oxygen or reactive oxygen species [2]. Many factors are responsible for inducing oxidative stress and enhancing the production of free radicals such as radiation or exposure to heavy metals and xenobiotics [3]. Antioxidants are responsible for the defense mechanism of the organism against the pathologies associated to the attack of free radicals, and thus, the intake of plant-derived antioxidants is involved in the prevention of degenerative diseases caused by oxidative stress, such as cancer, Parkinson, Alzheimer, or atherosclerosis [2]. The antioxidant activities of medicinal plants may be explained by the presence of phenolic compounds, containing the hydroxyl group that confers the hydrogen-donating ability [4].

Mimosa pudica L. has been used widely in traditional medicine [5]. Other names of *M. pudica* are touch me not or sensitive plant, and it is usually a short prickly plant with its branches growing close to the ground [6]. *M. pudica* belongs to the family Fabaceae and the Mimosoideae subfamily. It invites the attention of researchers worldwide for its pharmacological activities such as antidiabetic, antitoxin, antihepatotoxic, and wound healing activities. It is reported to contain alkaloid, glycoside, flavonoid, and tannin [7]. *M. pudica* contains an alkaloid called mimosine, which has been found to have potent antiproliferative and apoptotic effects [8]. It is used in the treatment of biliousness, leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, fatigue, asthma, leucoderma, blood diseases, etc. [9]. And also, *M. pudica* has antidepressant, anticonvulsant, and antivenom activities and diuretic effect [10].

In the present study, the phytochemicals were screened, and the free radical scavenging activity of the ethanolic and aqueous thorn extracts of *M. pudica* was analyzed.

METHODS

Collection and identification of plant material

The *M. pudica* plant was collected from Thirukalikundram, Kanchipuram District. The plant was authenticated by Dr. Sasikala Ethirajulu and Dr. Jega Jothi Pandian, Siddha Central Research Institute, Arignar Anna Government Hospital campus, Arumbakkam, Chennai. *M. pudica* thorns were thoroughly washed with fresh water. Then, the thorns were shade dried at room temperature, were powdered using pulverizer, and were used for extraction.

Preparation of ethanolic and aqueous thorn extracts

Ethanolic thorn extract

Successive extractions were made using 20 g of the powdered thorns of *M. pudica* with 250 mL of ethanol using Soxhlet extractor for 15 refluxes. The extract was condensed using rotary evaporator. Then, the thorn extract was labeled and stored at 5°C for further use.

Aqueous thorn extract

20 g of the powdered thorns of *M. pudica* were soaked in 250 mL double-distilled water and kept in an orbital shaker for 24 h in a closed Erlenmeyer flask for continuous agitation. Filtration of extract was needed to be done using Whatman No.1 filter paper. Using rotary vacuum evaporator, the solvent from the extract was removed. The extract obtained was labeled and then stored at 5°C for further use.

Phytochemical screening

Qualitative phytochemical screening of the thorn extracts of *M. pudica* was done using the standard methods [11,12]. Flavonoids, steroids,

tannins, saponins, glycosides, alkaloids, terpenoids, anthraquinones, and coumarins were the phytochemicals screened.

Antioxidant activity using 1, 1 diphenyl 1-2-picric hydrazine (DPPH) free radical scavenging activity

In vitro antioxidant activity was assessed by DPPH radical scavenging activity [13]. In the control tube (C), 0.1 mL of methanol was taken and 0.1 mL of varying concentrations (50–250 µg/mL) of ethanolic and aqueous thorn extracts of *M. pudica* was added in the tube (T), respectively. 2.0 mL of 0.1 mM of methanolic DPPH was added to all the tubes which include control, test, and standard. The standard used throughout the experiment was ascorbic acid. The tubes were incubated in the dark for 20 min and then were read at 517 nm by spectrophotometer. The percentage of inhibition was obtained by making use of the following formula and expressed as percentage scavenging of DPPH radical [14]. All the tests were carried out in triplicates.

$$\%\text{DPPH inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Antioxidant activity using 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS) free radical scavenging activity

ABTS*+ decolorization assay was used to determine the free radical scavenging activity [15]. ABTS radical cation was obtained by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (final concentration) and was stored for 12–16 h in the dark at room temperature before use. ABTS*+ solution was then diluted with absolute ethanol to obtain an absorbance of 0.70 (±0.02) at 734 nm. Reagent blank reading was taken (A_0). 2.0 mL of diluted ABTS*+ solution (A734 nm = 0.70 (±0.02)) was added to 20 µL of varying concentrations (50–250 µg/mL) of the ethanolic and aqueous thorn extracts of *M. pudica*, and the absorbance was measured exactly 6 min after initial mixing (A_t). Appropriate solvent blanks were run in each assay. Ascorbic acid was regarded as standard. The tests were replicated 3 times. Absorbance was measured at 734 nm. Using the formula, the scavenging activity was calculated as percentage inhibition.

$$\%\ \text{Inhibition} = [(A_{C(0)} - A_{A(t)})/A_{C(0)}] \times 100$$

Where $A_{C(0)}$ is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the sample (thorn extracts) at t = 6 min.

Nitric oxide free radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH was measured by Griess–Ilosvay reaction [16,17]. The reaction mixture of 3 mL containing 2 mL sodium nitroprusside (10 mm) in 0.5 mL phosphate buffer saline (0.025 M, and pH 7.4) and 0.5 mL of the ethanolic and aqueous thorn extracts of *M. pudica* of various concentrations (50–250 µg/mL) was incubated at 25°C for 150 min. A control experiment without the sample but with an equivalent amount of buffer was made in an identical manner. 1.5 mL of the reaction mixture was removed after incubation, and 1.5 mL of the Griess reagent (1% sulfanilamide, 2% orthophosphoric acid, and 0.1% napthylethylenediamine dihydrochloride) was added. Using ascorbic acid as standard, the same procedure was conducted. The absorbance of the chromophore formed was read at 546 nm. Percentage inhibition of nitric oxide scavenging activity was estimated using the formula. All the tests were performed out in triplicates.

$$\%\ \text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The samples were taken in triplicates and analyzed, and the results were reported in mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemicals such as flavonoids, saponins, glycosides, alkaloids, terpenoids, and coumarins were present in the ethanolic and aqueous thorn extracts of *M. pudica* [Table 1]. In the ethanolic thorn extracts of *M. pudica*, steroids were present. Tannin was present in the aqueous thorn extracts of *M. pudica.* It was reported in a study that the preliminary phytochemical screening of the *M. pudica* leaf extract showed the presence of bioactive components such as terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins, and coumarins [18]. Studies suggest that the preliminary phytochemical analysis of the chloroform extract of *M. pudica* Linn. leaves revealed the presence of phytoconstituents such as steroids, flavonoids, glycosides, alkaloids, and phenolic compounds [19]. It is also supported in a study that preliminary phytochemical screening of the ethanolic extracts of *M. pudica* Linn. revealed the presence of alkaloids, flavonoids, saponins,

Table 1: Phytochemical analysis of thorn extracts of *M. pudica*

Phytochemicals	Ethanolic thorn extracts	Aqueous thorn extracts
Flavonoids	+	+
Steroids	+	-
Tannins	-	+
Saponins	+	+
Glycosides	+	+
Alkaloids	+	+
Terpenoids	+	+
Anthraquinones	-	-
Coumarins	+	+

+: Present, -: Absent, *M. pudica: Mimosa pudica*

Table 2: Percentage inhibition by DPPH free radical scavenging method

Concentration (µg/mL)	Ethanolic thorn extracts of *M. pudica*	Aqueous thorn extracts of *M. pudica*
50	21.24±0.36	53.64±0.41
100	22.8±0.28	55.41±0.41
150	36.3±0.38	60.68±0.23
200	46.39±0.42	62.35±0.40
250	60.59±0.41	73.41±0.26

Data are mean±SD values; n=3, SD: Standard deviation, *M. pudica*: *Mimosa pudica,* DPPH: 1, 1 diphenyl 1-2-picric hydrazine

Table 3: Percentage inhibition by ABTS free radical scavenging method

Concentration (µg/mL)	Ethanolic thorn extracts of *M. pudica*	Aqueous thorn extracts of *M. pudica*
50	50.23±0.61	9.28±0.27
100	56.18±0.29	14.61±0.35
150	59.52±0.52	16.38±0.47
200	65.24±0.32	20.86±0.20
250	73.35±0.54	25.69±0.33

Data are mean±SD values; n=3, SD: Standard deviation, *M. pudica*: *Mimosa pudica*, ABTS: 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid

Table 4: Percentage inhibition by nitric oxide free radical scavenging method

Concentration (µg/mL)	Ethanolic thorn extracts of *M. pudica*	Aqueous thorn extracts of *M. pudica*
50	3.74±0.26	13.51±0.44
100	6.47±0.43	16.60±0.33
150	10.28±0.32	20.8±0.52
200	15.27±0.44	22.08±0.27
250	17.94±0.26	26.10±0.22

Data are mean±SD values; n=3, SD: Standard deviation, *M. pudica*: *Mimosa pudica*

terpenoids, tannins, and phenolics [20] The experimental results of previous studies suggest that the biologically active phytoconstituents such as flavonoids, glycosides and alkaloids present in the methanolic extract of plant *M. pudica* may be responsible for the significant hepatoprotective activity [21] and also supported by a study that the phytochemical analysis of the crude ethanolic extracts of leaves and roots of *M. pudica* indicated the presence of tannins, proteins, and steroids [22]. The results of the previous study suggested that phenolic and flavonoids may be the major contributors for the antioxidant activity [23].

Antioxidant activity by DPPH free radical scavenging method

To determine the antioxidant activity of drugs obtained from plants, DPPH method is used commonly. The aqueous thorn extracts exhibited higher values than the ethanolic thorn extracts of *M. pudica* [Table 2]. 73.41% radical scavenging activity was obtained at 250 µg/mL concentration of aqueous thorn extracts. The IC_{50} value of ethanolic and aqueous thorn extracts of *M. pudica* was found to be 211.25±0.51 µg/mL and 30.56±0.41 µg/mL, respectively. IC_{50} value of aqueous thorn extracts is lesser than the ethanolic thorn extracts of *M. pudica,* proving that aqueous thorn extracts have higher antioxidant activity. The hydroalcoholic extract of *M. pudica* Linn. (Mimosaceae) and L-Mimosine proved to have a significant antioxidant and anticancer activity [24]. It was reported in a study that the *in vitro* antioxidant activity indicates that petroleum ether leaf extract of *M. pudica* showed maximum antioxidant activity [25]. Another study suggests that the methanolic crude extracts of *M. pudica* leaves showed moderate antioxidant activity [26]. The ethanolic extract of *M. pudica* roots showed a very good antioxidant activity [27]. It was also found in previous studies that the whole plant, stems, leaves, and seeds of *M. pudica* Linn. showed strong antioxidant capacity, and moreover, the antioxidant activity of *M. pudica* Linn. *in vitro* could be related to the high concentration of flavonoids and phenolics [28]. A study reported that the methanol crude extract of the aerial parts of *M. pudica* showed moderate antioxidant activity using the DPPH free radical scavenging assay [29].

Antioxidant activity by ABTS free radical scavenging method

Among the ethanolic and aqueous thorn extracts, the ethanolic thorn extracts of *M. pudica* showed higher values [Table 3]. At 250 µg/mL concentrations of ethanolic thorn extracts of *M. pudica*, the radical scavenging activity was found to be 73.35%. Ethanolic thorn extracts of *M. pudica* exhibited IC_{50} value of 51.28±4.31 µg/mL, and the aqueous thorn extracts exhibited IC_{50} value of 567.84±11.71 µg/mL. The results of previous studies revealed that *M. pudica* leaves exhibited higher antioxidant activity by ABTS radical scavenging activity [30]. It was suggested in a study that the methanolic extracts of *M. pudica* were having maximum antioxidant efficiency in DPPH, ABTS, and FRAP assays [31]. The methanolic extracts of *Mimosa hamata* stem exhibited greatest antioxidant activity with DPPH and ABTS methods [32]. It was reported in a study that water extracts from *M. pudica* exhibited prominent ABTS radical scavenging activities [33].

Nitric oxide free radical scavenging method

Aqueous thorn extracts of *M. pudica* unveiled higher values than its ethanolic thorn extracts, using nitric oxide radical scavenging method [Table 4]. At 250 µg/mL concentrations of aqueous thorn extracts, 26.10% inhibition was resulted. The IC_{50} values of the aqueous thorn extracts of *M. pudica* were found to be lesser (642.68±20.82 µg/mL) than the respective ethanolic extracts (677.09±17.97 µg/mL). A study suggested that the hexane extracts of *M. pudica* Linn. revealed the significant scavenging effect on DPPH, hydroxyl, nitric oxide, and superoxide radicals [34]. It was reported in a study that the ethanolic extract of *M. pudica* (Mimosaceae) exhibited a significant inhibition in nitric oxide and DPPH free radical formation [35]. The ethyl acetate fraction of *M. pudica* exhibited high reduction capacity and powerful free radical scavenging against DPPH and NO radical inhibition assay [36]. Previous studies revealed that the chloroform extract of *M. pudica* Linn. leaves showed a significant antioxidant activity against free radical scavenging by DPPH, nitric oxide, superoxide dismutase, and reducing ability. Moreover, it was also reported that the *in vitro* antioxidant activity was due to the antioxidant principle phenolic compounds and flavonoids [19]. In earlier studies, it is reported that the nitric oxide activity may be due to the presence of antioxidant polyphenolic molecules [37].

CONCLUSION

The results of phytochemical analysis revealed the presence of flavonoids, saponins, glycosides, alkaloids, terpenoids, and coumarins in the ethanolic and aqueous thorn extracts of *M. pudica*. The aqueous thorn extracts of *M. pudica* exhibited higher antioxidant activity by DPPH and nitric oxide free radical scavenging method, whereas the ethanolic extracts of *M. pudica* thorns exhibited higher antioxidant activity by ABTS free radical scavenging method. The bioactive principles in the extracts of *M. pudica* might have contributed the free radical scavenging activity. Hence, the current study gives a roadmap for isolating and characterizing the potential compound from the extracts of *M. pudica* for therapeutic applications.

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AUTHORS' CONTRIBUTIONS

Mrs. R. Lakshmibai performed the experiment and wrote the manuscript, and Dr. D. Amirtham supervised the progress of the work and involved in the corrections of the manuscript.

CONFLICTS OF INTEREST

The authors confirm that there is no conflict of interest.

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