

The Phytochemical Potential of *Mimosa pudica* L Plants Under Enhanced Solar UV-B (280-320 Nm) Radiation

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Abstract: UV-B radiation can improve the quality of medicinal plants such as *Mimosa pudica* L by increasing the production of phenol, alkaloids, saponin and other secondary bioactive compounds. Qualitative analysis of these compounds using TLC revealed high concentrations of these components, with higher production in plants exposed to UVB radiation. The synthesis of these phytochemical compounds correlates with the antioxidant potential and increased FRAP activity. The antibacterial effect induced by UVB radiation is effective against pathogenic microorganisms such as *Proteus vulgaris*, *Staphylococcus epidermidis* and *Bacillus subtilis*, but less effective against *Salmonella typhi*. The long-term goal is to explore the potential applications of these improved bioactive chemicals in pharmaceutical research and development.

Keywords: Antimicrobial activity, alkaloids, *Mimosa pudica*, phenol, phytochemical compounds, TLC, UVB

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1. Introduction

In recent decades, the depletion of stratospheric ozone by anthropogenic pollutants such as halogenated hydrocarbons and other ozone-depleting chemicals entering the stratosphere has caused great concern [1,2]. Greenhouse gases that cause cooling of the stratospheric ozone layer contribute indirectly to ozone depletion by increasing UV-B radiation (280-320 nm) from solar radiation, which can cause high levels of biological damage even at lower doses and potentially alter the Earth's biochemical profile [3].

UV-B radiation can directly or indirectly alter the phenotype of plants by causing DNA damage and cell damage. This can lead to slower stem growth, dwarfism, smaller leaf size and lower photosynthetic efficiency. [4,5]. Exposure to UV-B radiation has been associated in previous studies with reduced leaf area and plant height in cucumber, sunflower and soya bean, as well as reduced biomass accumulation and production in several plant species. [6,7,8].

In addition, plants exposed to abiotic stress produced more reactive oxygen species (ROS) and many more of their bioactive components [9]. To scavenge excess ROS produced under UV-B stress, plants have developed

complex antioxidant defence systems comprising a range of enzymes and metabolites [5]. In addition, secondary metabolites produced by plants in response to various environmental situations are involved in the detoxification and control of ROS via non-enzymatic pathways [10].

These metabolites include various flavonoids, hydroxycinnamic acid esters and phenolic compounds; most of these substances have an antioxidant effect or the ability to scavenge free radicals. The direct absorption of UV-B wavelengths by these secondary metabolites, which may serve as part of a common defence mechanism in plants as sunscreens [11,12].

Recent studies suggest that it can promote secondary metabolism in medicinal plants [13,14]. The number of scientific reports on the effects of UV-B on medicinal plants is low or zero compared to cultivated plants. [15]. Therefore, we selected an important and popular medicinal plant: *M. pudica* is a common medicinal plant that grows as a roadside weed in tropical regions. Although the whole plant is used in traditional systems of medicine, the seeds, roots and leaves are the main parts that are most commonly used. It is reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids, with flavonoids shown to prevent the development of some cancers [16] in addition to the biological activities mentioned above, the various extracts of *M. pudica* are also known for their antibacterial activity. The study of

this aspect has become an important area of research in recent years due to the increasing problem of resistance among pathogenic bacterial strains.

The aim of the study was to investigate the UV-B-induced synthesis of bioactive compounds in *M. pudica* and to analyze their antioxidant potential and antibacterial activity against various pathogenic organisms.

2. Materials and Methods

2.1. Plant Materials

Certified seeds of *Mimosa pudica* L obtained commercial manufacture from Chennai. It was shown in experimental plots in the Pachaiyappa's College Botanical Garden, Chennai. One set of plants was grown under ambient solar radiation and other set of plants grown under 20% UV-B enhanced solar radiation.

2.2. Plant Growth and UV-B Treatment

The seeds were soaked 6 to 7 hrs in running water. Separate soil beds were prepared for control (ambient) and UV-B treatment and seeds were sown in these experimental plots. There were four experimental plots were prepared. The two experimental plots used for ambient and remaining two used for UV-B treatment. In each plot 20 seeds were sown. The plants were watered regularly and care was taken to avoid microbial or pest infection during the experimental period. Plants with the first foliage leaf stage were used for UV-B treatment. UV-B treatment was given to these plants for 4 hrs daily from 10 a.m. to 2 p.m. Treatment was continued under ambient solar radiation and 20% UV-B enhanced solar radiation supplemented by a Philips TL40W/12 sunlamp (Gloeelampen fabrieken, Holland). The first formed leaves were collected at various time periods and all the physiological and biochemical examinations were completed.

2.3. Measurement of Radiation

A Li-Cor Li-188B quantum/radiometer (Li-Cor., Inc., USA) with suitable photodetector was used to measure all the visible and photosynthetically active radiation. Radiation below 400 nm was determined by an IL 700 radiometer with a SEE 400 photodiode detector (International Light Inc., USA). This instrument used to measure quantity of UV-B radiation in the sunlight.

2.4. Extraction of Sample

The dried and powdered materials (5 g) were extracted successively with 250 mL of methanol by using a Soxhlet extractor for 8 hrs at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered by using Whatman filter paper (No: 1) and then concentrated in vacuum at the 40°C using Rotary evaporator. The residues obtained were stored in a freezer -20°C until further tests.

2.4.1. Thin Layer Chromatography of Methanol Extracts for Various Phytochemical

Thin layer chromatography (TLC) was carried out on precoated silica gel aluminium sheets (Merck TLC, silica gel 60 F254 (20 x 20 cm). The chromatogram was developed by placing the TLC plate in a TLC apparatus containing suitable solvent system. The developed TLC plates were dried at room temperature. The spots were observed under visible as well as UV light (254 and 365 nm), then exposing the plates to iodine vapours. The developed TLC plates were placed in iodine chamber [17,18]. The R_f values of the spots were recorded. The specific solvent system used for TLC such as Toluene: Ethyl acetate: Triethylamine (7:2:1; v/v/v) Chloroform: methanol (9: 1; v/v) for phenol, Toluene: Ethyl acetate (7:3; v/v) flavonoid, ethyl acetate: hexane (1:9; v/v) for saponins and Toluene: diethyl ether (1:1; v/v) for phytosterols.

$$R_f \text{ Value} = \frac{\text{Distance travelled by solute front (in cm)}}{\text{Distance travelled by the solvent front (in cm)}}$$

2.4.2. Determination of Antioxidant Activity (FRAP) Method

The FRAP assay [19] involved adding methanolic extract to a reagent, which was prepared by mixing sodium acetate buffer solution, TPZT, and FeCl_3 hexahydrate. The absorbance increase was measured at 593 nm in a UV-30 spectrophotometer, and the results were expressed in milligram equivalents of FeSO_4 per milligram of dry weight.

2.4.3. Antibacterial Activity

The antibacterial activity of plant extract was examined against selected pathogenic bacteria namely *Proteus vulgaris*, *Bacillus subtilis*, *Salmonella typhi*, and *Staphylococcus epidermidis* using agar disc diffusion method [20]. The zone of inhibition was calculated and compared with standard antibiotic, amoxicillin. The plant extract was subjected to sequential dilution using DMSO in the concentrations of 100, 250, 500 and 750 $\mu\text{g/mL}$. Whatman No. 1 sterile filter paper discs (6 mm diameter) were drenched and placed on inoculated agar. The working culture was prepared by inoculating a loopful of each tested bacteria in 10 mL Mueller-Hinton agar medium and all the plates were incubated at 37°C for 24 h. Antibacterial activity was assessed by measuring the diameter of zones of inhibition.

2.4.4. Determination of Minimum Inhibitory Concentrations (MIC)

The MIC of was performed by 96 well microdilution method [20]. Briefly, 50 μL of plant extracts at different concentrations (500 to 0.488 $\mu\text{g/mL}$ was obtained using a two-fold serial dilution) and 50 μL Mueller Hinton broth were poured in each well. Then, 50 μL of bacterial inoculum at 10⁶ CFU/ml was added to each well and incubated at 37°C for 24 hrs. After incubation, 20 μL of 0.5 mg/mL INT (p-iodonitro-tetrazolium or 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride) was added to each well and incubated at 37°C for 30 min. Ampicillin (30 to 0.029 $\mu\text{g/mL}$) was used as positive control. The MIC of leaf extract was observed by change in colour after the addition of INT dye. The red-pink colour indicated

the bacterial growth and inhibition of bacterial growth in the broth medium with plant extracts was noted by no colour change.

3. Result and Discussion

In ancient times, man relied on nature for medicinal purposes. For a while, man used allopathic medicine, but due to its negative effects, this medicinal plant was rediscovered by man. For this reason, we have conducted this research. The medicinal properties of medicinal plants are widely known, but the studies on their function and how to utilize them optimally are quite limited. This study is a small attempt to utilize the changes that can occur when medicinal plants are exposed to UV-B rays under natural conditions. The traditional medicinal plant *M.pudica* was exposed to UV-B rays, which not only accelerated plant growth but also promoted the synthesis of potential bioactive chemicals, as shown by our results when we used TLC for qualitative analysis.

3.1. Thin Layer Chromatography Analysis

The TLC studies proved the presence of secondary metabolites such as alkaloids, phenols present in the methanol extracts of *M. pudica* leaves.

TLC of leaf extracts of *M. pudica* revealed the presence of 7 compounds with different R_f values of 3.5, 4.3, 4.6, 5.0 when a solvent phase of ethyl acetate: triethylamine (70:20:10) was used. After an irradiation period of 40 days, the total content of alkaloid compounds in *M. pudica* grown under additional UV-B irradiation was almost 1.5 times higher than in a control plant (Figure 1). Bioactive compounds such as flavonoids, alkaloids, terpenoids, phenols, Ca-rotenoids, anthraquinones, sterols and lignin are UV-B absorbing compounds, and sufficient UV-B irradiation increases their synthesis [21]. Previous research has shown that UV-B radiation can stimulate secondary metabolism and increase the concentration of bioactive components in medicinal plants [22]. Recently, some studies have concluded that UV-B significantly affects plant defence mechanisms and can increase the content of bioactive substances over short periods of time [20,23,24].

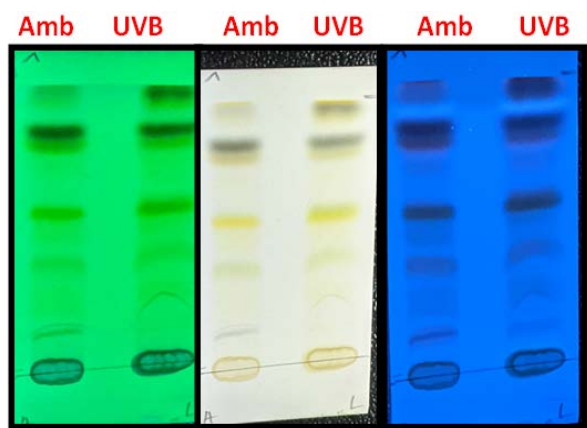


Figure 1. TLC profile of alkaloid pattern of Methanol extracts of dried leaves of *Mimosa pudica* a. Under 365 nm b. TLC visualized under visible light and c. Under 254 nm. 1= Control sample 2= treated sample Solvent system: Toluene: Ethyl Acetate: Triethylamine (70:20:10; v/v/v)

TLC of methanolic leaf extracts of *M. pudica* revealed the presence of 7 compounds with different R_f values of 3,3,5,4,5,5,5 when a solvent phase of chloroform: methanol (9:1) was used. The effectiveness of UV-B irradiation increases the production of phenolic content twofold compared to the control plants (Figure 2). The formation of phenolic compounds that protect both directly and indirectly against UVB-induced damage is enhanced when UV-B irradiation is increased, which improves PAL activity [25]. Similarly, *D. antarctica*, the most successful angiosperm in the maritime Antarctic region, resists UV-B irradiation due to its production and storage of phenolic-like compounds [26,27].

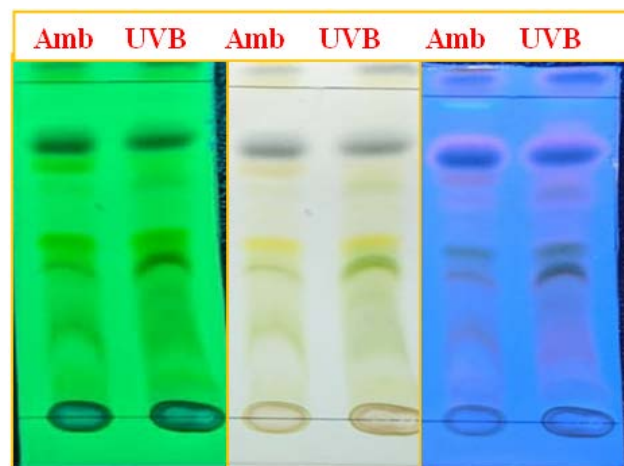


Figure 2. TLC profile of phenol pattern of methanolic extracts of dried leaves of *Mimosa pudica*. a. Under 365 nm b. TLC visualized under visible light and c. Under 254 nm. 1= Control sample 2= treated sample Solvent system: Chloroform: Methanol (9:1; v/v)

Table 1. Total no. of Phytochemical compound (phenol and alkaloids) by TLC solvent system for methanolic leaf extract of *Mimosa pudica*

TLC	Alkaloid	Phenol
Total No of Compounds	7	7
Solvent phase	Toluene: Ethyl Acetate: Triethylamine (70:20:10)	Chloroform: Methanol (9:1)

Table 2. R_f values of Phytochemical compound (phenol and alkaloids) by TLC solvent system for methanolic leaf extract of *Mimosa pudica*

TLC	Alkaloid	Phenol
R_f Values Obtained	3.5, 4.3, 4.6, 5.0	3,3,5,4,5,5,5
	4.3	3.5
	4.6	4.5
	5.0	5
		5'5

The FRAP test evaluates the reducing capacity of compounds, focusing on the ability of antioxidants to convert the colourless Fe_3^+ - TPTZ complex into the blue Fe_2^+ - TPTZ complex. The FRAP (plasma ferric reducing ability) test evaluates the overall antioxidant capacity and is used to study the potential effect of medicinal plants. Regarding the antioxidant capacity of methanolic leaf extracts of *Mimosa pudica*, the UV-B-treated plant showed the highest reducing power. The FRAP activity was slightly higher in the control plant in the early phase,

but in the late phase of the irradiated plant, the activity was higher, indicating that the irradiated phytochemical compound. The potential is high, showing that there is a correlation between these bioactive compounds and antioxidant activity. The recent study confirmed that the downstream signal transduction pathway triggers acclimation responses to UV-B conditions by overexpressing genes involved in the phenylpropanoid metabolic pathway. Plants benefit from the accumulation of phenols, which counteract the overproduction of ROS, resulting in higher antioxidant activity and greater health benefits [25]. In addition, the reducing power of the methanolic extract of the UV-B-treated plant was comparable to that of ascorbic acid. Previous studies reported that supplemental UV-B irradiation shows a linear correlation between FRAP and the scavenging of hydroxyl radicals or singlet oxygen, suggesting that these antioxidant capacities are the main components of FRAP [28].

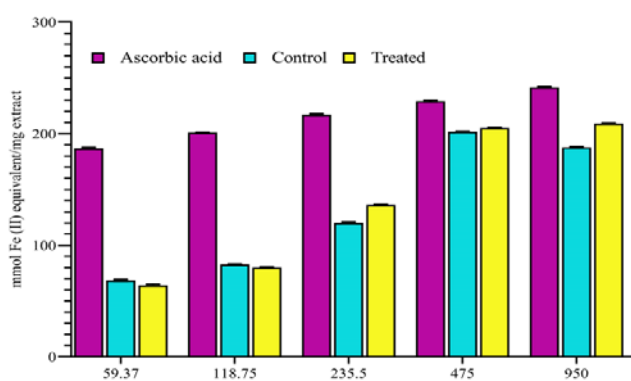


Figure 3. FRAP antioxidant activity of the methanolic extracts leaves of *Mimosa pudica* plants grown under ambient and enhanced UV-B radiation

The active bioactive compounds of herbal plants were a starting point for the development of modern medicine. These herbal remedies have been used to treat many diseases throughout human history, so the bioactive compounds in medicinal plants are useful as a source of antimicrobial agents to improve antimicrobial resistance. The results showed that the plant extract obtained from the

leaves of *M. pudica* was effective in inhibiting the growth of the test pathogens, as shown in Figure 3. In UV-treated plants, the activity was found to be very high for *Proteus vulgaris* and normal for the microorganisms *Staphylococcus epidermidis* and *Bacillus subtilis*. Thus, the bioactive compounds were potentiated by UV-B treatment and the activity in some microorganisms showed upright, while the activity of some microorganisms was normal. The induced chemicals have an antibacterial and antiviral effect in particular. In practise, however, UV-B induction technology is much simpler and more efficient than microbiological induction. Chalconoracin had a UV-B inducing content of 0.082% and a microbiological inducing content of 0.013%. Methanolic leaf extracts of *M. pudica* 250-1000 µl had a lower inhibitory effect against *Salmonella typhi* in both the control and UVB-treated plant. The absence of antimicrobial activity does not mean that the bioactive substances are not present in the plant or that the plant has no antimicrobial activity against microbes. The presence of insufficient amounts of active substances or components in extracts showing antimicrobial activity may be responsible for negative results. Many recent studies have demonstrated the antibacterial and antifungal activity of phenolic compounds from plants, including the main components of the extracts studied (rutin and isoquercitrin) [29,30].

The minimum inhibitory concentration (MIC) of methanolic leaf extracts was determined against some pathogenic microorganisms. After overnight incubation, the growth of a microbial pathogen is inhibited by the minimum inhibitory concentration, i.e. the lowest concentration of an antimicrobial agent, e.g. a methanolic plant leaf extract. At the lowest concentration, between 759 and 1.25 g, the MIC of UV-treated plants inhibits the growth of bacterial pathogens. Previous studies for *A. reptans* showed a similar pattern in terms of MIC and MBC [31]. According to Salvat et al. [32], herbal extracts with MIC values of less than/around 0.50 mg/ml exhibit good antibacterial activity. Consequently, the results presented here showed modest antibacterial activity.

Table 3. Antibacterial activity of methanolic extract by disc diffusion assay (Zone of inhibition in mm)

Sample name	Concentration (mg/mL)/ Bacterial strains	<i>Salmonella typhi</i>	<i>Proteus vulgaris</i>	<i>Staphylococcus epidermidis</i>	<i>Bacillus subtilis</i>
Control	25	0.4 ± 0.2	9.03 ± 0.40	8.8 ± 0.45	10.36 ± 0.25
	50	1.46 ± 0.20	11.66 ± 0.56	11.5 ± 0.3	11.33 ± 0.15
	75	2.63 ± 0.25	12.1 ± 0.2	11.63 ± 0.15	13.36 ± 0.35
	Standard (Amoxycillin)	3.26 ± 0.20	21.7 ± 0.26	20.56 ± 0.15	22.3 ± 0.3
	Negative control	-	-	-	-
Treated	25	1.73 ± 0.45	15.33 ± 0.35	8.53 ± 0.50	11.16 ± 0.15
	50	2.56 ± 0.20	16.03 ± 0.56	13.7 ± 0.3	12.16 ± 0.37
	75	3.23 ± 0.25	19.26 ± 0.30	14.5 ± 0.3	15.5 ± 0.2
	Standard (Amoxycillin)	22 ± 0.95	21.6 ± 0.36	24.46 ± 0.30	18.36 ± 0.25
	Negative control	-	-	-	-

Table 4. Antibacterial activity by disc diffusion assay by microdilution methods (Minimum inhibitory concentration)

Sample name/Bacterial strains	<i>Salmonella typhi</i>	<i>Proteus vulgaris</i>	<i>Staphylococcus epidermidis</i>	<i>Bacillus subtilis</i>
Control	93.75	96.8	93.75	76.8
Treated	81.8	46.8	55.75	46.8
Standard (Amoxycillin)	0.117	0.058	0.058	0.117

Values expressed as mean ± SD

4. Conclusion

Previous studies have shown that UV-B radiation affects plants in different ways and leads to various changes. UVB radiation increased the phytochemical molecule in *Mimosa pudica* through a defence mechanism, which makes this study interesting. In addition, potentiated phytochemicals have been shown to have higher antioxidant activity. The antimicrobial activity of the potentized phytochemical compounds against various pathogenic bacteria was also good. Due to their easy availability, cost-effectiveness, safety and bio-friendly restriction, these herbs are identified for potential therapeutic applications as pharmaceuticals, making them a preferred choice over synthetic substances in medicine and other fields.

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