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Bioactive compounds and their activity influenced by enhanced solar UVB (280-320 nm) radiation in *Mimosa pudica L.*

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Abstract:

Mimosa pudica, a medicinal plant in India, has been utilized for thousands of years for its health benefits. When exposed to UV-B radiation, the plant shows improved growth and a 30% rise in pigment content. Quantitative studies have shown that UV-B radiation doubles the concentration of therapeutic bioactive compounds, with phenols and flavonoids being the main compounds showing a doubling in proliferation in treated plants. The concentrations of alkaloids and saponins are more than one-fold higher than those in control plants. As additional validation, in GCMS results, the UV-B-treated plant showed two new peaks at 54.97 and 66.56 minutes, absent in plants not exposed to UV-B radiation. The mass spectrum of the LC peak appearing at 54.97 minutes showed a clear and distinctive peak at m/z 57, 316, 648, and 663. The mass peak observed at m/z 316 may be Rhamnetin, and the dimer of Rhamnetin with one methoxy group appeared at m/z 648. The peak at m/z 663 may be due to dimethoxy derivatives. The structure and activity of the unknown compound induced by UV-B will be explored in the future. Furthermore, the antioxidant activity of the bioactive molecule experiences a significant increase due to stress-induced free radicals, with DPPH and superoxide antioxidant activities markedly elevated. Although the IC50 value of the bioactive compound is higher than that of the control plant, it demonstrates similar antidiabetic activity to acarbose. This groundbreaking research challenges the traditional understanding of UV-B rays' antagonistic effects on plants, showcasing that UV-B rays can, in detail, enhance the medicinal properties of plants. Applying UV-B to medicinal plants proves to be a strategic approach to boost their medicinal properties, increase bioactive compound concentrations, and improve overall plant quality. This contributes to advancing scientific understanding and holds economic significance by elevating the plant's value.

Keywords: Bioactive compounds, ROS, DPPH, SO, GCMS, *Mimosa pudica.*

Introduction:

In recent decades, there has been a notable surge in research interest in harnessing the potential of medicinal plants. This increased interest is fueled by increasing trust in herbal medicine. This heightened attention is fueled by factors like a growing trust in herbal medicine. While allopathic medicine effectively addresses various health conditions, the significant costs and potential side effects frequently drive individuals to seek herbal alternatives, appreciated for their comparatively milder side effects [1]

Secondary metabolism in medicinal plants produces bioactive compounds with diverse global applications, including the treatment of infections. These compounds, categorized as terpenes and nitrogen-containing phenolic compounds play protective roles against diseases [2]. Phytochemicals such as alkaloids, carotenoids, flavonoids, terpenoids, saponins, phytosterols,

organic acids, essential oils, and protease inhibitors exhibit anti-allergy, antiproliferative, and anti-oxidative effects. While these compounds are highly valuable in the global market, their excessive use poses a risk of extinction. Research on these plants has received limited attention, particularly in the field of plant physiology. The current investigation emphasis on accepting the constancy of biochemical compounds in medicinal plants below abiotic stress conditions for maintainable use and conservation [3].

UV-B rays, as an abiotic stressor, induce significant changes in plant processes. While these rays can be highly damaging to agricultural plants, they yield interesting results in medicinal plants. When plants sense UV-B radiation, specific metabolites are produced either through direct screening or via their antioxidative potential. These metabolites play a crucial role in adapting to UV-B radiation [4] .Many of these specific metabolites are activated by abiotic stress and participate in plant defense mechanisms or scavenging of reactive oxygen species (ROS). Alkaloids, glucosinolates, and phenolic chemicals (or polyphenols) have gained attention in current years regarding plant defense and UV-B light adaptation. Thus, it is imperative to ascertain whether elevating one's exposure to UV-B radiation can serve as a simple and eco-friendly method of augmenting the concentration of bioactive components in medicinal plants [5].

The primary attention of this investigation is on the effects of UV-B radiation on the stages of bioactive components in *Mimosa pudica*. This plant, a perennial or annual herb that creeps, is known as Lajjalu in Ayurveda. Research has demonstrated its aphrodisiac, analgesic, antidepressant, and antiasthmatic qualities. Traditionally used for various conditions, including urogenital infections, tumors, alopecia, dysentery, diarrhea and insomnia, *M. pudica* possesses sedative, emetic, and tonic characteristics. Phytochemical research on *M. pudica* has identified alkaloids, fatty acids, flavonoids C-glycosides, sterols, terpenoids, non- protein amino acid (mimosine), and flavonoids [6]. In the Indian state of Manipur, a decoction of its leaves is believed to promote diuresis and treat urinary tract infections. The *Mimosa* plant is reputed for its various medicinal properties, including anti-hepatotoxic, contraceptive, hypolipidemic, anticonvulsant, antidepressant, and wound-healing effects. Furthermore, the seeds are purported to possess diuretic properties [7]. Within *Mimosa* roots, one can find crystals of calcium oxalate, ash, tannin, and the alkaloid mimosine [8]. The phytochemical components of *Mimosa pudica* have been the subject of several studies [9, 10, 11]. This study aims to investigate

changes in plant growth, physiological and biochemical responses, and levels of bioactive chemicals when field-grown *Mimosa pudica* is exposed to artificial UV-B radiation.

Materials and Methods:

Plant materials

Certified *Mimosa pudica* seeds were purchased from a commercial manufacturer in Chennai. In the Chennai Pachaiyapppa's College Botanical Garden, it was on display in test plots. Two sets of plants were developed using both ambient solar radiation and 20% UV-B enhanced solar radiation.

Plant growth and UV-B treatment

For six to seven hours, the seeds were immersed in running water. In these experimental plots, seeds were sown in distinct soil beds that were ready for the ambient control and UV-B treatment conditions. Four experimental plots had been set up. The remaining two experimental plots were employed for UV-B treatment, while two were used for ambient testing. Twenty seeds were sowed in each allotment. Throughout the trial period, the plants received regular irrigation and precautions were taken to prevent microbiological or pest infestation. For UV-B therapy, plants in the first stage of the foliage leaf stage were used. These plants received a daily 4-hours UV-B treatment after 10 a.m. to 2 p.m. The treatment plan included a Philips TL40W/12 sunlamp (Gloelampen fabrieken, Holland) in addition to ambient solar radiation and 20% UV-B enhanced solar radiation. The first fully grown leaves were collected at different intervals and all physiological and biochemical testing were completed.

Measurement of radiation

Total light intensity and photosynthetically active radiation were measured using a Li-Cor Li-188B quantum/radiometer (Li-Cor., Inc., USA) equipped with a light source. Radiation below 400 nm was measured using an IL 700 radio equipped with a SEE 400 photodiode detector (International Light Inc., USA). This meter measures the amount of UVB radiation in sunlight.

Determination of growth

The shoot length was measured in a specific interval (measurements taken every 10 days throughout the experimental period) shortly after the plants were uprooted. The average of 20 samples that were chosen at random was used for all measures.

Estimation of pigments

Chlorophylls

The first leaf is collected, cut into a disc and used for extraction. Total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids were determined after extraction of pigments in 80% acetone using the Wellburn and Lichtenthaler formula [12]

Chlorophyll a (mg/l): (12.21 x A663) – (2.81 x A646)

Chlorophyll b (mg/l): (20.13 x A646) – (5.03 x A663)

Total chlorophyll (mg/l): (7.18 x A663) + (17.32 x A646)

Carotenoids

Total carotenoid content in the 80% acetone extract was determined by measuring the absorbance at 480 nm. McKinney's formula was used to solve the problem of chlorophyll interference [13.

$$\text{Carotenoids (mg/l)} = \frac{1000\ A470 - (3.27\ \text{Chl a}) - (104\ \text{Chl b})}{229}$$

Flavonoids

Fresh leaf samples weighing 100 mg were separated into small pieces and immersed for a whole night at 4°C in the dark in 5 ml of methanol that had been 80 percent acidified (HCL) (79:20:1). The flavonoid content was described as µg/g leaf fresh weight after the absorbance at 315 nm was dignified and fragments was centrifuged off [14].

Anthocyanin

By pulverising the leaves in methanol that has been 80 percent acidified (79:20:1) with water and HCl, anthocyanin was recovered from the leaves. Mancinelli *et al.*,[15] state that the clear extract was centrifuged and the absorbance at 530 and 657 nm was used to calculate the anthocyanin concentration.

µg/g fresh weight: (A530) – (0.3 x A657)

Estimation of protein

Lowry *et al.*, ([16] state that protein content was determined with bovine serum albumin serving as the reference. Following the addition of 4 ml of the alkali mixture and 15 minutes of incubation, a known aliquot of the sample solution was raised to 1 ml using Milli Q water. Then, folin-phenol (1 N) was added, immediately vortexed, and allowed to sit at room temperature in the dark for ten minutes. Absorbance was measured at 640 nm.

Alkali mixture

Copper sulfate1%: Solution A

Sodium potassium tartarate 2%: Solution B

2% Sodium carbonate in 0.1 N NaOH: Solution C prepared when needed in the following proportions: A: B: C = 1:1:100.

Preparation of extracts

The control plant's fresh *M. pudica* leaves and the UV-B-irradiated plant leaves underwent separate 72-hour steeping times in ethanol (4:1) at room temperature. The extract was filtered with Whatman filter paper. Similar extracts were mixed and concentrated at 45°C under reduced pressure using a vacuum rotary evaporator. This was done two to three times. A preliminary phytochemical screening was performed on the concentrated crude methanol extract. Quantitative analysis of the phytochemical compound was performed using standard protocol method.

Antioxidant activity

Radical scavenging activity using DPPH assay

The effectiveness of plant extracts in neutralizing DPPH radicals was evaluated after the method was developed [17]. 100 µL of methanol containing 0.1 mM DPPH was mixed with 100 µL of leaf extracts at different concentrations. Then leave the mixture at room temperature for 30 minutes without light. After the incubation period, absorbance was measured at 517 nm. The calibration curve created by linear regression is used to determine the concentration of DPPH present in the reaction mixture. The method used in this study is ascorbic acid. To estimate the DPPH free radical scavenging activity, the percentage of free radical scavenging ability produced by the plant extract was determined and expressed as the IC50 value using the following equation.

% Inhibition = [(Control OD – Sample OD) / Control OD] × 100

Superoxide radical scavenging activity

The superoxide anion rummaging action was evaluated utilizing the nitro blue tetrazolium diminishment strategy as depicted by Thangaraj (17). At first, 100 µL of changing concentrations of leaf extricates were blended with 3 mL of a naturally arranged response blend containing 20 µg riboflavin, 12 mM EDTA, and 0.1 mg NBT in 50 mM sodium phosphate buffer (pH 7.6). The blend was at that point brooded at 25°C for 5 minutes, taken after by estimation of absorbance at 590 nm. Ascorbic corrosive served as the positive control. The superoxide radical rummaging capacity was calculated utilizing the equation:

Scavenging activity (%) = [(Control OD – Sample OD) / Control OD] × 100].

Scavenging activity (%) = [(Control OD – Sample OD) / Control OD] × 100]

In vitro antidiabetic activity

α-amylase inhibitory assay

Lowden *et al.*, [18] performed an α-amylase inhibition assay. Solutions containing 1% starch and various plant extracts in 20 mM phosphate buffer (pH 6.9 with 6 mM sodium chloride) were prepared at 25°C for 10 min. Each tube was then incubated for another 10 min at 25°C, and 100 µL of porcine pancreatic α-amylase (0.5 mg/mL) was added. The reaction was stopped by adding 200 µL dinitro salicylic acid reagent, and the mixture was incubated at 100 °C for 5 min. After cooling to room temperature, 50 µL of the reaction mixture in each tube was transferred to a 96-well microplate. The absorbance at 540 nm is stable after diluting the reaction mixture with 200 µL of distilled water in each well. Acarbose is well controlled. Therefore, α-amylase inhibitory activity of the plant extract has been suggested.

% Activity = Absorbance of extract / Absorbance of control X 100

α-glucosidase inhibitory assay

The method described by Lordon and colleagues [18] was used to measure inhibition of α-glucosidase. This method involves mixing 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in phosphate buffer with 50 µL of the same amount of leaf extract in 100 mM sodium phosphate buffer (pH 6.9) Mixture and then incubating the mixture at 37°C. It includes. °. at C for 5 minutes. Then, 100 µL of phosphate without 0.1 U/mL α-glucosidase was delivered to each well. After half an hour of incubation, the absorbance at 405 nm was recorded. A good control is acarbose. Based on these data, the inhibitory activity of plant extracts against α-glucosidase was calculated.

% Activity = Absorbance of extract / Absorbance of control X100

GC-MS analysis

GC-MS analysis using an integrated gas chromatography system 7890A (manufactured by Agilent, USA) equipped with an air detector. The size of the HP-5 MS fused silica column is 30.0 m × 250 µm and the film thickness is 0.25 µm. The system accepts µm. It communicates with the 5675C Inert MSD equipped with a three-axis detector. Helium was used as the gas and the flow rate was 1.0 ml/min. Operation is 1 ul. The injector is made in a separate mode with a ratio of 1:50, injection temperature 300 ° C, ion temperature 250 ° C, interface temperature 300 ° C and Pressure

test 16.2 psi, exit time 1.8 mm. Initially, the temperature was maintained at 36°C for 5 min and then increased to 150V at a rate of 4°C/min. Thus, the temperature was increased to 250°C at a rate of 20°C/min and held for 5 min, resulting in a total elution time of 47.5 min. Control management and data collection is done using MS solution software provided by the business owners. The relative percentage of each component was determined by relating the constant peak area to the total concentration.

Statistical analysis

Results are shown as the mean and standard error of the mean for each group's triplicates. ANOVA was used to statistically examine the data using InStat-Graph Pad, an open-source statistical programme. $P \geq 0.05$ was regarded as significant.

Results:

Since, the beginnings of time, medicinal plants have been employed by humans for their therapeutic characteristics. These plants can thrive in various stressful environments without specific requirements in their natural habitat. In our study, we cultivated a medicinal plant, *Mimosa pudica*, in an open field setting with artificial UV-B radiation. The improved solar UV-B amount over ambient radiation was 20%. We reviewed the samples taken at specific intervals and discovered many astounding findings.

The traditional medicinal plant, *Mimosa pudica*, thrived and responded favorably to UV-B radiation in our examination. Here, we describe how exposure to UV-B radiation alters the generation of bioactive molecules, their activities, as well as photosynthetic and non-photosynthetic pigments.

Changes in the photosynthetic pigment content

The plant morphology did not change as a result of UV-B radiation. Therefore, we decided to examine the attention of photosynthetic and non-photosynthetic pigments. Chlorophyll, a photosynthetic pigment, aids in photosynthesis by assisting plants in absorbing sunlight. UV-B rays enhance the creation of these crucial chlorophyll molecules, and we discovered that the production was 50% advanced than that in the control plant (Fig.1). Similarly, UV-B light causes this plant to contain more than 20% more carotenoids, a protective pigment (Figs. 1 & 2).

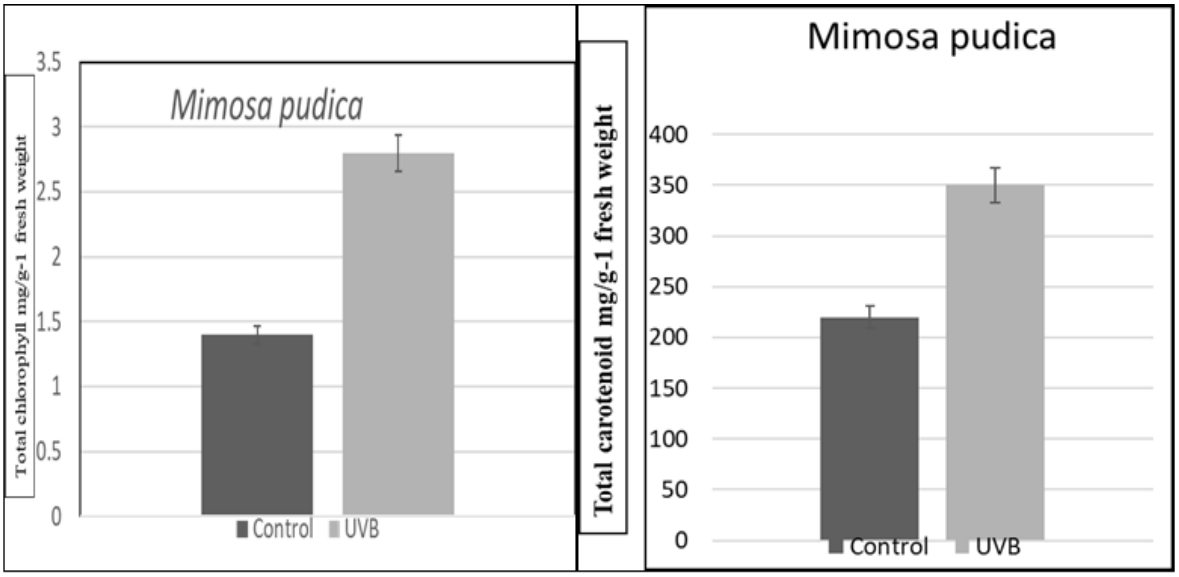

Fig.1&2 Modifications in the total chlorophyll concentration of *Mimosa pudica* cultivated in control and enhanced UV-B environments. The figures are the average of three different measurements. Mean ± SE, n=3.

Changes on non-photosynthetic pigment

Secondary pigments are pigments that do not undergo photosynthesis. Although many researchers say that UV-B radiation causes these pigments to increase, it turns out that these colors are produced more as a defense mechanism. To prevent harmful UV-B rays from interfering with the synthesis of photosynthetic pigments, the pigments block them. The study found that exposure to UV-B rays increased the plant's flavonoid content by 40 percent compared to the control plant. This result ensures the protection of the facility from hazardous radiation (Figure 3). Anthocyanin concentration is also increased by UV-B irradiation. The results showed that the yield was 30% higher than the control plant (Figures 3 and 4).

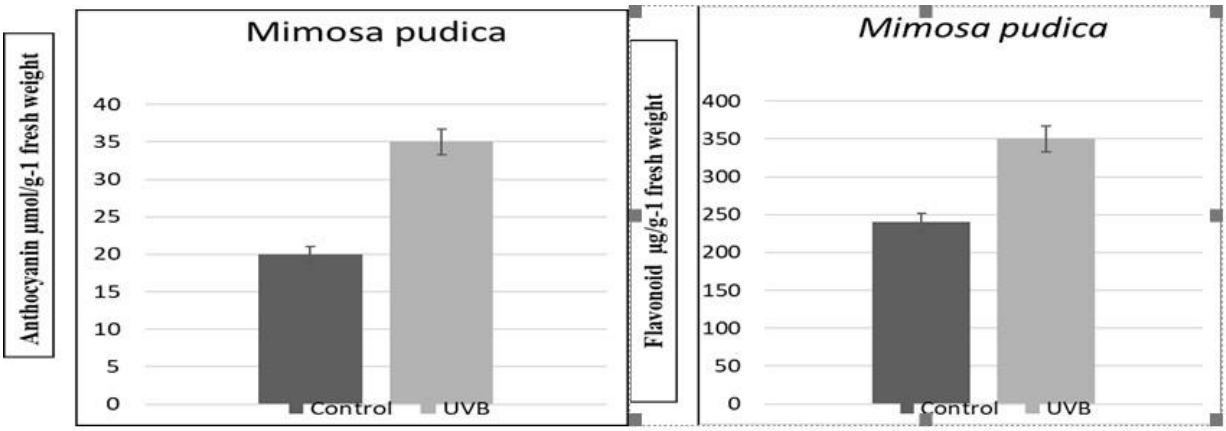

Fig. 3 & 4. Modifications in the anthocyanin and flavonoid contents of *Mimosa pudica* cultivated under control and enhanced UV-B. The numbers are the average of three separate measurements. Mean ± SE, n=3.

Total protein

In *Mimosa pudica*, the differences in total protein content were detected. Dropping protein levels is a common consequence of UV exposure. Because proteins absorb UV light, their amino acids break down, which is the source of this. But during our research, we found that UV-B-protein concentration had increased. Most likely, the production of protective proteins and enzymes is what has caused this increase. In general, protein concentrations were higher than the control level and rose by 30% with increasing levels of UV-B light (Fig.5).

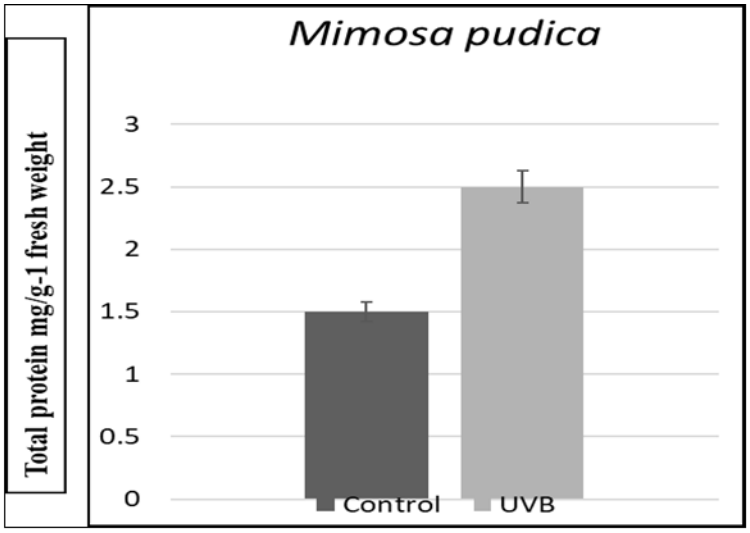

Fig.5. Modifications in the total protein content of *Mimosa pudica* plants cultivated under control and UV-B radiation. The figures are the average of three different measurements. Mean ± SE, n=3.

Phytochemical analysis of ethanol extracts of leaves of *Mimosa pudica*

In the natural environment, UV-B rays play an important role in the formation of secondary metabolites of medicinal plants. In this study, the plant was exposed to artificial UV-B radiation in addition to solar radiation under field conditions, resulting in a significant increase in

phenol,.alkaloids, flavonoids, phytosterols, glycosides, etc. It showed an increase in secondary metabolites as well as bioactive substances such as, saponins and carbohydrates. Scientific research shows that the production of these secondary metabolites is greater in UV-B irradiation compared to control plants. Especially in this study, phenol and saponin production doubled compared to control plants. In addition to providing twice the benefits of untreated plants, these alkaloids and flavonoids also increase plant resistance and accelerate plant growth (Tables 1 & 2).

Table 1. Qualitative analysis of various phytochemical of ethanol extracts of Fresh Leaves of *Mimosa pudica* Control and UV-B treated plants

Phytochemical test	**Ethanol extract**	
	MPM-Control	**MPM- treated**
Alkaloids Mayer's Test	+	++
Wagner's Test	+	++
Hager's test	+	++
Dragendorff' s test	+	++
Flavonoids Alkaline reagent test	+	++
Fixed oil test Spot test	+	+
Carbohydrate Fehling's test	+	++
Benedict's test	+	++
Glycosides Borntrages's test	+	++
Saponins Foam test	+	++

Phytosterols . Libermann-Burhard's test	+	++
Phenols Ferric chloride test	+	++
Gelatin test	+	++
Lead acetate test	+	++

Note: + = present ++ = Present strongly

Table 2. Comparative analysis of Total Phenol, Alkaloid and Saponin contents of *Mimosa pudica* plants of control and UV-B treated plants.

Total phenol mg/g DW MPMC of GAE		**Flavonoids mg/g DW MPMT of QE**	
Control	**Treated**	**Control**	**Treated**
120.02 ± 1.05	208.95 ± 2.01	103.08 ± 0.070	172.68 ± 1.23
Alkaloid (%)		**Saponin (%)**	
Control	**Treated**	**Control**	**Treated**
9.1 ± 0.63	13.18 ± 0.92	8.06 ± 0.60	17.24 ± 1.20

GC-MS analysis of control and treated sample

GC-MS has been used to examine the effects of UV-B stress. GC-MS is the most effective method for phytochemical analysis. The method is a useful option for figuring out how much and what kind of phytochemicals are in the treated and control samples. The gas chromatogram's peak area provides a quantitative breakdown of the sample's constituents. Shimadzu GC-MS apparatus (Model: QP2010 Plus) was used for the study, and mass fragments were found in the scan range of m/z 20 to 700. The gas chromatogram of control and treated samples is displayed in Figure 6.

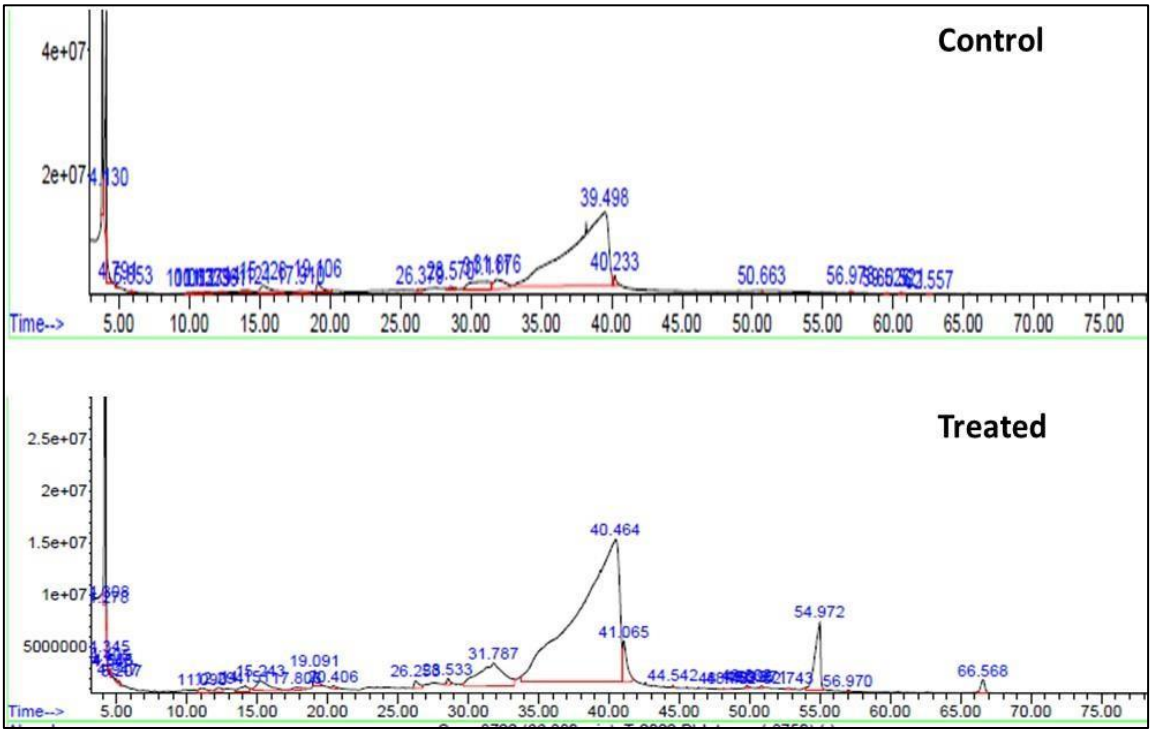

Fig.6. Gas chromatogram of control and treated sample

The same recording parameters were used for the GC-MS analysis of the treatment and control samples. Regarding retention time and mass spectrum, the gas chromatograms for the two samples were identical. The sample additionally displayed two brand-new peaks at 57.97 and 64.56 min. The gas chromatogram of both control and treated showed major peaks in the range of 4.18-4.23, 15.22-15.25, 19.00-19.20, 31.80-32.00, 39.50-40.50 and 40.50-41.07 min. The components relative abundances in the treatment and control groups are nearly the same. The component showed between 4.10 and 4.23 was discovered to be in 10%. The component at 31.78–31.88 minutes, however, increased 12.64 percent in the treated group as opposed to 3.96 percent in the control sample.

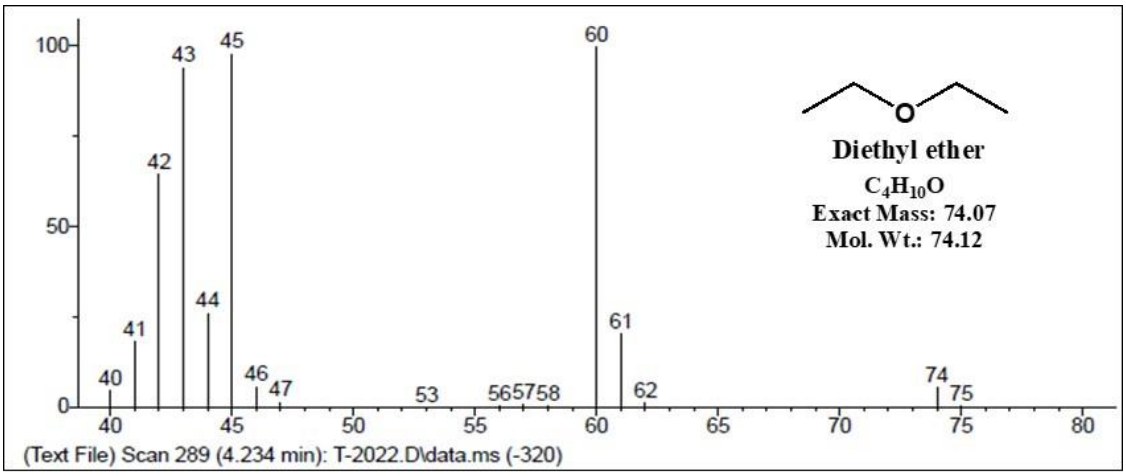

Fig.7. Mass spectrum and chemical structure of peak appeared at 4.18-4.23 min

Figure 7 displays the mass spectrum of the GC peak that occurred at 4.23 minutes in the treated sample. The spectra revealed a base peak at m/z 60 and a molecular ion peak at m/z 74, both of which point to the loss of the methylene group (>CH2). Another peak at m/z 45 showed the fragmentation of the ethyl group, which had a molecular weight of 29. The pattern of fragmentation shown in Figure 7 is comparable to the pattern of diethyl ether fragmentation. Diethyl ether, which has the chemical formula $C_4H_{10}O$ with a molecular weight of 74.07 g/mol, could be the substance.

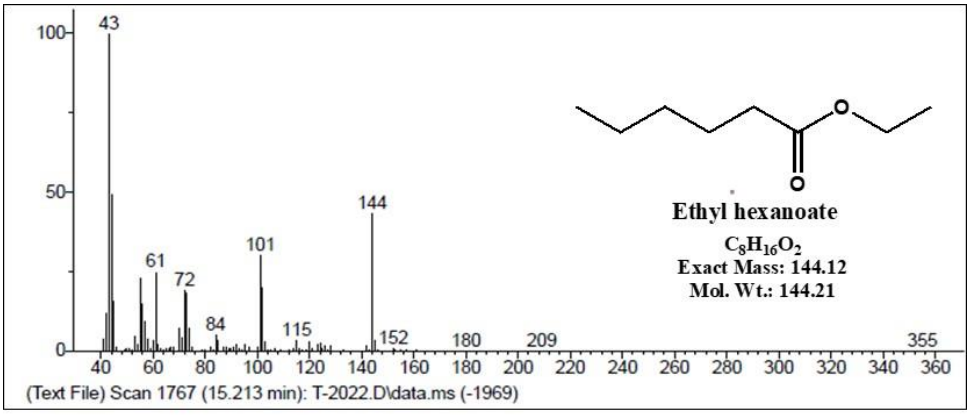

Fig.8. Mass spectrum and chemical structure of peak appeared at 15.22-15.25 min.

The mass spectrum of the first GC peak in the full sample is 15.21 min. As shown in Figure 8. The central peak at m/z 43 and the molecular ion peak at m/z 144 can be seen in the spectrum.

Removal of the ethoxy or propenyl group is indicated by the fragmentation signal at m/z 43. Additionally, the large peak at m/z 101 is formed by the separation of the ethoxy group from the molecular ion. The peaks at m/z 72 and 61 also indicate fragmentation of the molecule. Loss of the carbonyl group (molecular weight 44), while CO4 shows a peak at m/z 72. The molecular weight of the ethyl group is 29 and shows a peak at m/z 115. The fragmentation pattern shown in Figure 3 compared to ethyl hexanoate, which has a molecular weight of 144.12 g/mol and the chemical formula C8H16O2.

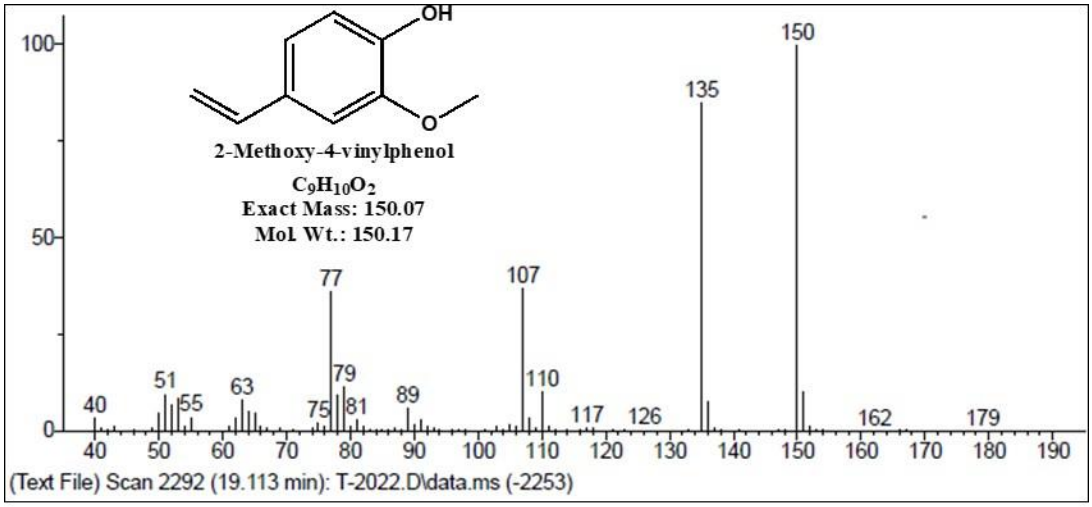

Fig.9. Mass spectrum and chemical structure of peak appeared at 19.00-19.20 min.

Mass spectra of the GC peak occurred at 19.11 min in treated and control samples. As shown in Figure 9. The spectrum shows a single molecular ion and a false peak at m/z 150 at 100% intensity. The molecule is very stable as shown by mass spectrometry. The major fragmentation signal at m/z 135 indicates removal of the methyl group. Importantly, methyl cleavage yielded another stable molecule at m/z 135. Moreover, the peak at m/z 107 resulted from the destruction of the weight 28 plastic group. The methyl and ethylene moieties of the compound have a vinyl group, a phenolic derivative. Figure 4 shows the cleavage pattern of 2-methoxy-4-vinylphenol, which has the chemical formula C9H10O2 and a molecular weight of 150.07 g/mol.

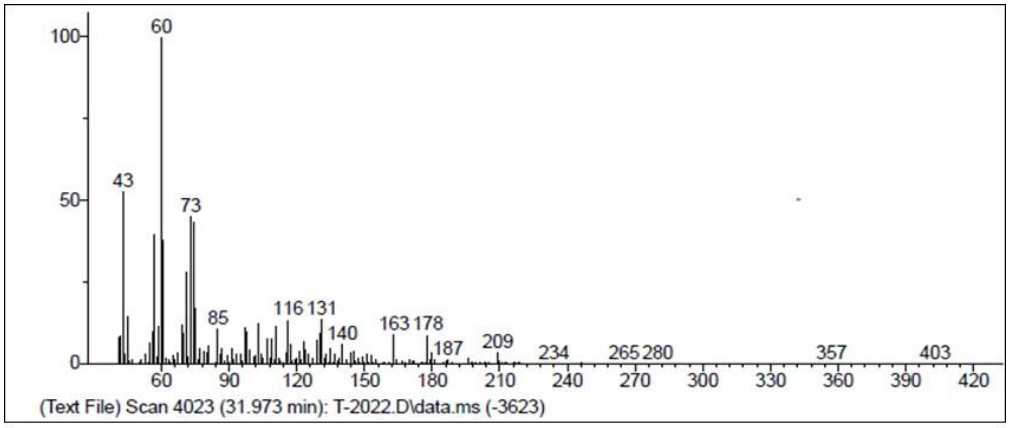

Fig.10. Mass spectrum and chemical structure of peak appeared at 31.80-32.00.

The corresponding 31.78 min GC peaks for treated and control samples are shown in Figure 10 mass. The mass spectrum shows that most of the methylene group in the alkyl chain is lost, as evidenced by the decrease in molecular weight at m/z 14. According to many studies, this product may be a fatty alcohol or a C14 fatty acid. -C16 alkyl chain. Methyl group losses.

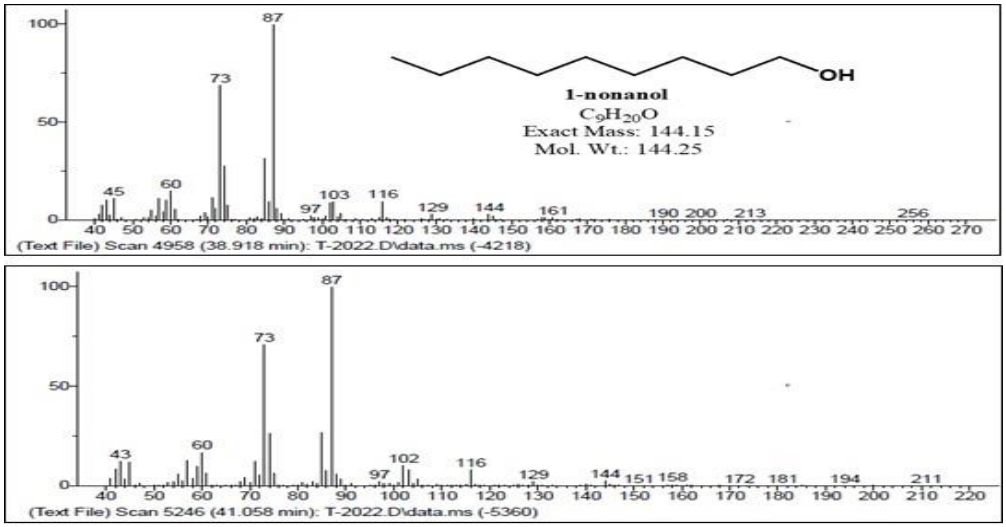

Fig.11. Mass spectrum and chemical structure of peak appeared at 39.50-40.50 and 40.50-41.07 min.

Figure 11 displays two further peaks with relative peak areas of 8.26 and 13.3 percent, occurring at 54.972 and 66.56 min. Furthermore, the area of the peak at 41.06 minutes has grown from the stated control, which was 0.85 percent. Compared to the control sample, 3.74 percent more of the treated sample is present. In the control sample, the peak was reached at 40.23 minutes.

The mass spectrum of the GC peak that emerged in the treated and control samples at 39.50-40.50 and 40.50-41.07 min is shown in Figure 12. The mass spectra of the GC peaks at 39.50-40.50 and 40.50-41.07 min are comparable. The mass spectrum of the peak that occurred at

41.065 minutes exhibited a base peak with 100% abundance at m/z 87. Following peaks at m/z 73 and 60 show removal of methylene group (-CH_2-) of alkyl chain with m/z 14 molecular weight. Furthermore, peaks at m/z 102, 116, and 129 emerge due to the loss of a methylene group containing nine carbon and oxygen atoms. The molecule might be 1- nonanol with the molecular formula $C_9H_{20}O$ and a molecular weight of 144.15 g/mole based on the fragmentation pattern.

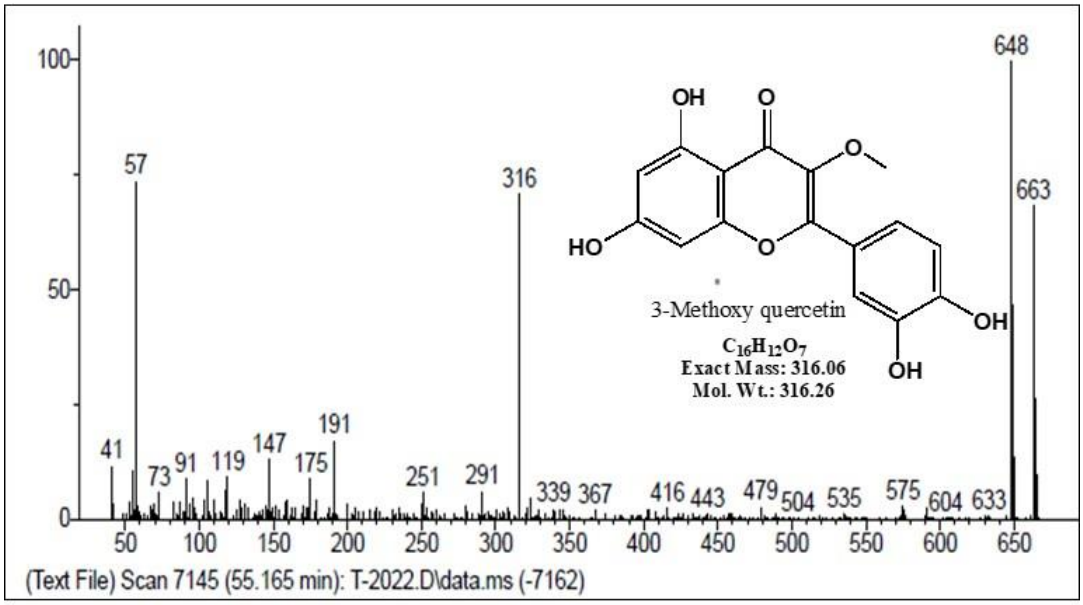

Fig. 12. Mass spectrum and chemical structure of peak appeared at 54.97 min.

At 54.97 and 66.56 minutes, two further peaks were visible on the treated sample's gas chromatogram. Figure 12 displays the mass spectrum of the GC peak, which occurred at 54.97 minutes and had distinct mass peaks at m/z 57, 316, 648, and 663. In this case, the molecule is stable and there is a notable abundance of the peak at m/z 648. The molecular formula and weight of 3-methoxy quercetin, whose mass and molecular weight are $C_{16}H_{12}O_7$ and 316 g/mole, respectively, are closely connected to the mass peak found at m/z 316.

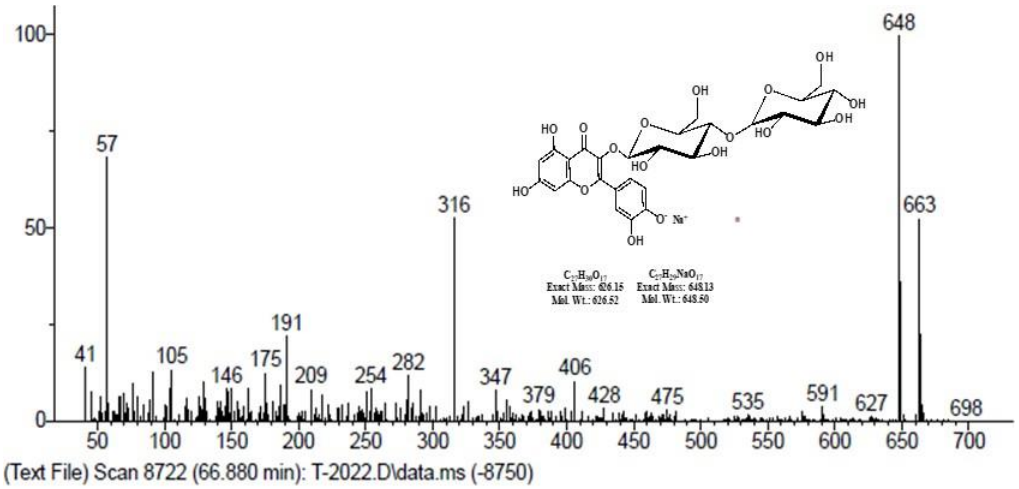

Fig.13. Mass spectrum and chemical structure of peak appeared at 54.97 min.

The GC peak of the treated sample at 66.56 min has the same spectrum as the peak at 54.97 min (Figure.13). Three peaks are observed in the mass spectrum at m/z 316, 648, and 663. The main peak observed at m/z 316 may be 3-methoxyquercetin, and the two glycosides are linked by the linkage between α-4,1. C3- of quercetin increases its molecular weight through glycosidic bonds. /z 648 followed by a peak at m/z 663 may arise from monosodium and methoxy derivatives of 3-methoxyquercetin. However further studies such as FT-IR, ^{1}H-NMR, and ^{13}C-NMR are necessary to establish structure.

Antioxidant activity:

Through a photoprotective mechanism, this bioactive compound that can be synthesized by exposure to UV-B radiation quenches the reactive oxygen species and nitrogen generated by this stress in the plant. Consequently, the antioxidant activity of the UV-B-treated plants was 10% more than that of the control plants.

DPPH and Super Oxide radical scavenging activity

The findings indicate that *Mimosa pudica* effectively produced DPPH and superoxide, enhancing their activity with the phytochemical substances induced by UVB light (Fig:14). The IC50 values of *M. pudica* extracts for scavenging DPPH radicals were 140.9917 g/ml for the UV-B treated extract and 149.5751 g/ml for the control extract (Table 3). For this activity, DPPH was utilized at doses of 31.25, 62.50, 125, 250, and 500 µg/mL of *M. pudica* leaf extract. Ascorbic acid, the authorized positive control, with an IC50 value of 143.86. With an IC50 value of 140.99 g/ml, the methanolic extract from the treated sample fraction showed the

maximum activity. For DPPH scavenging activity, the IC50 values of each extract varied significantly (P = 0.06489, one-way ANOVA). However, the UV-B treated plant's DPPH activity was 10% higher than the control plant and standard, which was a significant improvement.

Among the many oxygen species for free radicals, superoxide anion is one of the most powerful reactive radicals. The plant that was exposed to UV-B light was more active than the control plant. The UV-B IC50 value was 145.12, and 149.05 for the control plant. (Table 3). We found that the UV-B-induced plant, *M. pudica*, exhibited slightly higher activity when using a 950 µg/mL concentration of methanolic leaf extract compared to the control and the positive control (ascorbic acid). This increase, although modest, indicates a potential enhancement in the plant's bioactivity due to UV-B exposure.

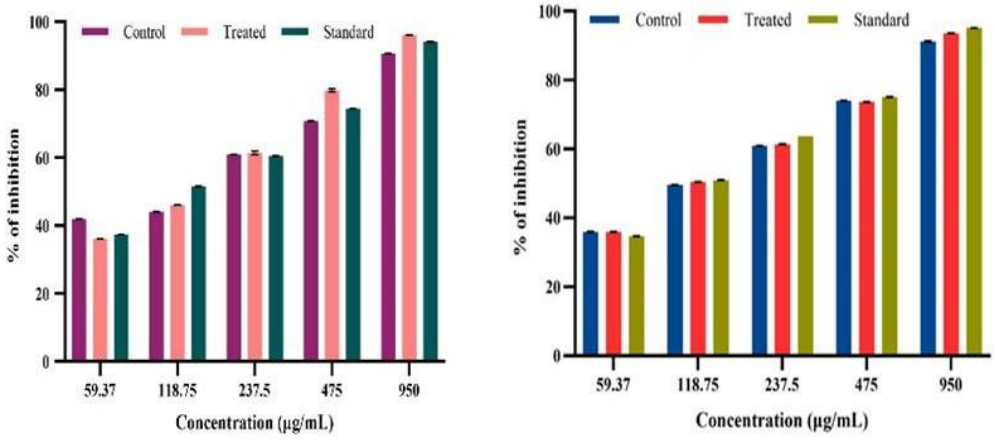

Fig.14. DPPH and Superoxide free radical scavenging activity in the methanolic extracts of the leaves of *M.pudica* plants cultivated under control and UVB treated conditions.

Table 3. Antioxidant activity of the methanolic extracts of the leaves of *Mimosa pudica* plants developed under ambient and enhanced UV-B radiation.

Extract	DPPH Scavenging Activity (IC_{50} (µg/ml)	Super oxide activity (IC50 (µg/ml)
Control	**149.5751**	**149.05**
UV-B treated plant	**140.9917**	**145.12**

Antidiabetic activity

Diabetes is a significant metabolic disorder caused by oxidative stress, and many medicinal plants, notably *Mimosa pudica*, have been traditionally used for its treatment. The antioxidant activity of the plant increases as two crucial compounds, flavonoid and phenol, become more

Exposed to UV-B rays. These compounds act as more potent α-amylase and α-glucosidase inhibitors, potentially helping to lower postprandial glucose levels—a crucial result for addressing this disorder (Table 4).

In this study, the IC50 value for alpha-amylase activity in control plants was found to be 97 ± 0.169 mg/mL, whereas UVB-treated plants exhibited a lower IC50 of 90 ± 0.74 mg/ml (Figs:15). This reduction in IC50 value indicates that the alpha-amylase activity in UVB-treated plants is higher compared to the control plants, as the lower IC50 value suggests that a smaller amount of the extract is needed to achieve the same inhibitory effect. Thus, the alpha-amylase activity in UVB-treated plants is approximately 10% higher than in control plants.

Regarding alpha-glucosidase activity, the UVB-treated plants had an IC50 of 100 ± 0.07 µg/mL, while the control plants exhibited an IC50 of 108 ± 0.55 µg/mL. Acarbose, a standard inhibitor used for comparison, had an IC50 of 92 ± 0.11 µg/mL (Figs.15). These results indicate that UVB-treated plants are more effective at inhibiting alpha-glucosidase activity than control plants, although they are slightly less effective than acarbose. Overall, the leaf extract of UVB-treated plants shows a greater tendency for inhibiting both alpha-amylase and alpha-glucosidase activities compared to the control plants. This suggests that UVB treatment enhances the inhibitory properties of the plant extracts, which could be beneficial for managing conditions such as diabetes by regulating carbohydrate metabolism. The marginal difference observed between the standard inhibitor (acarbose) and the UVB-treated plant sample highlights the potential efficacy of the plant extract as a natural alternative for enzyme inhibition.

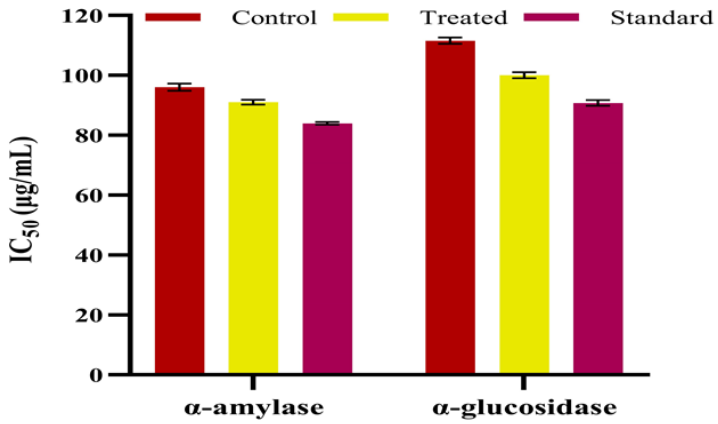

Fig: 15. α -Amylase activity and Alpha glucosidase of *M. pudica* plant leaves extracted with methanol grown under natural and enhanced UV-B radiation.

Table 4. Antidiabetic activity of the methanolic extracts leaves of *Mimosa pudica* plants developed under ambient and enhanced UV-B radiation.

Extract	Alpha glucosidase Activity (IC_{50} (µg/ml)	α-amylase inhibitory activity IC_{50} (µg/ml)
Control	**108 ± .55**	**97 ± .169**
UV-B treated plant	**103 ± .07**	**90 ±.74**

Discussion:

Low-latitude therapeutic plants are inherently resistant to a wide range of environmental factors. These therapeutic plants have been used for medical purposes for a very long time, and in their native habitat, they are rich producers of secondary metabolites. These medicinal plants showed interesting results when exposed to UV-B sunlight in a field setting. Under the impact of UV-B rays, they showed faster development and dramatically enhanced production of carotenoids and chlorophyll, reaching values 20–50% greater than those found in control plants. According to Agrawal *et al.*, [19], plants' levels of carotenoids and chlorophyll are influenced by their genetic composition, stage of growth, and length of exposure to ultraviolet-B light.

Research by Liu *et al.*, [20] indicated that UV-B light enhances chlorophyll production throughout the growth phase of *P. persicau.* When compared to plants that were not treated with UV-B, treated plants displayed higher levels of gene and miRNA expression. UV-B radiation significantly increased the concentration of carotenoids, the main pigments that defend against UV rays, in the leaves of *Mimosa pudica.* Carotenoids, as light-harvesting agents, shield chlorophyll molecules from photo-oxidative degradation when exposed to high excitation energy UV-B radiation [21, 22]. According to Tossi *et al.*, [23], these carotenoids instantly protect photosystems from UV exposure

The formation of non-photosynthetic pigments upon exposure to UV-B rays is closely linked to chlorophyll synthesis, as these pigments filter UV-B light. By increasing production, the chloroplast apparatus is protected, allowing thylakoids to generate a significant amount of chloroplasts without suffering harm. When exposed to UV-B light, plants produced twice as much anthocyanin and flavonoid as control plants. While anthocyanin accumulation has also been shown to be induced by UV-A, UV-B has been utilized to quantify the rise in anthocyanin levels [24].

Flavonoids protect inner epidermal cells from harmful radiation, forming a defense system common to all plants [25, 26]. These plant phenols absorb the majority of light between 280 and

340 nm, effectively blocking UV-B radiation and protecting plants' photosynthetic organs [27, 28]. It has been discovered that grape leaves (*Vitis vinifera*) and the leaves of silver birch (Betula pendula Roth) produce more flavonols when exposed to UV-B irradiation [29]. Furthermore, when stressed, plants can utilize flavonoids as antioxidants [30].

Protein deterioration is a result of oxidative stress. Numerous studies have found that when cells are exposed to oxidatively activating substances, their proteolysis rates increase [31]. This investigation showed that protein concentrations rose after UV-B treatments. This increase may have its roots in the production of defensive proteins and enzymes, which are probably generated during stressful situations [32]. Research indicates that oxidative stress increases the expression of defense genes that code for HSP proteins and antioxidant enzymes such as GR, PXs, CAT, and SOD in the Arabidopsis plant [33]. HSPs play a major role in the detoxification of broken-down proteins.

Moreover, it has been demonstrated that in wild types of Petunia, exposure to UV-B increases the expression of three important enzymes in the phenylpropanoid pathway: PAL, CHS, and CHI [34]. Proteolysis has revealed the intricacy of plant cellular regulation by aiding in the elimination of aberrant, misfolded proteins and the maturation, destruction, and repair of proteins in response to diverse exogenous stimuli [35]. According to Hieng *et al.,* [36], proteases support protein turnover in response to abiotic challenges, which is essential for the plant's reaction to environmental stress.

In order to lessen the effects of UV-B radiation on plants, it is advised to promote the synthesis of secondary metabolites like flavonoids, phenols, alkaloids, and phytosterols. In response to UV-B exposure, various enzymes within the phenylpropanoid pathway, such as Phenyl ammonium lyase and chalcone synthase (CHS), are activated. These compounds tend to accumulate within leaf vacuoles, serving to shield the photosynthetic tissue from UV-B-induced injury. For example, compared to untreated plants, *Mimosa pudica* plants exposed to UV-B radiation produced significantly more phytochemical components. Particularly, as comparison to the control plants, the production of phenols and saponins doubled, while that of alkaloids and flavonoids increased by more than twice. Studies conducted recently, such as those by Chen *et al.,* [37] and Kumari *et al.,* [38], indicate that UV-B radiation has a beneficial effect on the secondary metabolism of medicinal plants. Further research, such as those conducted by Sun *et al.,* [39]), Ning *et al.,* [40], and Chen *et al.,* [41], provides more proof that exposure to UV-B radiation

accelerates the accumulation of certain chemicals in medicinal plants. Results from earlier studies show that polyamines in cucumber (*Cucumis sativus*) and flavonoids in barley (*Hordeum vulg*are) are increased by UV-B radiation [42]. Similar to this, it has been discovered that grape and silver birch (*Betula pendula*) leaves (*Vitis vinifera*) are more likely to generate flavanols when exposed to UV-B irradiation [43].

Although the bioactive molecules produced in UV-B-treated and control plants were almost identical, we found that some compounds were produced by UV-B radiation. The treated plant's liquid chromatogram revealed two additional peaks at 54.97 and 66.56. The mass spectrum of the LC peak at 54.97 min showed a clear and distinctive peak at m/2 57,316,648,663. In this case, the peak at M/2 648 was found in high abundance and the compound is stable. The mass peak observed at M/Z 316 is closely related to the molecular weight of geranyl pyrophosphate, with a molecular formula and weight of 311 g/Mol respectively. The mass spectra of the treated sample's GC peak at 66.56 minutes resembled that of the GC peak at 54.97 minutes. Rhamnetin could be the mass peak seen at m/2 316. Rhamnetin dimer with a single methoxyl group emerged at m/z 648, and dimethoxy derivatives could be the cause of the peak at m/z 663. Future research will examine the structure and activities of the unidentified molecule caused by UV-B.

According to studies by Schreine *et al.,* [44] and Yadav *et al.,* [45], adding UV-B supplements may promote the production of secondary metabolites and provide defense against UV-B stress. Previous studies, such as those conducted by Lee *et al.,* [46], Chen *et al.* ,(47), and Son *et al.,* [48] , have indicated that exposure to ultraviolet A (UV-A) can augment the concentrations of advantageous phytochemicals in lettuce. Research on the Lamiaceae family, including works by Dolzhenko *et al.,* [49] Dou et al [50], and Nascimento *et al.,* [51], shows that exposure to UV-B radiation increases the concentrations of particular phytochemicals.

While UV-A and UV-B both contribute to the build-up of bioactive chemicals, research indicates that at comparable light intensity, UV-B has a more noticeable effect than UV-A. Additionally, it has been noted that phenols react quickly to UV radiation after harvest in a variety of horticultural products, such as Vaccinium and Brassica species [52]. Pluskota *et al.,* [53] discovered that exposure to UV light increases the activity of phenylalanine ammonia-lyase, which speeds up the synthesis of phenolic compounds. Terpenoids represent an additional class of secondary metabolites that contribute to the reduction of UVB-induced heat stress in plant leaves [54]. Research has shown that species that are important for commerce and medicine, such as

Artemisia annua, *Vitis vinifera*, *Cuminum cyminum*, and *Curcuma caesia*, exhibit their induction in response to UV-B radiation. [55,56,57,58]. According to Kumari *et al.*, [59], UV-B radiation modifies the makeup of active components and promotes volatile production. According to Kumari and Prasad [60], UV-B treatment had an impact on antioxidants, phenolics, and essential oils in *Coleus aromaticus*. Chang *et al.*, [61] found that *Ocimum basilicum* had an increase in volatile oils, such as linalool and eugenol. Furthermore, Afreen *et al.*, [62] discovered that whereas high-intensity UV-B decreased the concentration of glycyrrhizic acid, a triterpenoid saponin, suitable UV-B treatment increased the amount of it in *Glycyrrhiza uralensis*.

UV-B stress causes ROS levels in plants to rise, leading to cell damage and death. However, plants can develop secondary metabolites that can scavenge these ROS and benefit the plant. The *Mimosa pudica* plant responded favorably to UV-B radiation. Stress induced sufficient secondary metabolite synthesis and increased antioxidant activity, especially for the two antioxidants DPPH and superoxide. The IC50 values for the two antioxidant activities mentioned less those of control. The regulation of secondary metabolite biosynthesis heavily relies on enzymes like phenylalanine ammonia lyase (PAL), which show vital roles in plant replies to abiotic stress [63]. Plants produce these secondary metabolites either directly or indirectly to protect them from UV-B rays. Elevated UV-B irradiance causes an increase in PAL activity[64]. Antioxidants such glutathione reductase (GR), ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT) have different metabolisms when exposed to UV-B light [65]. Additionally, in the epidermal cell vacuoles of plants, phenol metabolites act as UV-B absorbers and barriers against UV-B penetration [66.67.68]. The heightened synthesis of these substances may contribute to increased antioxidant activity, as betalains and flavonoids exhibit significant antioxidant properties [69]. Apple fruits with elevated UV-B and flavonoid levels demonstrate enhanced antioxidant capabilities [70.71]. However, aside from flavonoid and betalain concentrations, the augmentation of antioxidant activity is linked to UV-B radiation triggering enzymes like peroxidases, which possess free radical scavenging abilities [72].

The primary cause of diabetes is the creation of too many free radicals, affecting people of all ages globally. The main factor contributing to this major metabolic condition is the pancreas inability to operate and create insulin. Consequently, the activity of two crucial digestive enzymes, alpha-amylase and alpha-glycosidase,is reduced. Phytochemical substances found in medicinal

plants play a critical part in removing these free radicals. phenols and flavonoids, particularly, play the most significant role in antioxidant activity to counteract oxidative stress.

According to this experiment, when exposed to UV-B radiation, the plant showed substantially stronger antidiabetic action, resulting in the production of phytochemical compounds at a rate twice as high as that of control plants. While the alpha-amylase and alpha-glucosidase activity in the control plant was initially higher, the UV-B-induced compounds showed greater activity as the concentration of phytochemical compounds grew. When compared with the standard sample, the value was higher in the UV-B-treated sample.

Previous studies have shown that plant phytoconstituents with strong antioxidant potential, such as alkaloids, glycosides, flavonoids, and terpenoids, are frequently associated with anti-diabetic effects [73, 74]. Research on *M. pudica* leaves in animals has shown that the ethanolic extract lowered blood sugar levels in Wistar rats who were diabetic due to alloxan [75]. Furthermore, in streptozotocin-induced diabetic Wistar rats, the ethanolic extract of the whole plant lowered blood sugar levels [76,77]. According to Subramani Parasuraman *et al.,* [78], in STZ-induced diabetes mellitus, the methanolic extract of *M. pudica* shows strong anti-diabetic and anti-hyperlipidemic properties. This efficacy may be attributed to the presence of flavonoids or alkaloids within the extract.

Conclusion:

Nature provides medicinal plants for treating various diseases, and there is growing awareness of their safety and potential side effects. A study found that stressed plants overproduce secondary metabolites to block UV-B radiation, alleviate oxidative stress, and promote plant development. UV-B-induced bioactive substances in plants not only promote growth and chlorophyll pigment synthesis but also demonstrate superior antioxidant and anti-diabetic activities compared to predictions. This research has significant socioeconomic implications, as the bioactive compounds induced by UV-B are of higher quality and nutritionally more valuable than those found in control plants, thereby leading to a higher market value. Consequently, UV-B treatment of plants could enhance their medicinal properties and economic worth, benefiting both health and the economy.

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Competing Interest:

The authors declare that they have no competing interests

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