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Drought Stress Modulates Antioxidant Defense, Phytochemical Composition, and Anti-Inflammatory Activity in Physalis minima Linn.

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ABSTRACT: Drought is one of the abiotic stress factors that may affect plant metabolism, antioxidant defense mechanisms and biosynthesis of biologically active substance. "In the present study, the effects of drought stress on the phytochemical characteristics, activity of antioxidant enzymes and anti-inflammatory properties of Physalis minima was investigated. Methanolic extract was prepared from both control and drought stressed plants subjected to the qualitative phytochemical screening, catalase (CAT) and superoxide dismutase (SOD) activity assays, in vitro anti-inflammatory test with membrane stabilization method and protein denaturation method. The phytochemical qualitative test showed the presence of tannins, flavonoids, proteins and phenolic compound in both control and drought treated plants. On the other hand, there was no qualitative difference observed for phytochemical constituents of drought treated and non-drought treated since no presence of saponins, alkaloids, steroids, terpenoids, quinones and cardiac glycosides was observed in both. Drought exposure led to higher catalase activity owing to lower half maximal effective concentration (EC_{50}) value (46.4 µg protein vs. 49.1 µg protein for control). A better ability to neutralize hydrogen peroxide exists in drought-exposed plants. Drought stress did show a slight decrease in SOD activity with the drought stress extract background to have a higher IC50 value of 56.3 µg/mL than the control extract which was 53.6 µg/mL. The membrane stabilization test revealed a little more anti-inflammatory activity in control plants compared with drought treated P. minima and albumin denaturation revealed more anti-inflammatory activity in drought stress P. minima at higher concentration. In general, drought stress triggered significant biochemical reactions in P. minima, which amounted to a rise in catalase activity and protein solubilization and not significant changes in the qualitative compound type of phytochemicals. Overall, the results indicate that P. minima possess a potent antioxidant and anti-inflammatory defense system that is activated during water deficiency and that the plant may be used for medical purposes in the future to help cope with abiotic stresses.

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1. INTRODUCTION

Drought stress is known to be one of the most important environmental stresses that limit plant growth, development, productivity and normal metabolic functions. The heat, drought or decline in annual rainfall due to climatic changes have caused a high incidence of water scarcity that has caused considerable difficulties during the production and cultivation of crops [Farooq et al.,2009]. Deficiency of water can disrupt various physiological processes of the plant such as photosynthesis, uptake of nutrients, metabolism, and plant growth. Water deficiency can induce excess production of reactive oxygen species (ROS) within the cells, like superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH) and singlet oxygen ($^1O_2$) [Sharma et al.,2012]. ROS, when present at adequate levels, can act as important signaling molecules, but upon excess accumulation, they result in oxidative damage of lipid, proteins, nucleic acids and membranes which leads to the disturbance of normal cellular homeostasis [Hasanuzzaman et al.,2020; Bashir et al.,2021].

Plants have highly developed defense mechanisms to protect them from the oxidative stress caused by drought, as both enzymatic and non-enzymatic antioxidant systems show a response. The key antioxidant enzymes which play this defense are such as: Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase, Glutathione reductase, and a number of peroxidases. The main function of SOD is the conversion of superoxide radicals to hydrogen peroxide (H_2O_2) and CAT further helps to eliminate hydrogen peroxide and detoxify (remove) it. [Gill & Tuteja, 2010] Coordinated action of these antioxidants systems is essential for a redox homeostasis within cell that safeguards the cellular machinery and structures from the damage of reduced water availability [Bashir et al.,2021]. Hence, varying or adjustment in antioxidant enzyme activities may be seen as a significant biochemical precondition that plants suggestively adapt to the drought induced stress.

Apart from the enzymatic defense system, the synthesis and accumulation of secondary metabolites, such as terpenoids, alkaloids, glycosides, phenolic compounds, flavonoids and tannin [Kumar et al.,2021, Yeshi et al.,2022] may be affected by drought. The secondary metabolites play several roles in plant stress response such as ROS scavenging, chelation of metals, stabilization of membranes and protection of important biomolecules. Some metabolic pathways (phe-nylpropanoid and flavonoid pathway) are activated on experiencing drought stress, leading to accumulation of bioactive compounds that may, therefore, play a role in better resistance to oxidative stresses [Yadav et al.,2021; Jogawat et al.,2021]. In addition, high flavonoids levels have been linked to higher drought tolerance for both their role in the antioxidant efficiency as well as their involvement in regulating plant stress-response pathways [Li et al.,2022].

The genus *Physalis* is a part of the Solanaceae family and is composed of several species which have significant medical and economic value. The species are rich in a variety of bioactive compounds such as flavonoids, phenolics, physalienes, withanolids, carotenoids and other secondary metabolites. Many of these compounds have important pharmaceutical functions such as anti-antioxidants, anti-inflammatory and antitumor properties [Lem et al., 2022; Liang et al.,2024]. In this group, *Physalis minima* species is known to have several pharmacological properties such as antioxidant, anti-inflammatory, and is a medicinal plant possessing withanolides with various bioactive properties.

The antioxidant activity of the extract of *P. minima* has also been found from previous research. Fatima et al. [2017] found that both the leaves and fruits of *P. minima* have significant antioxidant activity, indicating that the plant could be a valuable source of natural antioxidants. Finding several biologically active phytochemicals with medicinal applications in *P. minima* further validates the therapeutic properties and therapeutic potential of this species.

Although *Physalis* genus have medicinal value, but little information is available about the biochemical responses of *P. minima* under drought stress. Stress from water deficit may result in changes to the activities of antioxidant enzymes, accumulation of secondary metabolites, and properties of plant extract. Therefore, these responses need to be monitored to gain insight into biochemical mechanisms of drought adaptation and to assess if the drought exposure alters the plant's phytochemical profile and/or biological activity [Laxa et al.,2019; Alhaithloul et al.,2020].

Hence, the current study was conducted to investigate the biochemical changes in *Physalis minima* plants due to the drought stress, qualitative phytochemical screening, measuring catalase (CAT) and superoxide dismutase (SOD) enzyme and anti-inflammatory activity by albumin denaturation inhibition and human red blood cell (HRBC) membrane stabilization in vitro assay. Phytochemical composition, antioxidant defense mechanisms and anti-inflammatory properties of control plants and

drought stressed plants were compared to gain a better understanding of the biochemical responses exhibited by *P. minima* in water-deficit conditions.

2. MATERIALS AND METHODS

2.1 PLANT GROWTH AND EXPERIMENTAL TREATMENT

Physalis minima L. seeds were procured, identified as certified seeds and sown in the experimental field of Pachaiyappa's college, Chennai. Seed was soaked for 12 hours in running water, prior to sowing to ensure uniform and consistent germination. Normally plants were watered regularly during the experimental period, and proper measures were taken to lessen the chances of microbial infection and insect attack. Drought treatment was started during the 1st leaf stage. On the 5th day after seeding these seedlings were randomized between the various experimental groups as follows:

Control: Plant material in these rows had same treatments with respect to irrigation frequency throughout the whole experimental period.

Water Stress: Plants belonging to this group were watered every alternate day and created a drought stress.

2.2 PREPARATION OF PLANT POWDER AND EXTRACTION

Leaves of the fresh *Physalis minima* plants of both normally watered plants and droughted plants were cut off. The debris from the soil and other unwanted contaminants were removed from the leaves by being carefully washed with tap water and further washed with distilled water. All the samples were washed and gently soaked to remove any surface moisture, after which the washed samples were shade dried until getting a constant weight. This plant material was dried completely, then milled into powder by mill and sieves for the appropriate particle size were applied. The samples in the form of powders were processed, packed in appropriate containers and labeled before storing them for later for extraction procedures.

For extracting the material, 10 g of powdered plant material was extracted using 100 mL of 70% mehanol in the ratio of 1:10 (w/v). The extraction was carried out at room temperature for 24–48 h with agitation with rotary shaker. After the extraction was done, the mixture was filtered through whatman filter paper no.1. This filtrate was concentrated by using a rotary evaporator at temperature below 40°C. After concentrating, the weight of the crude extract obtained was recorded and the extraction yields was calculated.

A yield was defined as the quantity of substance extracted and was obtained from the following expression:

Extraction yield (%) = (Weight of dried extract / Weight of powdered plant material) × 100.

2.3 QUALITATIVE PHYTOCHEMICAL SCREENING

Qualitative phytochemical screening was conducted on all the prepared plant extracts for determining the types of secondary metabolites that were present in the greatest quantity. Conventional colour-producing and precipitation tests were done as described below to identify the various phytochemical tests.

2.3.1 Saponins

About 1mL of the plant extract was put inside an appropriate container of distilled water. The solution was then shaken up thoroughly. Formation of a stable and persistent froth or foam was taken as a positive indication of the presence of saponins.

2.3.2 Flavonoids

The stock plant extract contained about 1 mL which was treated with some drops of 2% Sodium hydroxide (NaOH). Presence of a yellow colour after the addition of the reagent was seen as evidence indicative of presence of flavonoids.

2.3.3 Alkaloids

A few drops of Dragendorff's reagent were added to the extract that was about 1 mL in volume. An orange-red colour formation and/or the appearance of the precipitates was accepted as positive and signs the presence of the alkaloids.

2.3.4 Proteins

A few drops of the "Bradford reagent" were added to about 1 mL of the plant extract. The addition of the reagent produced a blue colour as judged after which it was inferred that the extract contained proteins.

2.3.5 Steroids

About 1 mL of the plant extract was mixed with concentrated sulfuric acid (H_2SO_4; 10% as used for the assay). The reaction was considered positive when a green-coloured product appeared after the reaction.

2.3.6 Quinones

About 1 mL of the extract was put in the presence of aqueous ammonia and shaken well to facilitate the reaction. The appearance of pink, red or violet colour in aqueous layer was presumed as evidence of the presence of quinones.

2.3.7 Terpenoids

About 1 mL of the extract of the plant was mixed with chloroform to which concentrated H_2SO_4 was slowly added. A reddish-brown ring was seen at the interface of the layers indicating positive results of terpenoids presence. The presence of terpenoids was considered as positive for the appearance of reddish-brown ring at the interface between the layers.

2.3.8 Cardiac Glycosides

About 1 mL of the above-mentioned plant extract and 0.4 mL of glacial acetic acid were mixed with a few drops of ferric chloride solution. A drop of concentrated H_2SO_4 (sulfuric acid) was then gently placed down the inside wall of the test tube. Presence of a violet-coloured ring under a brown ring was considered a good indicator of the presence of cardiac glycosides.

2.3.9 Phenols

Some drops of the ferric chloride ($FeCl_3$) solution, (0.1%) were added to the approximation 1 mL of the extract. The development of blue colour served as a positive reaction which meant that there must be phenolic compounds present.

All the qualitative phytochemical tests were carried out and the results reported as either positive (+) or negative (−). Classification was based on the specific color change which occurred or precipitate formed or the froth formed in the specific phytochemical group.

2.3.10 Catalase (CAT) Activity Assay

A screening method for catalase activity was performed according to the decomposition of hydrogen peroxide (H_2O_2), by measuring the dichromate–acetic acid activity as described by [Sinha, 1972]. A solution of potassium dichromate was made up to 5% (w/v) in distilled water. Then, 150 mL of glacial acetic acid was gradually added to 50 mL of the dichromate solution to create the dichromate/acetic acid solution. In the assay phosphate buffer (0.01 M, pH 7.8) was used along with H_2O_2. H_2O_2 stock solution was prepared in the following way: 34 µL of H_2O_2 were diluted in 100 mL of distilled water for the preparation of 10 mM H_2O_2 stock solution. The stock solution of H_2O_2 was prepared according to the following steps; 34 µL of H_2O_2 were taken and diluted in 100 mL of distilled water to obtain 10 mM H_2O_2 stock solution.

The volume of the assay reaction mixture was 2.5ml of phosphate a buffer, 2.0 ml of H_2O_2 and 0.1ml of the plant sample extract. 1.0 mL of the reaction mixture was then pipetted into 2.0 mL of the dichromate–acetic acid reagent and the mixture was then again incubated. The tubes were then submerged in a boiling water bath, and heated for 10 minutes with the tubes remaining hot until cool. The absorbance was then measured at 570 nm in a suitable reagent blank. Catalase activity was expressed in units' min^{-1} mg^{-1} protein.

2.3.11 Superoxide Dismutase (SOD) Activity Assay

The superoxide dismutase (SOD) activity was estimated based on the degree of plant extract which inhibits the photochemical reduction of nitro blue tetrazolium (NBT) according to Alici and Arabaci [2016]. To the corresponding tubes assay solutions were added with different concentrations of the plant extract and blank was used as the reaction mixture without the plant extract. All reaction mixtures were made up to 3 mL. Ascorbic acid was used as reference compound.

The prepared reaction mixture was then subjected to 'white' light for 15 minutes at ambient temperature. The reaction mixture was then allowed to interact with 'white' light for 15 minutes at room temperature. After the incubation period, the absorbance at 560 nm was measured. The activity of SOD was expressed as % inhibition of the NBT photoreduction, and calculated using the following formula:

SOD inhibition (%) = [($A_{(}$blank$_{)}$ − $A_{(}$sample$_{)}$) / $A_{(}$blank$_{)}$] × 100

$A_{(}$blank$_{)}$ is absorbance of the blank reaction mixture while $A_{(}$sample$_{)}$ is absorbance of the reaction mixture containing plant extract.

2.4 *IN VITRO* ANTI-INFLAMMATORY ACTIVITY

2.4.1 Membrane Stabilization Assay

The plant extract's membrane stabilizing effect was measured by adopting the technique of Dragan et al. [2016] which involves human red blood cell (HRBC) membrane stabilization. An equal volume of Alsever's solution (which consists of 2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) was mixed with fresh blood in the ratio of 1mL of fresh blood 1mL of Alsever's solution. The mixture obtained was centrifuged for 5 minutes at 3000 RPM and erythrocytes packed were taken up and stored for further analysis.

Collected erythrocytes were washed two times with 0.85 % isosaline followed by centrifugation at 3000 rpm for 5 min. The washed erythrocytes were then resuspended in isosaline to get 10% HRBC suspension. The erythrocytes were suspended in 9 mL isosaline and samples were withdrawn for observation of the suspension.

The plant extract was taken from different concentrations in different test tubes and made upto 1mL with distilled water. The remaining contents of each tube were then filled with 1 mL of phosphate buffer, 2 mL of hypotonic saline (which contained 0.36% NaCl) and 0.5 mL of the HRBC suspension prepared. The reaction mixtures were then incubated at 37°C for 30 min and then centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant obtained by centrifugation was measured at 560 nm. Diclofenac sodium (1mg/mL) was used as the standard and all assay components were heated in its absence. Diclofenac sodium (1mg/mL) was the reference standard and all the assay components were heated in absence of plant extract.

membrane protection (or stabilization) percentage was calculated as follows:

Protection (%) = 100 − [($A_{(}$sample$_{)}$ / $A_{(}$control$_{)}$) × 100]

where $A_{(}$sample$_{)}$ and $A_{(}$control$_{)}$ represent the absorbance values of the sample and control, respectively.

2.4.2 Inhibition of Albumin Denaturation

The anti-inflammatory activity of this plant extract was also evaluated in terms of albumin denaturation inhibition assay. Different concentrations of the extract were made with phosphate buffered saline (PBS; pH 6.4) each final volume 1 mL. After this, 5% (w/v) aqueous B.S.A. was added to each of the reaction tubes. The resultant-mixtures had their pH adjusted to 6.3 by adding 0.1 N HCl and they were well mixed.

The reaction mixtures that were prepared were incubated at 37°C for 20 min followed by heating at 57°C for 3 min. These tubes were then immediately removed and cooled to room temperature for 10 min and centrifuged at 3000 rpm for 20 min. A clear supernatant (after centrifugation) was obtained and its absorbance was measured at 660 nm. Diclofenac sodium (1mg/mL) was added to the standards and the control reaction was made without adding the plant extract for the test.

The percentage inhibition of albumin denaturation was calculated using the equation:

Inhibition (%) = [($A_{(}$control$_{)}$ − $A_{(}$sample$_{)}$) / $A_{(}$control$_{)}$] × 100

where $A_{(}$control$_{)}$ is the absorbance of the control and $A_{(}$sample$_{)}$ is the absorbance of the sample.

3. RESULTS

Physalis minima response to drought stress was explored by analyzing its phytochemical compounds, antioxidant enzyme for drought responses and in vitro anti-inflammatory activity. These biochemical and physiological changes in wounding under drought stress conditions were evaluated by making comparison to the growth in control plants.

Table 1. Qualitative Phytochemical Screening of Control and Drought-Treated

Physalis minima.

S.No	CONTENTS	Test	Control
1.	Tannins (T)	+	+
2.	Saponin (S)	-	-
3.	Flavonoids (F)	+	+
4.	Alkaloids (A)	-	-
5.	Proteins (P)	+	+
6.	Steroid (St)	-	-
7.	Terpenoid (Ter)	-	-
8.	Quinones (Q)	-	-
9.	Cardiac glycosides (CG)	-	-
10.	Phenol (Ph)	+	+

Notes: (+) Positive and (-) Negative

The qualitative phytochemical screening of methanolic extract from control and drought-stressed plant of *P. minima* revealed that both plant extracts contain similar phytochemicals as indicated in Table 1. Both treatments showed the presence of such bioactive ingredients as tannin, flavonoids, proteins and Phenolic which is confirmed under normal and drought condition. Saponins, alkaloids, steroids, terpenoids, quinones and cardiac glycosides, however, were not found in both the samples.

There were no visible qualitative differences in phytochemicals of the two treatments in comparison with the control. Drought stress also resulted in no changes in the classes of phytochemicals added or subtracted from the plants. The overall results suggest that regarding phytochemical constitution, the *P. minima* is qualitatively preserved under the drought stress, but the shifts in metabolite contents should be analyzed further adopting quantitative analytical techniques.

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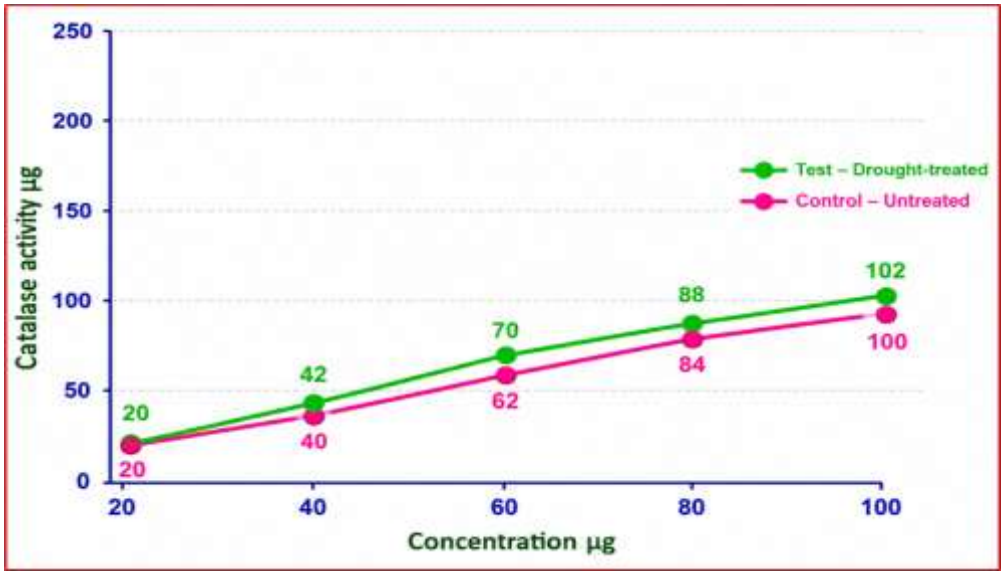

Fig.1. Catalase Activity in Control and Drought-Treated *Physalis minima*.

The enzyme activity (Fig.1) was used for calculation of the inhibition concentration at which catalase activity shows half (EC_{50}) which was obtained by linear interpolation [Table.2]. Drought stressed plants gave a lower EC_{50} of 46.4 µg protein as compared to control plants 49.1 µg protein. The result indicated that the lower EC_{50} was indicative of drought-stressed plants requiring less protein to synthesize 50% of the maximum catalase activity in comparison with drought non-stressed plants which was more efficient. This improvement means there is an upregulation of the antioxidant defense system caused by drought stress—seen in catalase activity improving the ability to remove reactive oxygen species (ROS). Catalase also facilitates removal of the Reactive Oxygen Species (ROS) byproduct of oxidative stress, H_2O_2, into oxygen and water, protecting from Oxidative Damage to Nucleic Acids, Proteins and Cell Membranes. This increase in catalytic activity under drought conditions is deemed to be a metabolic protective mechanism to maintain cellular redox homeostasis, defense of the photosynthetic apparatus and tolerance of drought conditions in *P. minima*.

Table.2. EC_{50} values of catalase activity in control and drought-treated *Physalis minima*

Treatment	**EC_{50} (µg/mL)**
Control – Untreated	**49.1±1.1**
Drought-treated	**46.4±0.5**

Antioxidant activity was dose dependent, increased with increase in concentration both for Control and Stress Extracts of *P. minima* (as analyzed by SOD assay). The IC_{50} for the control extract was 53.6 µg/mL and for drought-stressed extract was slightly higher (56.3 µg/mL), indicating a slightly higher drought-stressed extract's coefficient for 50% inhibition as compared to the control extract. This shows that the activity to scavenge superoxide radicals decreased under drought condition for *P. minima* when compared to control.

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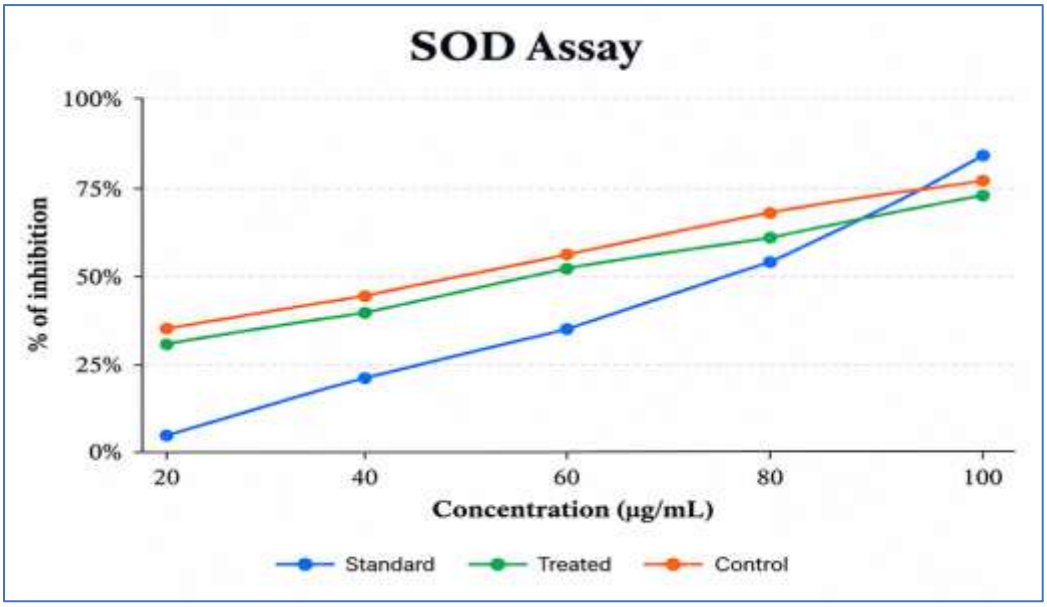

Figure 2. Superoxide Dismutase (SOD) Activity of *P. minima* under control and Drought Stress: Comparative Analysis of Standard, Drought-Treated, and Control Samples.

The SOD assay demonstrated a concentration-dependent increase in superoxide radical-scavenging activity in both control and drought-stressed *Physalis* extracts. The control extract showed 35%, 44%, 56%, 68%, and 77% inhibition at 20, 40, 60, 80, and 100 µg/mL, respectively, whereas the drought-stressed extract exhibited 31%, 40%, 53%, 61%, and 73% inhibition, respectively. The standard showed 5%, 20%, 35%, 54%, and 84% inhibition at the corresponding concentrations.

The estimated IC_{50} values were 50.0 µg/mL for the control, 55.4 µg/mL for the drought-stressed extract, and.47.8 µg/mL for the standard. The lower IC_{50} value of the control indicates greater superoxide radical-scavenging activity compared with the drought-stressed extract. Although drought-stressed *Physalis* retained substantial antioxidant activity, the increase in IC_{50} from 50.0 to 55.4 µg/mL suggests a slight reduction in SOD-mediated antioxidant potential under drought stress. Nevertheless, both extracts exhibited progressive antioxidant activity with increasing concentration, demonstrating their ability to scavenge superoxide radicals.

Table 3. IC_{50} values of SOD assay in control and drought-treated *Physalis minima*

Treatment	**IC_{50} (µg/mL)**
Standard	**47.8 ±0.7**
Control	**50.0±1.2**
Drought-treated	**55.4±0.07**

When inspected via membrane stabilizing method, anti-inflammatory effect as % inhibition was gradually rising with increase of concentration of both control and drought extract of *P. minima*. The extract of the control plants showed significantly higher membrane stabilizing effect than the extract from the plants under drought stress at all the concentrations tested.

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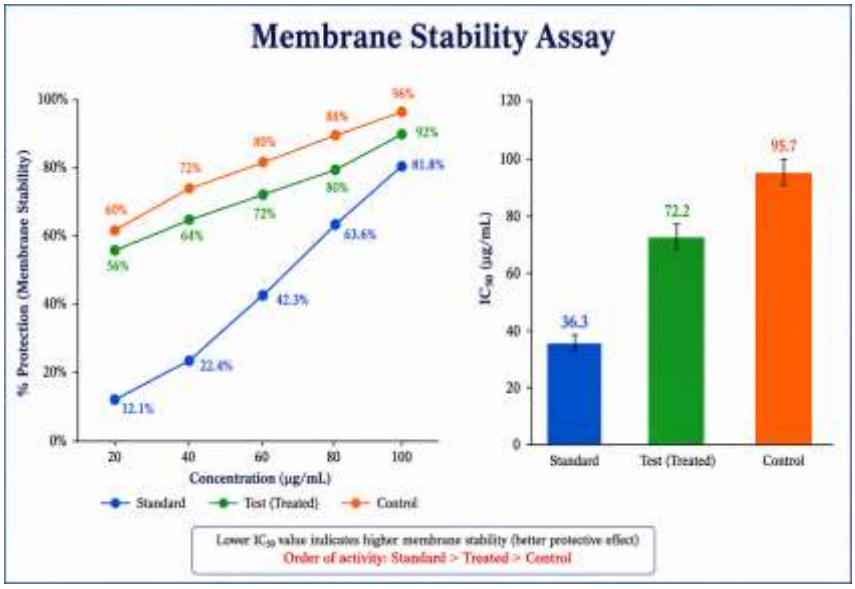

Figure 3. Membrane Stability Assay of *Physalis minima*: Comparative Analysis of Standard,

Drought-Treated, and Control Samples.

The control had 60%, 72%, 80%, 88%, and 96% inhibition at 20, 40, 60, 80, and 100 µg, while the drought-stressed extract had 56%, 64%, 72%, 80%, and 92% inhibition. The extract showed fairly good anti-inflammatory activity (92% at 100µg) while the membrane stabilizing activity (MSA) reduced marginally as compared to the control. Both extracts exert significant anti-inflammatory properties which are dose dependent as they appear to prevent the cell lysis of erythrocyte membranes, indicating that there exist potent anti-inflammatory properties, as well. However, in the control plants the greatest activity was achieved suggesting a slight reduction in the bioactivity of the compounds responsible for membrane stabilization, but that the anti-inflammation activity predominately remained.

Inhibition of Albumin denaturation method was used to explore the in vitro anti-inflammatory effect of *P.minima* extracts. Both the anti-inflammatory activity of the extracts obtained from untreated condition and drought stressed were checked in the denaturation of albumin which revealed the concentration dependent augmentative activity of anti-inflammatory in both the conditions.

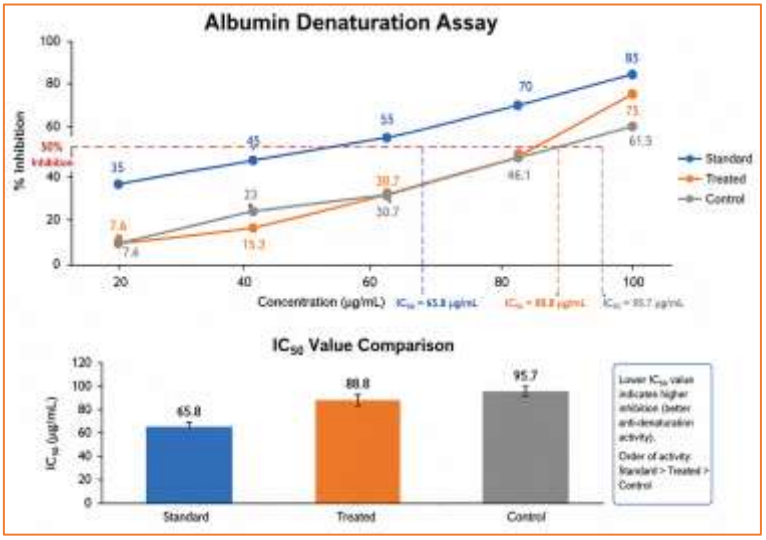

Figure 4. Albumin Denaturation Inhibition Assay of *Physalis minima*: Comparative IC_{50}

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Analysis of Standard, Drought-Treated, and Control Samples.

The increase in the percentage inhibition with the increase in concentration of extracts of the standard plants and drought stressed plants clearly indicated that an increase was indicative as compared with the controls. The percentage inhibition in the standard extract ranged from 37% at lower concentration to 85% at higher concentration with IC_{50} value of 65.8µg/mL. The percentage inhibition has increased from 7.6% to 75% in the drought stressed extract as the concentration was increased and the IC_{50} value is 88.8µg/mL. In contrast, the IC_{50} value for the control extract increased by 61.5% from 76 to 95.7% and thus showed the highest inhibition. The control extract displayed the highest inhibition at 76-61.5% at IC_{50} value of 95.7 µg/mL. The drought-stressed extract and control extract had similar inhibitory activity, with the drought-stressed being the second highest among all extracts, while the standard extract had the highest inhibitory activity.

The lower IC_{50} value of the extract obtained from the drought treated plants validate its good anti-inflammatory activity when compared to the non-treated plants. It seems that under drought stress, the bioactive molecules such as phenolics and flavonoids that prevent protein denaturation were bio produced resulting in the superior IC_{50} value." However, the results revealed that treated extract has less activity than the standard drug, but similarly drought stress has a positive effect on making the extract of *P. minima* more potent to fight inflammation.

The protein denaturation assay showed the following results: an increase of denaturation inhibition in all the experimental groups was found in a concentration-dependent manner. The standard showed inhibition values of approximately 13.5%, 26.5%, 40%, 60%, and 74% at concentrations of 20, 40, 60, 80, and 100 µg/mL, respectively. The drought treated *P. minima* showed 60%, 50%, 40%, 30% and 17% inhibition whereas the control showed 50%, 43.5%, 33.5%, 20.0% and 10 % inhibition at the above-mentioned concentrations respectively.

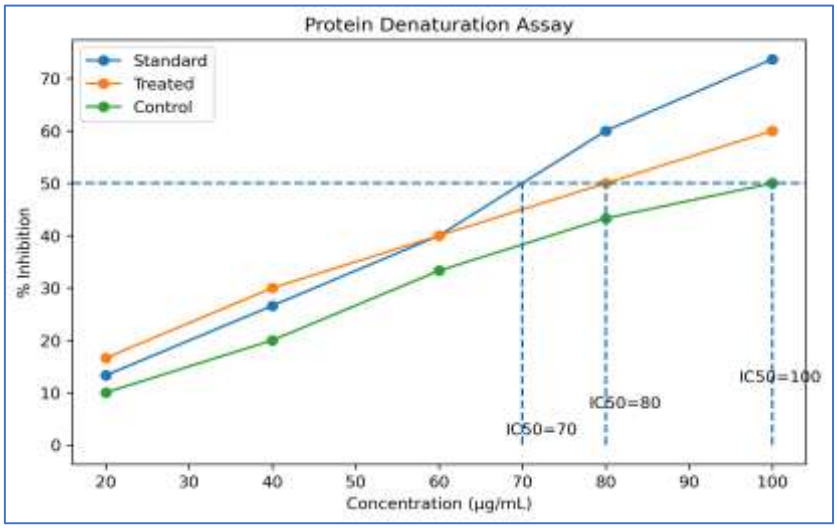

Figure 5. Protein Denaturation Inhibition Assay of *Physalis minima* L.: Concentration-

Dependent Response and IC_{50} Values of Standard, Drought-Treated, and Control

Samples.

The IC_{50} values were 70, 80, and 100 µg/mL for the standard, treated, and control samples, respectively. The UV-B-treated extract exhibited greater inhibitory activity than the control, as evidenced by the lower IC_{50} value (80 µg/mL) compared with the control (100 µg/mL). At 100 µg/mL, the standard, treated, and control samples showed 74%, 60%, and 50% inhibition, respectively. These findings indicate that UV-B treatment enhanced the protein denaturation inhibitory activity of the extract.

4. DISCUSSION

Response of various biochemical and biological reactions in *P. minima* under drought stress was suggested by the study. The antioxidant activities of the plant enzymes did not significantly change under both the drought stressed and control plants,

whereas the in vitro anti-inflammatory activities showed significant differences. "No noticeable difference was found in the qualitative phytochemical composition in regard to the two treatments. The present findings indicate that during water stress *P. minima* exhibits biochemical changes, which enable the cells to maintain a state of cellular homeostasis and protect themselves against oxidative stress during drought. Plants are well-known to produce ROS under drought stress, and to initiate antioxidant and secondary metabolism responses [Gill & Tuteja, 2010; Bashir et al.,2021].

4.1 Qualitative phytochemical profile

While doing qualitative phytochemical screening, it was observed that extracts of both the drought-stressed and control plants of *P. minima* were found to contain compounds such as tannins, flavonoids, proteins and phenolic compounds, but not saponins, alkaloids, steroids, terpenoids, quinones and cardiac glycosides were present in all the extracts examined. Therefore, there seems to be no apparent qualitative alteration for the major groups of phytochemicals studied, owing to drought stress. For, the mere presence or absence of any group of phytochemicals does not mean that the said group is concentrated. Drought stress might lead to obvious modification of the secondary metabolism thus altering synthesis or accumulation of protective metabolites without anything being changed qualitatively in terms of the phytochemical profile.

The presence of flavonoids and phenolic compounds in both treatments is of utmost significance because of their capability to remove ROS and assist in protecting cellular material from ROS-induced damage, a crucial antioxidant defense mechanism. Metabolic reprogramming that occurs under drought stress may lead to an increase in plant production of secondary metabolites (Mutanda et al., 2026). Hence, unchanged qualitative profile in the current study could be hiding the quantitative variations in certain flavonoids and phenols. Some quantitative assessment and chromatographic profiling will be required to do that.

4.2 Catalase activity

Catalase activity was significantly increased in both plants as the protein concentration increased while plant growing under drought stress. At different levels of protein, the drought stress groups exhibited slightly higher activity than control plants indicating an increase in detoxification of H_2O_2 under water stress. Catalase was also associated with regulating oxidative stress as it exhibits an important role in the enzymatic antioxidative system, catabolizing H_2O_2 to yield water and oxygen [Gill & Tuteja, 2010; Bashir et al.,2021].

An increase in CAT activity occurs in stressed plants of *P. minima* and may be considered to be an adaptation to increased production of H_2O_2 due to drought.

Increase of antioxidant activity level can alleviate the harm caused by oxidative stress on membranes, proteins, and other components of the cell. Despite this, the results of this study revealed that the activity of CAT increased in the experimental treatments, which can be interpreted as the positive response of *P. minima* to drought stress level.

4.3 Superoxide dismutase activity

SOD was observed to have a slight decrease in activity following drought treatment as illustrated by the lower IC_{50} value in comparison with CAT. However, SOD helps in the antioxidant defense mechanism by converting superoxide into H_2O_2. CAT and other peroxidases then can break down H_2O_2 [Gill & Tuteja, 2010].

The lack of SOD activity recorded under drought may suggest that the plant maintains a balance between antioxidant activity and ROS production and a prolonged drought interferes with this balance. This is because the drought may not induce the same response in each antioxidant enzyme; their regulation will be dependent on the type of stress, stress duration, developmental stage and the metabolic processes of the plant. This accounts for the registered reduction of the SOD activity and the increase of the CAT activity that resulted in different regulation of antioxidant systems, as opposed of uniform activation or deactivation processes. The increase in the effectiveness of CAT may help lessen the influence of the decreased SOD activity through effective removal of generated H_2O_2 [Jańczak-Pieniążek et al., 2025).

4.4 HRBC membrane stabilization activity

The anti-inflammatory activity of *P. minima* extract was evaluated at different concentrations using the HRBC membrane stabilization assay. The results showed a dose-dependent increase in membrane stabilization, with higher concentrations of the

extract producing greater membrane-stabilizing activity. However, the response of the extract following drought treatment differed from that of the control, indicating a variable effect of drought stress on the anti-inflammatory potential of *P. minima*.

The results of the membrane stabilization test indirectly suggest anti-inflammatory potential as membrane stability to hypotonic lysis implies that bioactive compounds have biological activity. In addition, the film composite comprising of various phenolic and flavonoid components in the material

The high antioxidant and membrane protective properties of *P. minima* might also enable this plant to do the same. The presence of noticeable effects in membrane stabilization even after the dry condition implies that the biological effects happened to be unaffected by the water stress, rather than influenced by it, and that the composition of the substances may have been altered by water stress due to the same active compounds in the membrane stabilization of the models.

This slight decrease in membrane stabilization should thus be taken with a grain of salt. The observed difference in the present study is in crude extract and in qualitative phytochemical screening which does not make it possible to attribute it to a single chemical compound (Mehravi etal.,2025). Measuring the quantitative amounts of phenolics flavonoids and other secondary metabolites will give more insight into the biochemical means of such a response.

4.5 Inhibition of albumin denaturation

The results of denaturation of the proteins clearly indicated that there is a direct relationship between the extract used and the level of inhibition when the extracts used in the control and drought treatment were increased. The standard successfully showed inhibiting values of about 13.5%, 26.5%, 40%, 60% and 74% at 20, 40, 60, 80, and 100 µg/mL, respectively. Drought-treated extract suppresses with inhibition values of 17%, 30% 40%, 50% and 60%, while the control has values of 10%, 20%, 33.5%, 43.5% and 50%, respectively. Thus, it is feasible to conduct tests of samples with higher concentrations of extracts, effectively.

Certainly, the exposure to drought condition yield extract with IC_{50} value of 80 µg/mL, while the control yield 100 µg/mL. It is concluded that this particular extract was able to exert stronger inhibitory effect after the drought treatment than the control. The drought extract proved to be more potent than the control extract while the standard extract had the highest potency with an IC_{50} of 70 µg/mL. The results regarding the reduction of IC_{50} by 20% in comparison with the control suggest that the extract has higher protein-stabilizing effect after drought treatment.

Increased inhibition of albumin denaturation might be linked with the drought-induced changes of secondary metabolism. Under drought stress condition, the metabolic process might be induced to accumulate phenols and flavonoids and other protective metabolites which participate in antioxidant protection [Yadav et al.,2021; Jogawat et al.,2021]. The current study's qualitative analysis showed that the same type of phytochemicals was present in both treatments studied, but there were some quantitative differences in some of these chemo term groups that may account for the better activity of the drought extract. Thus, it does not appear that the increased protein stabilizing activity is related to the development of novel types of phytochemicals, but instead the changes in the quantity of the bioactive components and/or their composition.

It not only corroborates the anti-inflammatory activity of *P. minima* but is also supported by additional phytochemical research. Some of the withanolides and withaphysalins extracted from *P. minima* have shown anti-inflammatory effects in experimental research. Anti-inflammatory withanolides were found in the whole plant of *P. minima* by Wu et al. [2021]. As shown by Liu et al. [2022] withanolides obtained from *P. minima* were able to inhibit nitric oxide production. A similar anti-inflammatory activity of withaphysalins isolated from *P. minima* was also reported by Hu et al. [2022]. Hence, the evidence for the anti-inflammatory effect observed in the present study may have been biochemical and a result of these bioactive compounds, although the identified chemicals in the extracts used in the present study that are responsible for the anti-inflammatory action still need to be pinpointed.

4.6 Integrated response to drought stress

Drought stress did not affect all biochemical processes equally as evidenced by differential responses of CAT and SOD activity, membrane stabilization tests and classification of albumin denaturation tests, hence, there is differential biochemical regulation occurring in *P. minima*. The rise of the CAT activity might be because of an increased capability to detoxify H_2O_2, while the small reduction

in SOD activity might be because of differential regulation of ROS-scavenging pathways under drought stress. In addition, the loss of membrane-stabilizing activity and the gain of anti-denaturation activity for the albumin indicate that the two anti-inflammatory assays may consist of different combination of bioactive components.

The capacity of *P. minima* to maintain its antioxidant and anti-inflammatory activity in drought condition could be due to its antioxidant biosynthesis and regulation of the production of ROS. Other signalling nutrient involved pathways that are triggered due to drought may shed light on secondary metabolite biosynthesis and tolerance to oxidative stress and adaptation of drought in the plant [Yadav et al.,2021; Jogawat et al., 2021]. The bioactive withanolides detected in *P. minima* also reinforce the pharmacological possibilities of this species and probably its biochemical stress-response is related to its medicinal use [Liang et al.,2024].

5. CONCLUSION

These study results support the adoption of a rather flexible concept of drought stress and indicate that significant biochemical plasticity exists in *P. minima* plants but that the general qualitative phytochemical profile remains unchanged. This is supported by the observation that catalase activity was enhanced and the slight decrease in superoxide dismutase activity did not result in a reduction of plant defence against water-deficit conditions; rather, there was an improvement in the anti-inflammatory activity. The decrease in IC_{50} from around 100 to 80 μg/mL supports the notion that drought stress could help the metabolites in *P. minima* become more bioactive. All its observations indicate that *P. minima* possess good biochemical flexibility for adaptation to drought stress, and it can be used as a medicinal plant that maintains or even enhances its bioactive properties under the unfavourable environmental condition. Further studies using metabolomic, transcriptomic and proteomic approaches would be beneficial to ascertain which specific metabolites are responsible for drought-induced bioactivity, as well as revealing the mechanism(s) and pathways through which drought stress is being handled.

6.AUTHORS' CONTRIBUTIONS

A. Linga Malar: Investigation, methodology, data collection, and initial manuscript preparation. R.Vinoth Kumar: Data analysis, interpretation of results, and manuscript review. N.Shanthi: Conceptualization, supervision, validation, critical revision, and overall manuscript editing. All authors read and approved the final manuscript.for all the elements of this manuscript.

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8.MANDATORY DISCLOSURE ON USE OF ARTIFICIAL INTELLIGENCE

During the preparation of this work, the author(s) used artificial intelligence tools to check the accuracy and quality of the English translation. After using this tool, the author(s) reviewed and edited the content as necessary and take full responsibility for the content of the publication.

9.CONFLICTS OF INTEREST

The authors have no financial involvement, interest, benefit or competing interest with this research activity.

10.DATA AVAILABILITY

The information/data used in this overview on *Physalis minima* are cited in references and would be available for public access where necessary.

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