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Comprehensive analysis of effects of abiotic stress on the *Spondias dulcis* plant and evaluating the antibacterial properties

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Abstract

Plant stress is the term used to describe environmental factors that cause abrupt changes in respiration, photosynthesis, senescence, flowering, seed germination and development, cellular metabolism, gene expression, and growth in plants, all of which reduce crop yields and productivity. The detrimental effects that abiotic influences have on plant tissues are known as abiotic stress. In contrast to biotic stress, which is triggered by living organisms, abiotic stress is caused by non-living elements. This study investigates the morphological changes, and quantitative and qualitative evaluations of the phytochemical composition of *Spondias dulcis* under abiotic stress conditions such as drought, high salinity, flood, and nutrient deficiency, in addition to its antibacterial properties. The morphological changes of the stress-induced plants exhibited symptoms of abiotic stresses such as leaf yellowing, wilting, and sparser foliage. The qualitative assays indicated the presence of phytochemicals such as alkaloids, tannins, coumarins, phenols, cardiac glycosides, terpenoids, steroids, even under different stress conditions and indicated the absence of flavonoids and saponins. Drought had a considerable impact on total phenolic content, total antioxidant capacity and total protein content of the quantitative analysis. The total antioxidant capacity increased in response to high salinity. High salinity, nutrient deficiency, and flooding contributed to an increase in total flavonoid content. Methanolic extract of *S. dulcis* exhibited antibacterial activity, suppressing the growth of *Escherichia coli* and *Staphylococcus aureus*. The results of the investigation showed that Spondias dulcis has potential in medicine due to its antioxidant and antibacterial properties, as well as its ability to survive abiotic stressors in various habitats.

Keywords: *Spondias dulcis* leaves, Abiotic stress, Phenolic compounds, Antibacterial activity, Antioxidant activity

1. Introduction

External stresses that impact the plant's growth, yield and its life cycle are considered as plant stress. Plant stress can be divided into two major groups as biotics and abiotic stress (Figure 1). Biotic stress is a biological damage a plant undergoes such as disease or insects. Abiotic stress is a result of chemical or physical factors such as (light, water, or salt) which harm the plant and its surrounding environment. Stress triggers plants to exhibit abnormalities in their development and metabolic processes. When stress is minimal in plants they recover quickly. However, plants experiencing prolonged or excessive stress exhibit inhibition of their developmental processes, which leads to plant mortality.[1]

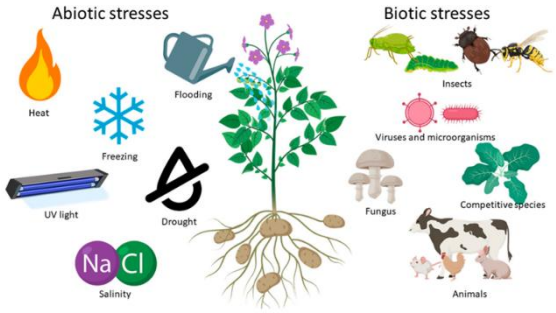


Figure 1. Several types of biotic and abiotic stresses that can affect plants.[2]

1.1 Abiotic stresses. The antagonistic effects of abiotic factors on a plant in a particular environment is referred to as abiotic stress. The stress influences biological processes like gene expression and cell metabolism that impacts growth and development.[3] Abiotic stressors include extreme temperature, changes in water

supply, extreme salt conditions, heavy metal contaminations, and nutritional stress. Different stressors elicit different effects like increase in reactive oxygen species and decrease in photosynthetic activity, plant growth and yield as shown in the Figure 2 and 3.[4]

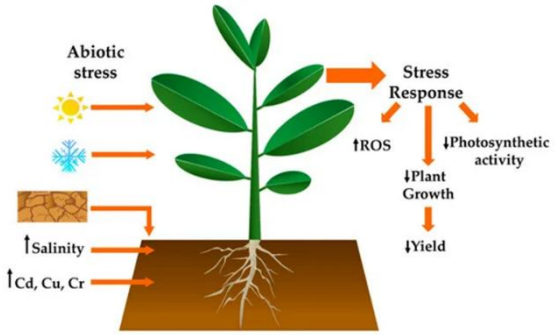


Figure 2. Abiotic stresses in plants and their stress responses.[5]

Water is crucial for plant survival and nutrient delivery. Since environmental conditions create a decrease in water in the soil, plants experience drought stress, resulting in an ongoing loss of water through transpiration or evaporation. This stress causes cellular water loss, plasmolysis, and cell death.[6]

Insufficient drainage infrastructure, increased soil deprivation and climate change has contributed to an increase in floods over the past few years.[7] Therefore, the flooding stress causes hypoxia and anoxia in plant tissues leading to cell death. Waterlogging stress in which the plants leaves and stems are partially submerged and submergence stress in which the plant is fully submerged, are the two types of flooding stress.[8]

Macronutrients such as Nitrogen (N), Phosphorous (P) and Potassium (K) and micronutrients such as Boron (B), Zinc (Zn) and Manganese (Mn) affects the plants functions in agriculture and natural ecosystems. The shortage of macronutrients and micronutrients negatively influences the growth of the plant leading to a nutrient deficient condition. Excess macronutrients have an adverse effect on the soil. Moreover, nutrients are responsible for antioxidant production. Absence of antioxidants can disrupt the plants system.[9]

Increased salt content in the soil causes salinity stress. This stress is a global and a life-threatening issue to the agricultural biosphere. Plant growth is hindered by high salt concentrations. The greater osmotic potential and specific ion toxicity damages the plant's development stages and inhibits seed germination, which adversely affect the quality and quantity of plant production.[10]

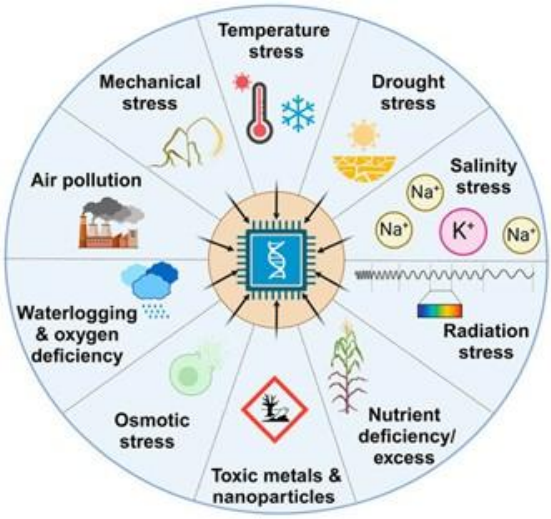


Figure 3. Types of abiotic stresses in plant tissues.[11]

1.2 Spondias dulcis. The genus *Spondias* (*Anacardiaceae*) consists of seventeen species; ten of which are indigenous to tropical Asia. This plant consists of edible fruits, tiny white flowers, and glossy leaves. *Spondias dulcis* is a fast-growing equatorial tree popular in Sri Lanka known as "Ambarella" while its commonly called as golden apple or hog plum.[12] The fruit can be eaten raw or used to make jams and juice drinks. Mature leaves are used in salads while young leaves and the fruit are cooked as a vegetable. Tribal members in Cambodia uses the bark of the plant to treat diarrhoea. The fruits and the leaves of *Spondias dulcis* have been utilized to treat eye infections, improve vision, reduce itching, internal ulcers, sore throats, and skin inflammation. The plant *Spondias dulcis* is more commonly used as a fruit in all parts of the world, which has numerous benefits due to the nutrient dense composition (Figure 4). The leaves also exhibit important properties such as antimicrobial, antioxidant, enzymatic inhibition and thrombolytic. High incident diseases like Alzheimer's, cancer, diabetes, and obesity have been studied as a novel treatment with the use of *Spondias dulcis* leaves.[13]

Figure 4. Ambarella leaves**.**

From sea level to 700m, the plant thrives in the subhumid, frost-free topics. *Spondias dulcis* can withstand 12-35℃, it thrives in regions with yearly daytime temperatures between 22-27℃ in response to abiotic stress conditions the leaves show signs of curling/wilting with yellowing and decreased lateral branching. While the plant can withstand temperatures as low as -3℃ when dormant, 0℃ can harm young plants. The plant can withstand 600-2200mm of rainfall per year but prefers 900-1800mm of rain and the leaves show slight wilting/ curling in response to the stress.[14] *Spondias dulcis* thrives on acid sand and soils formed from limestone, but the soil needs to be well drained. It can tolerate a pH between 4.5 and 8, preferring a pH between 5.5-6.5 for the plant growth. Although the plant may momentarily lose their leaves when in stress conditions, mature plants can withstand drought conditions.[15]

In recent years, scientists have used plants as sources of medications and bioactive substances which includes well known medicinal species but also plants are used as traditional medicines and food in several countries. The public interest in natural products and the significance of medicinal plants in the healthcare sector have increased due to the low toxicity, strong pharmacological action, and commercial availability. Moreover, plants contain bioactive constituents such as polyphenols, carotenoids, antioxidants, and proteins each of which exposes important biological activities.[16] To increase agricultural productivity and sustainability, it is crucial to investigate the effect of abiotic factors including heat, salinity, and drought on plants. Food security is at risk due to these pressures, which restrict crop yields, particularly considering climate change. Understanding how plants react to these circumstances can help us create crops that are more resilient, use water more efficiently, and manage resources effectively by promoting ecosystem protection.

Therefore, this study aimed to analyse the effects of abiotic stress on *Spondias dulcis* plant and evaluate the phytochemicals, antioxidant concentration and, antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus.*

2. Methodology

2.1 Inducing of abiotic stress for Spondias dulcis. Healthy *Spondias dulcis* plants were collected from Seed & Plant Retail Shop, a plant nursery located in Colombo 07. A total number of healthy forty-two plants were randomly selected and transported to the BMS campus premises for further processing to ensure consistency in the analysis. Seven *Spondias dulcis* plants were assigned to each of the drought, flood, nutrient deficiency, and high salinity conditions and the stress was induced according to Table 1. A batch of 7 plants were also maintained as control plants without inducing stress and 100 mL of distilled water were added to the plants every morning and evening.

Table 1. Abiotic stress inducing process.

Stress	Methodology
High salinity	For fourteen days, the plant was treated with 100 mL of 200 mM sodium chloride (NaCl) solution every morning and evening.[17]
Flood	After adding 5600 mL of water to a container, the plant roots and a part of the stem were immersed completely in water for 14 days.[18]
Nutrient deficiency	The plants original soil was replaced with autoclaved sand. 100 mL of distilled water was added to the plant

	each morning and evening for 14 days.[19]
Drought	For 14 days, 100 mL of 10% Polyethylene glycol (PEG 4000) were added each morning and evening.[20]

2.2 Spondias dulcis aqueous extract preparation. The leaves were collected, weighed, and cleansed with tap water, followed by distilled water to get rid of impurities. Leaves were patted and air-dried for 30 minutes and were cut into small pieces and evenly spread on an aluminium foil. Leaves were then placed in dry oven (Meditry instrument, China) at 50℃ for 48 hours. Once dried, they were crushed and weighed. Distilled water was added in a 1:10 ratio. The aqueous extract was then placed in dry oven at 90℃ for 15 minutes. After cooling to room temperature, the extract was transferred to a 50 mL falcon tube and centrifuged (Gemmy industrial corporation, Taiwan) at 4000 rpm for 10 minutes. The extract was filtered using Whatman No.1 filter papers. The filtrate was collected in a 50 mL falcon tube and stored in a refrigerator at 4℃.[21]

2.3 Spondias dulcis methanolic plant extract preparation. The leaves were dried as explained in 2.2. After drying, leaves were weighed to obtain 5 g using an analytical balance. The measured powder was transferred to a beaker, and 35 mL of methanol was added. The mixture was left to macerate in a watch glass at RT for three days. After maceration, mixture was filtered using Whatman No.1 filter papers to remove plant debris, and the filtrate was transferred to a beaker. The beaker was then placed in a dry oven set at 40°C. Once the solvent had evaporated, the concentrated residue was dissolved in 2 mL of methanol. The dissolved extract was transferred into a clean, sterile falcon tube and stored in refrigerator at 4℃.[22]

2.4 Determination of moisture content. The moisture content of plant leaves was found by following the below equation.[23]

$$\text{Moisture Content} = (w-d)/w \times 100$$

w= Fresh weight

d= Dry weight

2.5 Qualitative assays for phytochemicals. Qualitative tests were conducted to determine the presence of phytochemicals as shown in Table 2.

Table 2. Methodology for Qualitative assays

Test	**Methodology**
Alkaloids - Wagner Test	A drop of Wagner's reagent (Iodo-potassium iodide) was added to 1 mL of plant extract.[24]
Flavonoid - NaOH test	A mixture containing 0.5 mL of plant extract, few drops of 2% NaOH, and 2–3 drops of 1% HCl were mixed.[25]
Tannins	1 mL of 5% ferric chloride was added to 0.5 mL of plant extract.[26]
Saponins	A combination of 0.5 mL of plant extract and 5 mL of distilled water was shaken thoroughly.[27]
Coumarins	0.5 mL of 10% NaOH was added to 0.5 mL of plant extract.[28]
Phenols	A mixture consisting of 1 mL of plant extract, 1 mL of distilled water, and few drops of 10% $FeCl_3$ was added.[29]
Cardiac glycosides - Keller Kiliani test	A mixture containing 1 mL of plant extract, 0.5 mL of glacial acetic acid, 0.5 mL of $FeCl_3$, and 0.5 mL of concentrated H_2SO_4 was added.[30]
Terpenoids	A combination of 2.5 mL of plant extract, 1 mL of chloroform, and 1.5 mL of concentrated H_2SO_4 was added.[31]
Steroid	A mixture of 0.5 mL of plant extract, 0.5 mL of chloroform, and few drops of concentrated H_2SO_4 was added.[32]

2.6 Determination of Total Phenolic Content (TPC) using Folin-Ciocalteu method. Gallic acid (1 mg/mL in distilled water) was used as the standard solution. A standard series of 0.01, 0.05, 0.1, 0.25, and 0.5 mg/mL was prepared for the standard curve. Plant extract dilutions were

made by adding 100 µL of each sample with 500 µL of distilled water in test tubes. Reaction was initiated by mixing 100 µL of Folin-Ciocalteu reagent, followed by incubation in dark for 6 minutes. 1 mL of 7% sodium carbonate was added, with 500 µL of distilled water. The mixture was incubated at room RT for 90 minutes. Absorbance was measured using a UV-Visible spectrophotometer at 760 nm, and a standard curve was plotted. TPC was expressed as Gallic acid equivalents (mg GAE/g).[33] Distilled water was used as blank.

2.7 Determination of Total Antioxidant Capacity (TAC) using phosphomolybdate method. Ascorbic acid (1 mg/mL in distilled water) was used as the standard solution. A standard series of 0.01, 0.05, 0.1, 0.25, and 0.5 mg/mL was prepared for the standard curve. Plant extract dilutions were formulated by adding 0.1 mL of each sample into 0.5 mL of distilled water to a test tube. The reaction was initiated by mixing 1 mL of reagent solution, consisting of 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate, which had been thoroughly mixed and set aside. The test tubes were capped with aluminium foil and incubated in a water bath at 95°C for 90 minutes. After incubation, the samples were allowed to cool to RT. Absorbance was measured at 765 nm using a UV-Visible spectrophotometer, and a standard curve was plotted. TAC was expressed as Ascorbic acid equivalents (mg AAE/g).[34] Distilled water was used as blank.

2.8 Determination of Total Flavonoid Content (TFC) using aluminium chloride assay. Quercetin (1 mg/mL in distilled water) was used as the standard solution. A standard series of 0.01, 0.05, 0.1, 0.25, and 0.5 mg/mL was prepared for the standard curve. Plant extract dilutions were formulated by adding 100 µL of each sample (15x) with 500 µL of distilled water in test tubes. To each mixture, 100 µL of 5% sodium nitrate was added, and the solution was allowed to stand for 6 minutes. 150 µL of 10% aluminium chloride solution was added and left to stand for 5 minutes. Afterwards, 200 µL of 1 M sodium hydroxide was added, and the mixture was gently stirred to ensure complete reaction. Absorbance was measured using a UV-Visible spectrophotometer at 510 nm, and a standard curve was plotted. TFC was expressed as Quercetin equivalents (mg QE/g).[33] Distilled water was used as blank.

2.9 Determination of Total Protein Content (TPrC) using lowry assay. Bovine Serum Albumin (BSA) (1 mg/mL in distilled water) was used as the standard solution and a standard series of 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, and 1 mg/mL was prepared for the standard curve. Plant extract dilutions were formulated by mixing 5 mL of reagent solution to 20X diluted sample. The reagent solution was prepared by mixing 48 mL of 2% sodium carbonate in 0.1N sodium hydroxide with 1 mL of 0.5% copper sulphate and 1 mL of 1% sodium potassium tartrate. The reaction mixture was allowed to stand at room temperature for 15 minutes. 0.5 mL of freshly prepared Folin-Ciocalteu reagent (1:1 dilution with water) was added and mixed well to ensure thorough reaction with the protein in the sample. The test tubes were incubated in the dark for 30 minutes. Absorbance was measured using a UV-Visible spectrophotometer at 660 nm, and a BSA standard curve was plotted. TPrC was expressed as BSA equivalents (mg BSA/g).[35] Distilled water was used as blank.

2.10 Determination of antimicrobial effect of Spondias dulcis using well diffusion-Antibiotic susceptibility test. Mueller-Hinton agar (MHA) was prepared by boiling the agar for few minutes until completely dissolved, followed by autoclaving at 121°C for 15 minutes. The agar was then cooled before being poured into petri dishes. A volume of 20 mL of agar was poured into each petri dish and allowed to solidify. Bacterial suspensions of *Escherichia coli* and *Staphylococcus aureus* was spread onto the MHA plates using a sterile cotton swab. Four wells were created in each MHA plate using a sterile micropipette tip, ensuring that the wells were evenly spaced and did not overlap. Wells 1 and 2 were filled with methanolic plant extract, while well 3 (negative control) was filled with methanol, and well 4 (positive control) was filled with Ciprofloxacin (100 µg/mL) solution (Figure 5). The plates were incubated at 37°C for 24 hours. Following incubation, the zones of inhibition around the wells were observed and measured to evaluate antibacterial activity.[36]

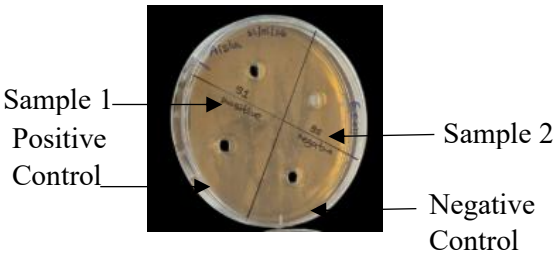


Figure 5. ABST plates before streaking before incubation

2.11 Statistical analysis. All values are expressed as mean ± Standard error. Microsoft Excel 2023 was used to calculate the standard error of the mean value.

3. Results and Discussion/Analysis and Findings

3.1 Morphological changes of control and abiotic stress. Observed morphological changes of the control plant and abiotic stress induced plants during the 14 days is shown in Table 3.

Table 3. Morphological changes of the plant control plant and in stress-induced plants

Condition	Morphological changes
Control	• More branching • Broader and healthier leaves • Long and bushier growth • Thicker stems • Even distribution of leaves
High salinity	• Sparser foliage • Thinner stems • Leaf wilting and damage • Less bushy appearance
Flood	• More elongated growth • Less compact foliage • Leaning structure • Slightly wilting/ curling leaves
Nutrient deficiency	• Elongated stems • Reduced lateral branching. • Smaller leaf size and number • Leaf yellowing
Drought	• Elongated stems • Reduced lateral branching. • Smaller leaf size and number • Leaf yellowing • Leaf wilting and damage

3.2 Qualitative phytochemical assays. Qualitative phytochemicals assay results conducted for control and stress-induced plants are shown in Table 4 as Presence (P) and Absence (Ab) of phytochemicals.

Table 4. Phytochemicals for qualitative assays

Tests	Control	Drought	High salinity	Heavy metal	Nutrient Deficiency	Flood
Alkaloid	P	P	P	P	P	P
Tannins	P	P	P	P	P	P
Phenolic Compound	P	P	P	P	P	P
Saponins	Ab	Ab	Ab	Ab	Ab	Ab
Flavonoid	Ab	Ab	Ab	Ab	Ab	Ab
Coumarins	P	P	P	P	P	P
Terpenoids	P	P	P	P	Ab	Ab
Cardiac Glycoside	P	P	P	P	P	P
Steroids	P	P	P	P	P	P

3.3 Total Moisture Content (TMC)

The plant aqueous extract obtained from the control had the highest TMC and the lowest was obtained in the drought condition. At the 10% error margins, the error bars of high salinity, nutrient deficiency, flood, and drought overlapped with control plants, whereas the drought condition did not overlap (Figure 6).

Therefore, the decreased moisture content in drought was statistically significant compared to control plants.

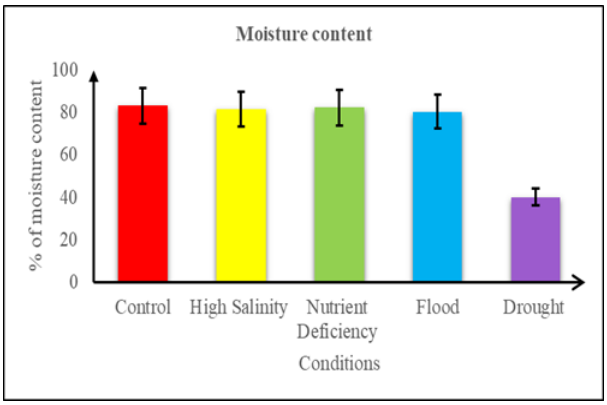


Figure 6. Bar chart of moisture content in each test group

3.4 Total Phenolic Content (TPC)

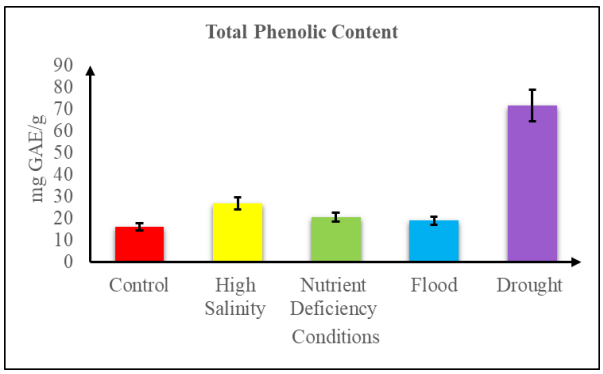


Figure 7. Total phenolic content in each test group

The plant aqueous extract obtained from the drought condition had the highest TPC and the lowest was obtained in the control plant. At the 10% error margins, the error bars of flood and nutrient deficiency overlapped with the control plants whereas high salinity and drought did not overlap (Figure 7) Therefore, the increased total phenolic content in high salinity, and drought were statistically significant compared to control plants.

3.5 Total Antioxidant Capacity (TAC). The plant aqueous extract obtained from the drought condition had the highest TAC and the lowest was obtained in the flood condition.

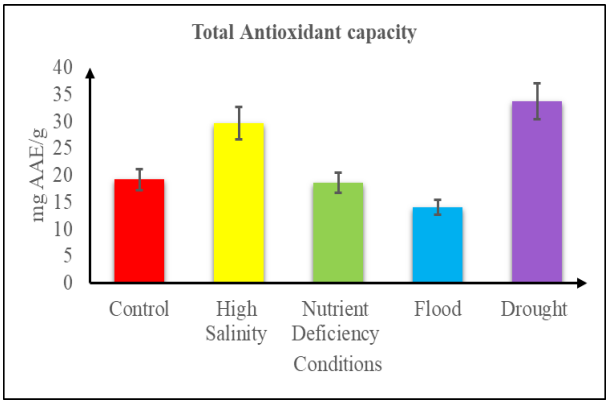


Figure 8. Total antioxidant capacity in each test group.

At the 10% error margin, the error bars of nutrient deficiency overlapped with control plants whereas high salinity, flood, and drought did not overlap (Figure 8). Therefore, the increased total antioxidant capacity in high salinity, drought and decreased TAC in flood conditions were statistically significant compared to control plants.

3.5 Total Flavonoid Content (TFC)

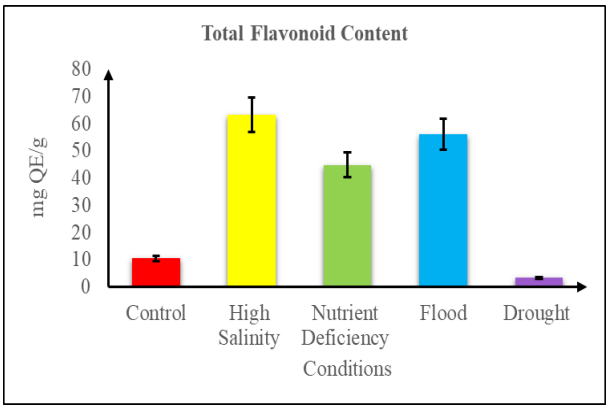


Figure 9. Total flavonoid content in each test group.

The plant aqueous extract obtained from the high salinity condition had the highest TFC and the lowest was obtained in the drought condition. At the 10% error margins, the error bars of high salinity, flood, nutrient deficiency, and drought did not overlap (Figure 9). Therefore, the increased total flavonoid content in high salinity, flood, nutrient deficiency, and decreased TFC in drought conditions were statistically significant compared to control plants.

3.6 Total Protein Content (TPrC)

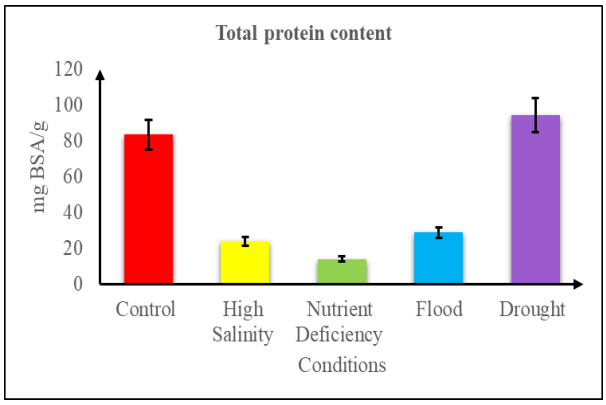
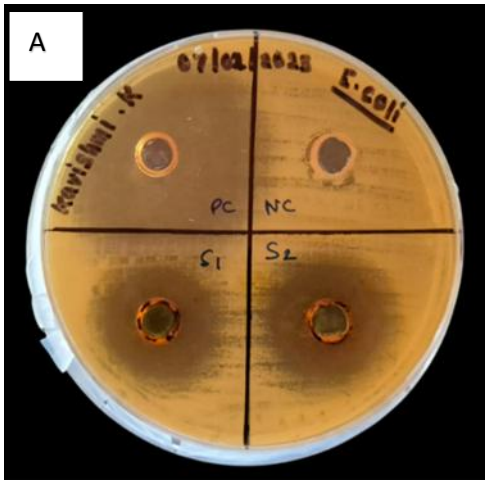


Figure 10. Total protein content in each test group.

The plant aqueous extract obtained from the drought condition had the highest TPrC and the lowest was obtained in the nutrient deficiency condition. At the 10% error margins, the error bars of drought overlapped with control. Whereas the error bars of high salinity, nutrient deficiency, and flood conditions did not overlap (Figure 10). Therefore, the decreased total protein content in high salinity, flood, nutrient deficiency, and increased TPrC drought conditions were statistically significant compared to control plants.

3.7 Antibacterial activity



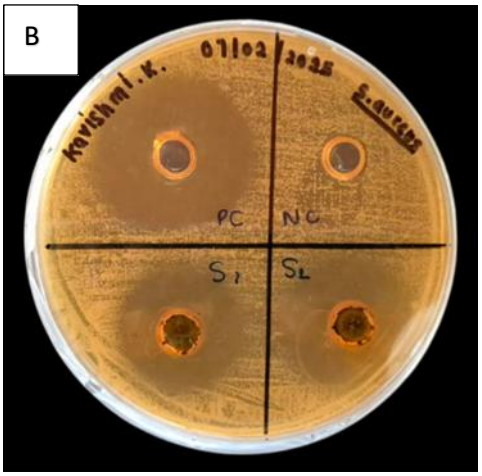


Figure 11. Antibacterial results of *Spondias dulcis* activity against *Escherichia coli* (A) and *Staphylococcus aureus* (B)

The antibacterial activity of *Spondias dulcis* activity against *Escherichia coli* (Figure 11A) and *Staphylococcus aureus* (Figure 11B). The methanolic extract of *Spondias dulcis* showed strong inhibitory activity against both *Escherichia coli* and *Staphylococcus aureus* as indicated by the clear zones of inhibition (Table 5).

Table 5. Zones of inhibition against bacterial species

Bacteria	**Zone of inhibition**	
	Sample 1	Sample 2
Escherichia coli	10 mm	9 mm
Staphylococcus aureus	10 mm	11 mm

4. Discussion

To maintain agricultural sustainability in different environment conditions triggered by climate change, this research focused on abiotic stresses like high salinity, flood, nutrient deficiency, and drought on *Spondias dulcis* plants due to the growing use of medicinal plants and evaluating the adaptation mechanism exhibited by the plant. Qualitative and quantitative analyses of the aqueous plant extract were done following antimicrobial potential of the methanolic plant extract of the *Spondias dulcis* leaves.

Due to various environmental circumstances, plants exhibit substantial variations in growth and morphology. Under optimal conditions control plants show greater branching, broader and healthier leaves, lengthy and bushier growth, thicker stems, and even leaf distribution, all of which represent the ideal water balance and nutrient availability (Table 4).[37]

In the high salinity condition, 200 mM of NaCl solution was chosen to add to the soil, due to the concentration having a depressive effect on the plant while giving a suitable condition for the salt stress.[38] Plants grown in high salinity environments resulted in thinner stems, withering leaves, and less bushy appearance due to osmotic stress brought on by salt accumulation, which interferes with water absorption and lowers leaf turgor while preventing lateral growth.[39] Moreover, a greater uptake of Na^+ and Cl^- ions from the soil lowers the photosynthetic efficiency, which has a detrimental effect on the leaves causing sparser foliage.[40]

In flood conditions, when stems and roots are submerged, plant experience temporary hypoxia until water subdues and normoxia returns. This gives a suitable condition for waterlogging stress in *Spondias dulcis* leaves and the plant showed curled and withering leaves.[41] In reaction to hypoxia in the root zone, flooded plants exhibit extended growth, as a tolerance mechanism,[42] less compact foliage, a leaning structure due to the stems elongation to access better aerated areas.

Elongated stems, less lateral branching, smaller leaves, and leaf yellowing (chlorosis) are all symptoms of nutrient deficiency condition which was obtained from autoclaving sand, free from nutrients and microorganisms.[43] Observations in the plant leaves were due to the lack of vital nutrients like Potassium and Nitrogen which are necessary for cell division and the synthesis of chlorophyll.[44] The plant development and yield causing less lateral branching and smaller leaves are caused due to magnesium deficiency.[45]

Drought stress was facilitated by 10% PEG 4000 as an osmotic agent to stimulate water deficit conditions.[46] Plants under drought stress resulted in longer stems, yellowing and wilting of the leaves due to a lack of water, promoting stomatal closure, limiting photosynthesis, and decreased cell growth to save water.[47] As the stress increases the plants showed reduced stature which affects the plants' yield. Cellular free radical metabolism is disturbed resulting in the buildup of free radicals and ROS in plants.[48]

Moisture content (MC) of the *Spondias dulcis* plant extract in the control condition was high compared to the abiotic stress induced plants. In the abiotic stresses, nutrient deficiency was the highest (82.2%) due to stunted growth, which retains more water within its tissues, and the lowest was obtained from the drought (40%) causing low absorption of water in the soil for roots. The determination of MC in nutrient deficient environments align with previous research articles.[15] confirming that due to the Nutrient Deficiency stress plants like *Spondias dulcis* (drought tolerant) maintain higher moisture content. In general drought stress reduces the MC of the plant due to loss of turgor and reduced uptake of water.[49] However, in *Spondias dulcis* plant, strategies such as leaf shedding and wilting are observations to control moisture loss during drought, the scientific literature currently lacks precise quantitative information on the leaf's MC. To measure the alterations in specific to the plant, more empirical research is required.

According to the results from the qualitative phytochemical analysis, the control plant parameters are consistent with other studies that have found alkaloids, tannins, coumarins, phenols, cardiac glycosides, terpenoids and steroids presence in *Spondias dulcis*.[12,13] These phytochemicals contain antibacterial, antidiarrheal, antiviral properties in addition to the antioxidant properties.[50] However, the presence of flavonoids and saponins were reported to be present in *Spondias dulcis* in previous research articles.[51] In comparison, in the present study both the phytochemicals were absent from *S. dulcis* leaf

extract. Different environmental factors, the plant parts used, the development stage variation, different extraction techniques employed and concentration below a detectable limit could be the reasons for the negative results obtained.[51]

The abiotic stresses and their phytochemical composition produced consistent results for the presence of alkaloids, tannins, coumarins, phenols, cardiac glycosides under all stress conditions. However, the absence of saponins and flavonoids were observed to be consistent throughout the abiotic stresses. Terpenoids were present in high salinity and drought conditions and were absent in flood and nutrient deficient conditions. Steroids were present only in drought conditions and were absent in high salinity, flood, and nutrient deficiency conditions. The variation could be a result of adaptive mechanism displayed by the plant on different stress conditions. During extreme conditions, the phytochemical composition changes due to metabolic pathways that help plants survive, which can affect synthesis of secondary metabolites.[52] Qualitative data on analysis of abiotic stress conditions phytochemical composition are currently lacking in the scientific literature therefore further investigations is required.

According to the TPC results, the highest TPC was caused drought stress (71.69 GAE g^{-1}). Since phenolics function as antioxidants to lessen the harm promoted by ROS, the rise is the result of oxidative stress.[53] A moderate increase in salinity (26.69 GAE g^{-1}) causes oxidative stress which in turn increases the phenols.[54] Conversely nutrient deficiency (20.59 GAE g^{-1}) and flood stress (18.83 GAE g^{-1}) resulted in slight increase indicating no significant difference compared to the control which was the baseline for the comparison of TPC.

According to the TAC results, the highest was caused by drought stress (33.765 AAE g^{-1}) indicating a protective mechanism for the oxidative stress by ROS .[55] Moderate increase in high salinity (29.76 AAE g^{-1}) is reflected by the plant's response to prevent oxidative damage and preserve cellular homeostasis.[56] The low levels of TAC in flood (14.145 AAE g^{-1}) are due to the hypoxia condition leading to root damage thus lowering the ascorbic acid levels.[57] Conversely nutrient deficiency (18.705 AAE g^{-1}) resulted in slight increase indicating no significant difference compared to the control which was the baseline for the comparison of TAC.

According to the TFC results, the highest was caused by high salinity stress (63.405 QE g^{-1}), indicating a protective mechanism for the oxidative stress by ROS, reducing the ion toxicity and altering the osmotic pressure.[58] Moderate increase in flood (56.19 QE g^{-1}) and nutrient deficiency (44.895 QE g^{-1}) is reflected by the plant's tolerance and the stress signalling pathway.[59] The low levels of TFC in drought (3.39 QE g^{-1}) are due to the hypoxia condition leading to root damage thus lowering the quercetin levels.

According to the TPrC results, the highest was reported by drought (94.36 BSA g^{-1}), indicating a protective mechanism for stress by producing proteins and proteases which help in increasing the protein content.[60] In high salinity (29.76 BSA g^{-1}) denaturation of proteins take place causing the protein levels to decrease. In flood (14.145 BSA g^{-1}) and nutrient deficit (18.705 BSA g^{-1}) conditions, oxygen deprivation and low levels of nutrients hinders protein synthesis thus lowering the protein content.[61]

Methanolic extract of *S. dulcis* indicated antibacterial activity in the antibiotic sensitivity testing using well diffusion against a gram-negative bacteria *E. coli* and a gram-positive bacteria *S. aureus*. The positive control (ciprofloxacin) inhibited the bacterial growth with inhibition zone while the negative control (methanol) no inhibition zone suggesting the methanol did not affect the growth. Inhibition of *E. coli* and *S. aureus* in the *Spondias dulcis* plant confirms the presence of antimicrobial activity, also in previously mentioned in research articles.[12] Investigating plants under abiotic stress is essential for enhancing

agricultural resilience, securing food supply, and responding to climate changes. It assists scientists in understanding plant responses, developing stress-tolerant plants, promoting ecological restoration and driving biotechnological advancements for sustainable agriculture.

5. Conclusion

In conclusion, this research examined abiotic stresses like high salinity, flood, nutrient deficiency and drought responds to S. dulcis plant. The phytochemical composition of the plant suggests presence of alkaloids, tannins, coumarins, phenols, cardiac glycosides, terpenoids, steroids, under different stress conditions and indicated the absence of flavonoids and saponins. The quantitative biochemical analysis showed, drought induced plants had a significant impact on TPC, TAC and TPrC. High salinity increased TAC and TFC. Nutrient deficiency and flooding also increased TFC. Methanolic extract of *S. dulcis* displayed antibacterial activity against *E. coli* and *S. aureus.* The study's findings have shown Spondias dulcis to have potential health benefits as antioxidant, and antibacterial properties, while withstanding abiotic stresses in different environments. Plant research under abiotic stress conditions contributes to the development of resilient plants, improved food security, and sustainable agriculture during environmental changes.

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