

Effect of Concurrent Extreme Temperatures and Water Deficit on the Phytochemistry, Antimicrobial and Antioxidant Activities of *Portulacaria Afra* Jacq Using Four Extraction Solvents

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The study focused on *Portulacaria afra*, a plant native to South Africa which is renowned for its traditional medicinal applications against oral and skin infections. This study aimed to explore the phytochemical composition, antimicrobial, and antioxidant capabilities of the leaves, stems, and roots under concurrent extreme temperatures (hot and cold) and water deficit conditions to further validate its medicinal significance. To achieve this, the study subjected *Portulacaria afra* to various temperatures ranging from 0/5°C to 35/45°C combined with water deficit and evaluated its antimicrobial and antioxidant properties. Phytochemical compounds were extracted from different solvent extracts of *P. afra* and analysed. Antioxidant activity was assessed using metal chelating activity, hydrogen peroxide scavenging, and 2,2-diphenylpicrylhydrazyl (DPPH) free radical assays, while antimicrobial efficacy was determined using the agar-well diffusion method against three microbial strains: *Staphylococcus aureus*, *Escherichia coli*, and *Streptomyces griseus*. Results revealed an enhanced presence of 13 phytochemical groups in *Portulacaria afra* extracts compared to controls, with peak accumulation of phenolic and flavonoid compounds observed under extreme hot temperatures (35/45°C) with water deficit. Extracts from these conditions exhibited superior inhibitory effects against *Escherichia coli*. Moreover, antioxidant activity peaked at hot temperatures (30/40°C) for 2,2-diphenylpicrylhydrazyl (DPPH) and metal chelating assays, while the most effective hydrogen peroxide scavenging activity was observed under hot temperatures (35/45°C) with water deficit. Antioxidant activity peaked at mid-range hot temperatures (30/40°C) for DPPH and metal chelating, while the best hydrogen peroxide scavenging activity was observed during concurrent extreme hot temperatures (35/45°C) with water deficit. The findings suggest that extreme temperature variations combined with water deficit significantly impact the biological activities of *Portulacaria afra*, highlighting the plant's adaptability and resilience while retaining its therapeutic potential.

Keywords: Antioxidant Activity assays; Extreme temperatures; Phytochemical screening; *Portulacaria afra*; Water deficit; Whole plant.

Abiotic factors encompass a spectrum of challenging environmental elements that significantly influence ecological systems. These factors include, but are not limited to, temperature variations, drought occurrences, salinity levels, heavy metal concentrations, water stress, cold

temperature exposure, anoxic conditions, high-intensity light exposure, and imbalances in nutrient availability¹.

The impact of abiotic factors, notably temperature fluctuations and water deficits, presents a significant impediment to the reproductive

processes of medicinal plants. This interference consequently disrupts biosynthetic pathways, which in turn affects secondary metabolites accumulation and subsequent alterations in phytopharmacological activities².

Elevated temperatures induce heat stress, while reduced temperatures result in cold stress. The substantial impact of high temperatures extends to the composition of phytochemicals³. Elevated temperatures typically correlate with heightened biosynthesis of secondary metabolites in plants, thereby enhancing the production of secondary metabolites across various plant species⁴. Conversely, high temperatures inducing stress were observed to result in reduced levels of secondary metabolites in plants⁵.

Water deficit results in a decrease of water absorption and potentials within plants, consequently negatively impacting various physiological processes and altering the biosynthesis of secondary metabolites⁶. Earlier research has demonstrated the profound impact of water deficit or drought stress on the composition of secondary metabolites in herbal plants, leading to increased accumulation. An instance is the increased proportion of flavonoids and phenolics observed in plants as a response to the stress-induced by water deficit⁷.

In adverse environmental conditions, plants exhibit resilience against diverse abiotic stresses through the activation of adaptive mechanisms⁸. These mechanisms encompass the synthesis of chemicals facilitating the assembly of proteins and cellular structures, osmotic adjustments, maintenance of general homeostasis, and modulation of the antioxidant system. Additionally, this response potentially leads to heightened production of secondary metabolites such as phenolics¹.

According to recent climate change models, it is more likely than previously thought that plants will experience novel or rigorous concurrent abiotic stresses in the years to come⁹.

Earlier research findings have reported that plant responses to stresses caused by combined antagonistic abiotic factors, are phenomenal compared to when exposed to single ones⁹. The long-term effects of these abiotic factors may significantly affect the quality and biochemical productivity of medicinal plants such as *P. afra* by

disrupting the phytopharmacological activities, metabolic, developmental, and physiological processes⁹. These have in turn contributed to the need to study abiotic stress combinations within medicinal plant species.

Portulacaria afra is a succulent evergreen plant indigenous to South Africa, Kenya, and Mozambique. The taxonomic classification of *Portulacaria afra* falls within the family *Portulacaceae* or alternatively recently categorized under *Didiereaceae*. According to ethnobotanical knowledge, and a few studies, *P. afra* leaves and leaf juice is renowned for its curative properties against skin diseases and oral infections¹⁰ and phytochemical studies on *P. afra* tissue extracts revealed a strong presence of various phytochemicals¹¹. Also, it was previously demonstrated that *P. afra* extracts exhibited high levels of therapeutic properties in all plant parts, with a strong presence of coumarins known for anti-cancer properties in the methanolic leaf and stem extract¹². Basson¹³, also reported how *P. afra* leaves showed significant increase in phytochemicals and biological activities after exposure to elevated CO₂. The lack of comprehensive scientific documentation regarding the impact of combined environmental factors on *P. afra* is a notable limitation. This prompted the need to investigate on the impact of concurrent extreme temperatures (hot and cold) with water deficit on the phytochemistry, antimicrobial and antioxidant activities in *P. afra*.

MATERIALS AND METHODS

Materials

In accordance with standard laboratory protocols, analytical-grade chemicals and solvents were used throughout the experimental procedures. The specific substances, namely gallic acid, Folin-Ciocalteu reagent, methanol, *n*-hexane, ethyl acetate, Mueller-Hinton agar, Baird Parker agar, dimethyl sulfoxide (DMSO), 2,2-diphenylpicrylhydrazyl (DPPH), and iron (II) chloride, were procured from Sigma-Aldrich, USA, bearing the corresponding CAS numbers 1162-658.

In this study, the walk-in convirons (Conviron, Winnipeg, Canada; model No-PGW40) with specifications meticulously detailed bearing serial N0-170028 and manufactured in China (280033R01) were used. It operates at 230/400

Volts, 50 Hz frequency, and a three-phase system. The total input amperage is 29.2, with the compressor drawing 65.5 Amps, while designed to function under high pressure at 400 PSI and low pressure at 200 PSI. Control, lighting, heater, fan, and drier systems require 2.0, 9.4, 4.3, 2.6 HP, and 10.9 Amps respectively. Additionally, specific details regarding receptacle, glycol pump, motor, and auxiliary amperages were included. The system's minimum circuit ampacity (MCA) is 31.2 Amps, with a maximum overcurrent protection (MOP) set at 32 Amps, and a robust short circuit withstand capacity of 5000 Amps RMS.

Plant Material collection

In July 2021, cuttings of *Portulacaria afra* were cultivated at the University of the Witwatersrand in Johannesburg, South Africa (26.1899° S, 28.0319° E) for propagation. The plant specimen was verified by a botanist and a voucher specimen (IRM 001) was placed in the institute's herbarium centre.

Green cuttings with non-lignified stems were carefully chosen from a pathogen-free parent plant of *P. afra*. To hold the stem cuttings for roots, 225 pots measuring 2 to 2.5 litres each were filled with professional cutting mix soil from Culterrean Potting.

The plants underwent a three-month acclimatization period and root system development in the Oppenheimer Life Sciences greenhouse facilities within the premises of University of the Witwatersrand in Johannesburg, South Africa (26.1899° S, 28.0319° E). The plant growth was observed and sustained by administering 500 ml of water per potted plant every 48 hours due to their low water requirement.

Sample size per harvest: n= 15/harvest

The conviron simulations settings used for the treatments were in accordance with the South African Department of Environmental Affairs predictions¹⁴, IPCC¹⁵, and Mbokodo¹⁶ records on the variations in the current and anticipated climatic conditions. The treatments were selected in terms of temperature ranges and the span of heat and cold waves. Control temperature ranges were based on records from the South African Weather Service. The different temperature settings used were (25/27°C), (0/5°C), (10/15°C), (30/40°C), and (35/45°C). Each plant was placed in the controlled climate simulation chamber (Convicon chambers,

PGW40) with the high and low temperatures set concurrently with water deficit while other parameters such as CO₂ (400 ppm), humidity (60%), light (160 nits/level 1) pH, salinity, were kept at ambient. The lightning was programmed to operate for a duration of 12 hours daily, spanning from 6 am to 6 pm¹⁷.

Five plants were harvested episodically 3 times every 48hrs for up to 6 days: (48hr, 96hr, 144hr) (Table 1). Samples were subjected to oven drying at 40°C for a period of 4 days post-harvest. This procedure was repeated three times for each of the treatments. Where, n is [15 plants per harvest period-(48hrs, 96hrs, 144hrs) =45 plants per treatment]; n=45 for control (25°), n=45 for (0/5°C), n=45 (10/15°C), n=45 (30/40°C), n=45 (35/45°C) with a total sample size of 225.

Sample preparation and extraction

The leaves, stems, and roots were systematically gathered and subjected to a sequential process: initial harvesting, followed by thorough washing using deionized water, and subsequent drying in a hot air dryer maintained at 40°C for a duration of four days. Post-drying, the plant materials were finely ground into a crude powder using an electric grinder. This powdered form was then enclosed in foil and secured in an airtight container. Subsequently, it was stored in a dark cupboard at room temperature (32°C) until required for subsequent testing procedures¹⁸.

Preparation of extracts

The plant extract of the leaves, stems and roots were produced through sequential extraction using: 80% methanol, *n*-hexane, ethyl acetate, and distilled water, boiled at 60°C. Each extraction involved placing 3g of powdered plant material into individual glass bottles with cover, with the addition of 30 ml of the respective solvents¹⁹. The methanol, *n*-hexane, ethyl acetate extracts were placed on a shaker, the water extract underwent preparation using a sonicator at a specific temperature (60°C) for a duration of 45 min. The shaking of all extracts lasted 48 hrs, except for the water extracts. The water extracts underwent centrifugation aimed at reducing their viscosity, followed by filtration through filter paper into vials. The residual supernatant was removed, and subsequently, the vials were enveloped in foil and refrigerated at 4°C until the commencement of the tests.

Preliminary Phytochemical Screening Tests

The chemical constituents within the leaves, stems, and roots extracts underwent evaluation using standard colour test methods. A qualitative analysis was conducted in order to determine the presence or absence of quinones, coumarins, phenols, carbohydrates, terpenoids, flavonoids, glycosides, steroids, saponins, amino acids, phytosteroids, and volatile oils, through visual observation for alterations in colour or precipitation with the adoption of the procedure described by Roghini and Vijayalakshmi²⁰. The observation for each test was then documented.

Evaluation of Total Phenolics and Total Flavonoids Contents

Folin-Ciocalteu reagent assay was used for the approximation of the total phenolics and flavonoids content in *Portulacaria afra* extracts using the procedure outlined by Teffo²¹. Standard solutions of gallic acid at varying concentrations were used to construct a calibration curve. The concentrations of total phenolic content within the extracts were extrapolated from this curve, using the equation: $y=0.9287x+0.0658$, $r^2=0.9952$. Denoting the findings as gallic acid equivalents per gram (mg GAE/g) of the extract's dry weight.

Similarly, the total flavonoid contents of the plant parts were assessed using a quercetin-based standard curve, using the equation: $y=0.2388x-0.0019$, $r^2=0.9997$ with the results expressed as quercetin per gram (mg RE/g) of dry weight.

Antibacterial activity assay

The antibacterial inhibitory activity of *Portulacaria afra* extracts was assessed by following the agar-well diffusion procedure described by Teffo²² with a few modifications. The bacterial strains were obtained from Thermo-Fisher's laboratory specialists in Johannesburg (Pty) Ltd, South Africa, and the Mueller-Hinton (MH) agar was purchased from Sigma Aldrich Ltd. Briefly, Petri dishes were first sterilized and autoclaved prior to the addition of Mueller-Hinton agar and Baird Parker agar. Once the agar solidified, sterile cotton swabs were used to uniformly inoculate the surfaces of the agar with specific test microorganisms. Baird Parker agar plates were inoculated with Gram+ *Staphylococcus aureus* (ATCC 25923), whereas Mueller-Hinton agar

plates were inoculated with Gram- *Escherichia coli* (ATCC 25922), and Gram+ *Streptomyces griseus* and were incubated at 37°C for 24 hours.

Six wells were created in the inoculated media with successful growth of pathogens, using a 6 mm sterile borer. Subsequently, 100 μ l of plant extracts were introduced into five of these wells, while the sixth well served as the negative control (dimethyl sulfoxide (DMSO)). The plates were then covered and refrigerated for 30 minutes to allow for diffusion before being transferred to an incubator set at 37°C for 24 hours. Following the incubation period, the diameters of the zones of inhibition were measured in millimetres using a ruler. The presence of an inhibitory zone indicated positive antibacterial activity. The experiment was conducted in triplicate, and the data were analysed using Microsoft Excel to express the results as mean \pm standard deviation. $P < 0.05$ was used to determine the statistical significance of the findings.

In vitro antioxidant activity assessment

The assessment of antioxidant potential was conducted using the metal chelating activity assay, hydrogen peroxide scavenging (H₂O₂), and the 2,2-diphenylpicrylhydrazyl (DPPH) free radical assay for the respective plant parts as described below:

DPPH scavenging activity assay

A 2, 2 diphenyl picrylhydrazyl (DPPH) free radical assay described by Basson²³ was used with some modifications. In the determination of the scavenging potential of DPPH, 50 mg of DPPH was dissolved in 100 ml of 80% methanol to create a stock solution. From this stock solution, a working solution was derived at a ratio of 1:5 with 80% methanol. Each extract had 5 concentrations, ranging from 10 to 50 μ L, and was mixed with 700 μ L of the working solution which was then adjusted to a final volume of 1 ml with 80% methanol to create a reaction mixture. A control solution was prepared using the working solution and 80% methanol, while a blank was generated using solely 80% methanol. These mixtures were then incubated in darkness for 45 minutes, after which the absorbance was measured at a wavelength of 517 nm using a spectrophotometer. The inhibition (%) was expressed with the following equation:

DPPH Scavenging Effect (%) = [(Absorbance of sample – Absorbance of blank)/Absorbance of control] x 100

Metal chelating activity

The metal chelating antioxidant assay was carried out by following the procedure of Adusei²⁴ with few modifications. 2 ml of extracts was combined with 0.25 ml of a 250 mM iron (II) chloride solution with an addition of 0.25 ml of ferrozine. The resulting mixture was vigorously shaken and left at room temperature (32°C) for a duration of 10 minutes. Following this, the absorbance was quantified at a wavelength of 562 nm. Methanol was used as the blank, while a control group (without extracts) was used. The percentage of metal chelation was determined with the formula below:

$$\text{Metal chelation (\%)} = \{(A_0 - A_1) / A_0\} \times 100$$

A₀ represents the absorbance of the control while A₁ stands for the absorbance of the test samples.

Hydrogen peroxide scavenging (H, O₂) assay

The hydrogen peroxide scavenging activity was determined by adapting the methods of Bouabid²⁵. 1 ml of extracts was combined with 3.4 ml of phosphate buffer (50 mM, pH 7.4). Following this, 600 μ l of 40 mM hydrogen peroxide (30%) was introduced into the solution. The resulting mixture was left at room temperature (32°C) for a duration of 40 minutes. Subsequently, the absorbance of the solution was determined using a spectrophotometer at a wavelength of 230 nm. The hydrogen peroxide scavenging activity was quantified using the provided formula:

$$\text{Scavenged H}_2\text{O}_2 \text{ (\%)} = [(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100$$

Statistical Analysis

The concentration values were plotted against the % inhibition values in Microsoft Excel to derive a trend line equation used for calculating the IC₅₀. A one-way analysis of variance (ANOVA) was used to determine the significant differences. All experiments were conducted thrice, and data were presented as mean \pm standard error (SE) for n = 3.

P < 0.05 was used to determine the statistical significance of the findings and Tukey HSD post hoc tests in R Studio was used to determine where the significance lies. The significant differences (P < 0.05) were indicated by different letters (a, b, c, d) which were compared with the control within the same group.

RESULTS AND DISCUSSION

Qualitative screening of phytochemicals

The aqueous (60°C), methanol, *n*-hexane, and ethyl acetate extracts of *P. afra* subjected to concurrent extreme hot and cold temperatures and water deficit were screened for their phytochemical constituents. For the qualitative phytochemical screening, the leaves, stems, and roots extracts were evaluated for the presence of saponins, flavonoids, glycosides, quinones, phenols, terpenoids, steroids, phytosteroids, volatile oil, carbohydrate, amino acids and coumarins. In general, phytochemicals in the plant parts varied according to the different solvents mentioned above. Increasing trends were noticed in the phytochemical presence under concurrent cold & hot temperatures with water deficit condition as shown in the heat map (Tables 2 and 3). Table 2 shows the fluctuations in the presence of phytochemical contents of the leaves, stems, and roots of *P. afra*, when plant parts were subjected to concurrent cold & hot temperatures (10/15°C and 30/40°C) with water deficit conditions. Table 3 summarizes the results of the preliminary phytochemical screening under concurrent cold & hot temperatures (0/5°C and 35/45°C) with water deficit conditions.

Quantitative analysis of phytochemical

Quantitative results for the total phenolics (TPC) and total flavonoid content (TFC) in Figure 2 – 9, show the variations in the phytoconstituents across all the plant extracts, under concurrent cold and hot temperatures [(10/15°C), (30/40°C)] and [(0/5°C), (35/45°C)] with water deficit condition. The highest TPC and TFC in the plant extracts in comparison with the control samples were found in cold temperatures (10/15°C) with water deficit settings (Figures 2 and 3 respectively). The above were recorded in the aqueous stem extracts after a 144-hour treatment period and aqueous leaf extracts after a 48-hour treatment period (9921.55 \pm 8.91 mg GAE/g and 7529.09 \pm 3.12 mg QE/g; Figures 2 and

3 respectively) with control samples values for TPC (1820.67 ± 25.08 mg GAE/g) and TFC values (592.70 ± 49.99 mg QE/g) in the aqueous stem and leaf extracts respectively. ANOVA results showed a significant difference ($P < 0.05$) in *P. afra*, Tukey test in R Studio revealed where the significance lies among plant parts.

Antibacterial activity assay

Observed variation in the inhibitory effect of *Portulacaria afra* extracts against the tested microorganisms are presented in Table 4 – 7. The highest level of inhibitory effect of 21mm against gram-negative *Escherichia coli* in comparison with the control samples was observed in the methanolic root extracts after a 48hr-treatment period, upon exposure to combined extreme hot temperatures ($35/45^\circ\text{C}$) and water deficit, (Table 7). This was also followed by a considerable effect of 14mm against *Escherichia coli* in the methanolic stem extracts (Table 7). Furthermore, the inhibitory activities observed in the ethyl acetate extracts were intermediate, ranging from 9-12mm, with an increasing trend when compared with the control samples (Table 7). No inhibitory activity was observed against *Escherichia coli* across all the plant extracts exposed to hot temperatures ($30/40^\circ\text{C}$), however, intermediate inhibitory activities ranged from 11-13mm against gram-positive *Streptomyces griseus* and *Staphylococcus aureus* (Table 6). Under the cold temperatures settings ($10/15^\circ\text{C}$) with water deficit condition (Table 4), a slight inhibitory effect of 11mm was

observed only in the *n*-hexane and ethyl acetate root extracts against gram-positive *Streptomyces griseus* and *Staphylococcus aureus* after a 48hr and 96-hour treatment period respectively, with a decline in activity when compared with the control samples. However, there were no inhibitory activities observed across all the plant extracts exposed to cold temperatures ($0/5^\circ\text{C}$) with water deficit condition (Table 5). From these findings, the examined plant extracts exposed to temperatures [($30/40^\circ\text{C}$) and ($35/45^\circ\text{C}$)] with water deficit condition showed better antimicrobial activity against all the tested microorganisms. ANOVA results showed a significant difference ($P < 0.05$) in *P. afra* extracts. The findings indicate that the antimicrobial activity of *P. afra* extracts is subject to the plant part, temperature and water deficit.

Antioxidant Activity

The *in vitro* antioxidant potential of all the extracts was determined by a 2, 2 diphenylpicrylhydrazyl (DPPH) free radical assay, hydrogen peroxide scavenging (H₂O₂), and metal chelating activity assay, where IC₅₀ value is the amount of extract required to decrease the absorbance of antioxidant radicals by 50%. Lower IC₅₀ values indicate stronger antioxidant scavenging ability of the extracts. Figure 10 – 21 graphically show the various changes observed in the antioxidant activities of *Portulacaria afra* extracts upon exposure to hot and cold temperatures and water deficit, when compared with the control samples. Under the cold temperatures settings [



Fig. 1. *Portulacaria afra* leaves, stems, and roots

(10/15°C) and (0/5°C)] and water deficit (Figure 10 –15), a significant increasing trend of antioxidant capacity was observed across all plant parts when compared to the control samples. Similarly, under hot temperatures [(30/40°C) and (35/45°C)] with water deficit conditions (Figure 16 – 21), an increase in antioxidant activity was observed in some of the extracts in comparison with the control samples. The highest DPPH and metal-chelating antioxidant activity was observed in the ethyl acetate root extracts after a 96-hour treatment period and the methanolic leaf extracts after a 144-hour treatment period (0.26±0.065 mg/ml; Figure 16) and (0.40±0.078 mg/ml respectively; Figure 17), under concurrent hot temperatures settings (30/40°C) with water deficit. However, *n*-hexane stem extracts under concurrent hot temperatures settings (35/45°C) with water deficit conditions showed the strongest hydrogen peroxide scavenging activity (0.14±0.048 mg/ml; Figure 21) after a 144-hour treatment period.

Data available here reveal excellent antioxidant activity of all plant parts. The methanolic leaf extracts showed the strongest antioxidant activity against ferric ion radical, ethyl acetate root extracts also showed a very strong scavenging ability against DPPH radical, while *n*-hexane stems extract scavenged hydrogen peroxide radical much better, indicating the strongest antioxidant activity of *Portulacaria afra* across the three different assays. All IC₅₀ values were less than 1 mg/ml with extracts showing significant differences (P<0.05).

Components such as root, stem, leaf, flower, fruits, and seed are categorised according to the synthesis, accumulation, and distribution patterns of phytochemicals²⁶. In specific organs, tissues, and cells, distinct regulatory pathways and transport routes facilitate the synthesis of

diverse secondary metabolites across various parts of medicinal plants. This phenomenon arises due to the complexity and diversity inherent in these secondary metabolites²⁶.

Extraction solvents are important in the extraction of potential antioxidant compounds, and are determined by variations in chemical characteristics, polarities, and solubility of these compounds²⁷. Hence, the solvents options for extraction of phytochemicals in this study were chosen based on the polarity of the solute, for better accuracy in dissolving the solute²⁷. The presence of phytochemicals in the leaves, stems, and roots extracts of *P. afra* exposed to various treatments, when compared with the control, showed that the methanol extracts, across all the parts, in both hot & cold conditions showed the most presence of phytochemicals, followed by the aqueous extracts. This is in accordance with earlier and current research as they are mostly recommended for phytochemical extractions²⁸, due to their high polarity index of 5.1 and 10.2 respectively²⁷, which also validates the use of hot water extraction in traditional medicine.

Previous findings have documented that methanol and water target sugars, amino acids, and glycosides during phytochemical extraction. On the other hand, alkaloids, aglycones and glycosides are commonly extracted with ethyl acetate while *n*-hexane extracts waxes, fats, fixed oils and volatile²⁹.

Tannins are complex polyphenolic biomolecules with abundant hydroxyls and carboxyl groups, they form strong complexes with macromolecules. They are known for anti-inflammatory and curative properties, used in treating piles, burns, and venereal diseases, and serve as insecticides and regulators of plant

Table 1. Experimental Design

	Control	Treatment A	Treatment B	Treatment C	Treatment D
Water deficit	500ml of water every 2nd day.	No water	No water	No water	No water
Temperatures (°C night/day)	25/27	0/5	10/15	30/40	35/45
Episodic harvesting frequency(hrs)	Once off	48, 96, 144	48, 96, 144	48, 96, 144	48, 96, 144

Table 2. Heat map for the phytochemical tests of *Portulacaria afra* extracts exposed to temperatures (10/15°C and 30/40°C) and water deficit

TEMP (°C)	LEAVES															
	Tannins	Saponins	Flavonoids	Glycosides	Quinones	Phenols	Terpenoids	Steroids	Phytosteroids	Volatile oils	Coumarins	Carbohydrates	Amino acids			
	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4	1 4 9 4 C 8 6 4			
LEAVES																
10/15	W	M	H	E	W	M	H	E	W	M	H	E	W	M	H	E
30/40	W	M	H	E	W	M	H	E	W	M	H	E	W	M	H	E
STEMS																
10/15	W	M	H	E	W	M	H	E	W	M	H	E	W	M	H	E
30/40	W	M	H	E	W	M	H	E	W	M	H	E	W	M	H	E
ROOTS																
10/15	W	M	H	E	W	M	H	E	W	M	H	E	W	M	H	E
30/40	W	M	H	E	W	M	H	E	W	M	H	E	W	M	H	E

ABSENT (-)	PRESENT (+)	MODERATELY PRESENT (++)	STRONGLY PRESENT (+++)
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Temp °C, temperature; C, control samples; 48, 48hr treatment; 96, 96hr treatment; 144, 144hr treatment; W, hot water extract (60°C) extract; M, methanolic extract; H, *n*-hexane extract; E, ethyl acetate extracts.

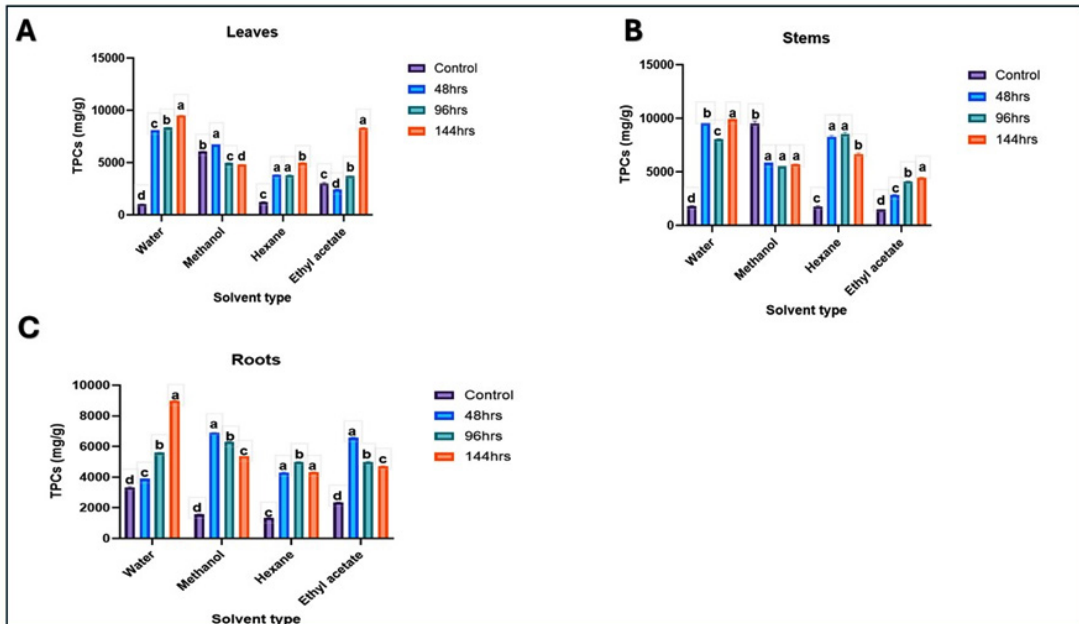


Fig. 2. Total Phenolic content in *Portulacaria afra* leaves (A), stems (B) and roots (C) extracts using four solvents under 10/15°C and water deficit. All values were presented as mean ± SE, n=3, and were quantified in terms of gallic acid (mg/g of GAE). Significant differences ($P < 0.05$) were indicated by different letters (a, b, c, d) and were compared with the control within the same group.

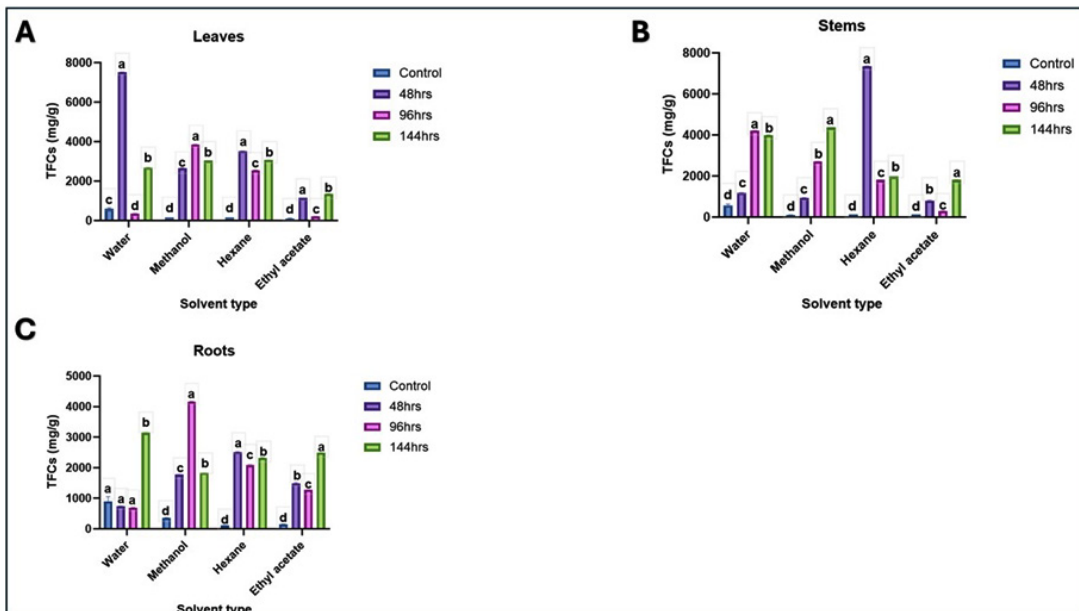


Fig. 3. Total Flavonoid content in *Portulacaria afra* leaves (A), stems (B) and roots (C) extracts using four solvents under 10/15°C and water deficit. All values were presented as mean ± SE, n=3, and were quantified in terms of quercetin concentration (mg/g of quercetin). Significant differences ($P < 0.05$) were indicated by different letters (a, b, c, d) and were compared with the control within the same group.

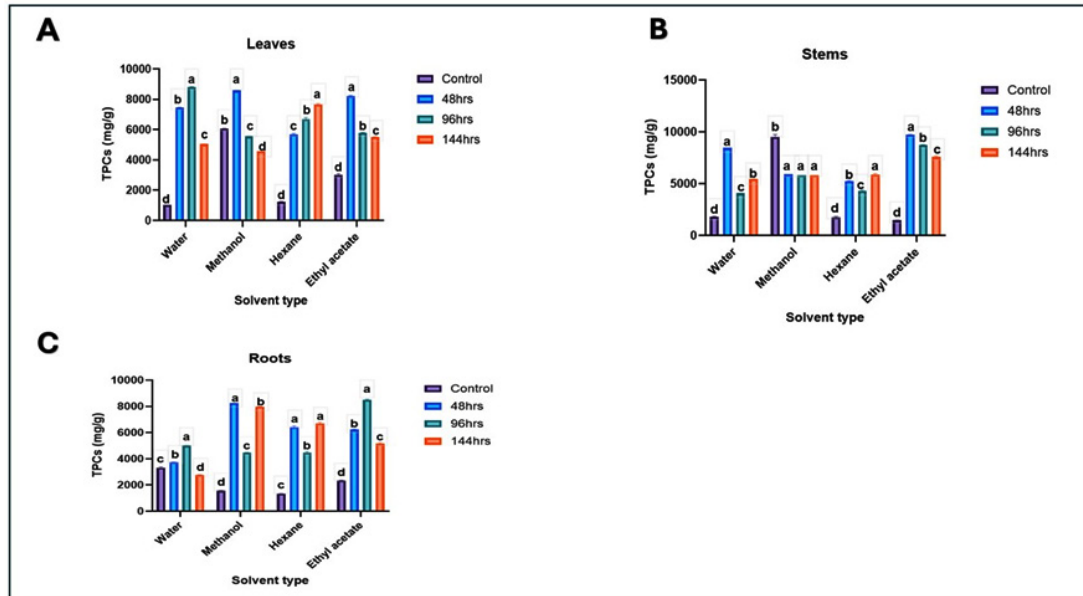


Fig. 4. Total Phenolic content in *Portulacaria afra* leaves (A), stems (B) and roots (C) extracts using four solvents under 30/40°C and water deficit. All values were presented as mean \pm SE, $n=3$, and were quantified in terms of gallic acid (mg/g of GAE). Significant differences ($P < 0.05$) were indicated by different letters (a, b, c, d) and were compared with the control within the same group.

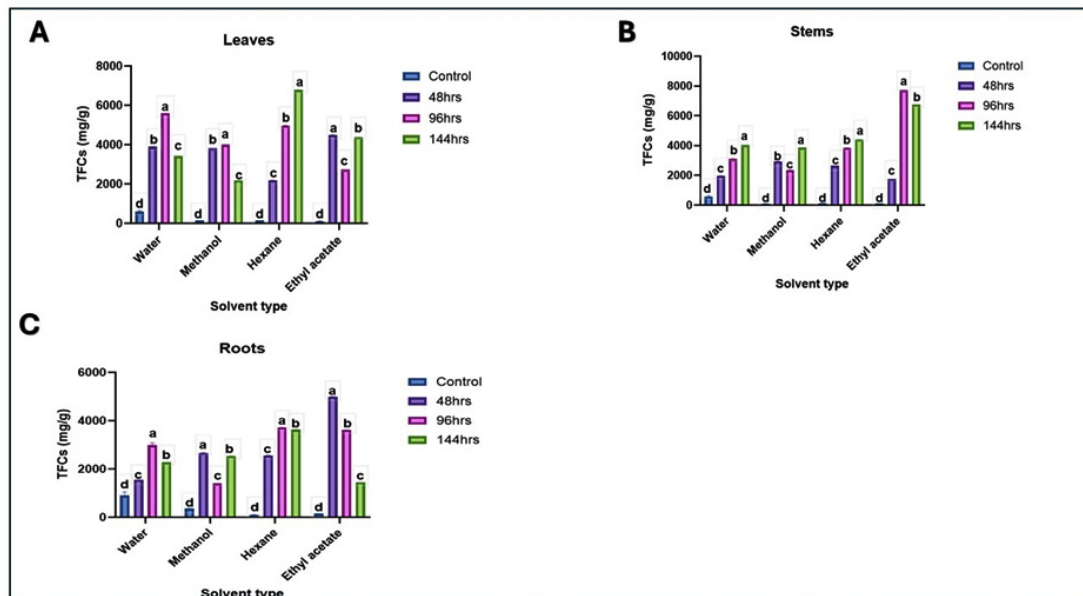


Fig. 5. Total Flavonoid content in *Portulacaria afra* leaves (A), stems (B) and roots (C) extracts using four solvents under 30/40°C and water deficit. All values were presented as mean \pm SE, $n=3$, and were quantified in terms of quercetin concentration (mg/g of quercetin). Significant differences ($P < 0.05$) were indicated by different letters (a, b, c, d) and were compared with the control within the same group.

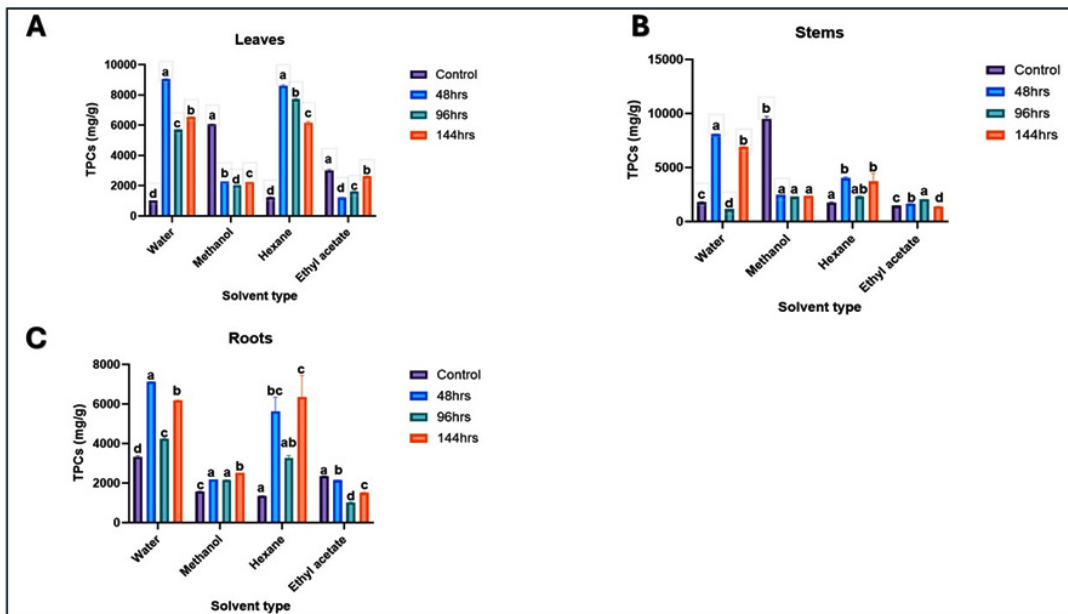


Fig. 6. Total Phenolic content in *Portulacaria afra* leaves (A), stems (B) and roots (C) extracts using four solvents under 0/5°C and water deficit. All values were presented as mean \pm SE, n=3, and were quantified in terms of gallic acid (mg/g of GAE). Significant differences ($P < 0.05$) were indicated by different letters (a, b, c, d) and were compared with the control within the same group.

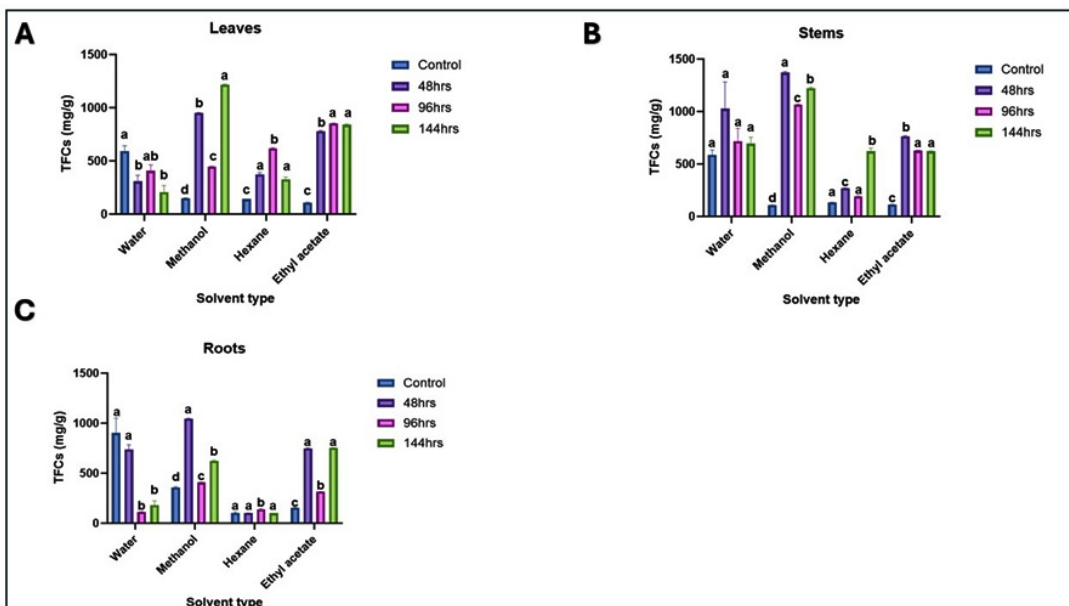


Fig. 7. Total Flavonoid content in *Portulacaria afra* leaves (A), stems (B) and roots (C) extracts using four solvents under 0/5°C and water deficit. All values were presented as mean \pm SE, n=3, and were quantified in terms of quercetin concentration (mg/g of quercetin). Significant differences ($P < 0.05$) were indicated by different letters (a, b, c, d) and were compared with the control within the same group.

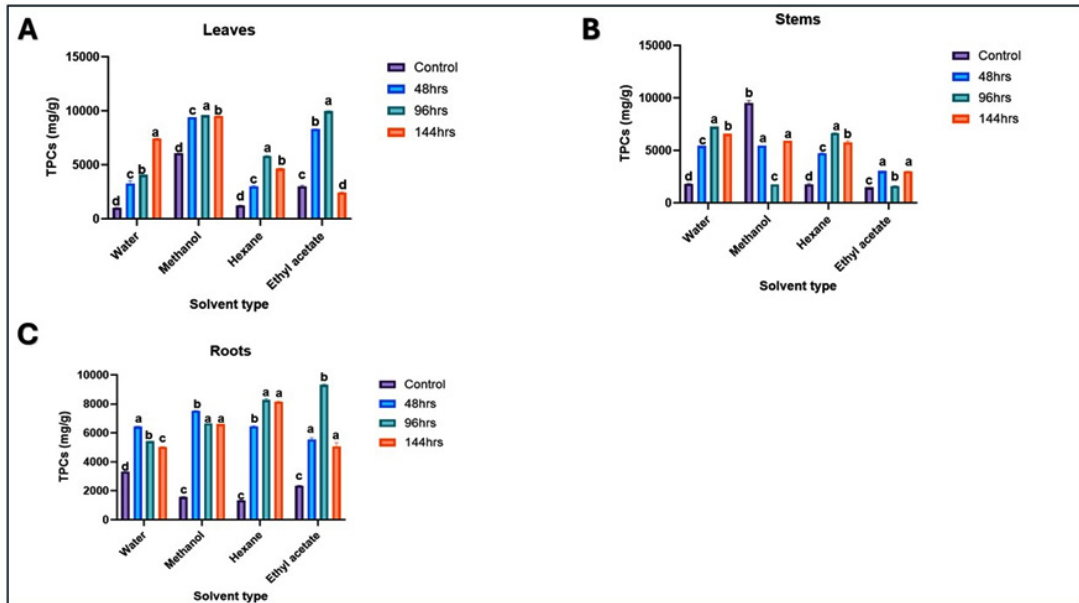


Fig. 8. Total Phenolic content in *Portulacaria afra* leaves (A), stems (B) and roots (C) extracts using four solvents under 35/45°C and water deficit. All values were presented as mean ± SE, n=3, and were quantified in terms of gallic acid (mg/g of GAE). Significant differences (P < 0.05) were indicated by different letters (a, b, c, d) and were compared with the control within the same group.

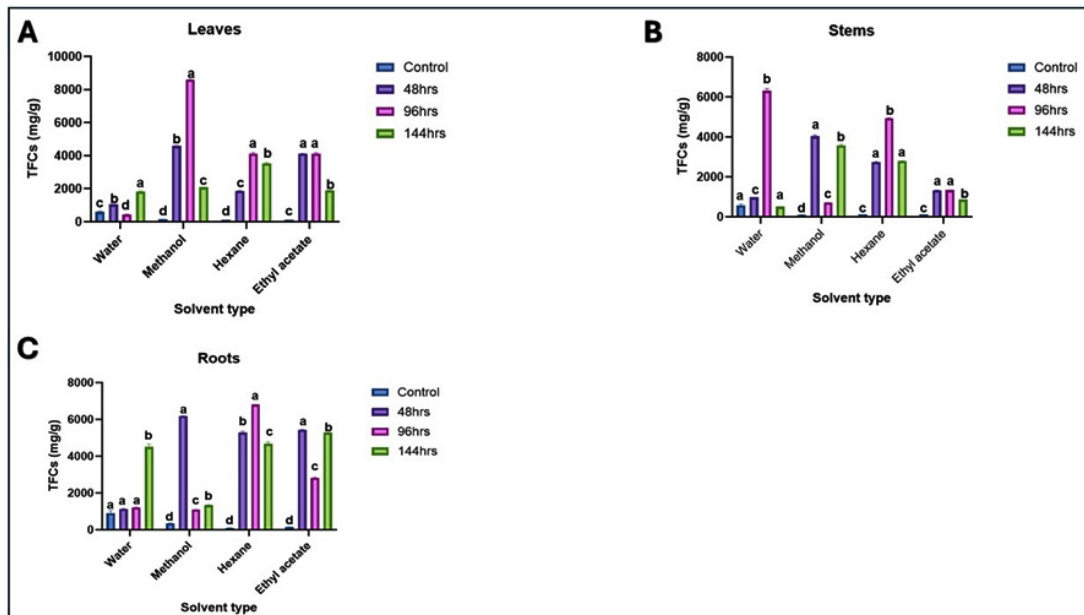


Fig. 9. Total Flavonoid content in *Portulacaria afra* leaves (A), stems (B) and roots (C) extracts using four solvents under 35/45°C and water deficit. All values were presented as mean ± SE, n=3, and were quantified in terms of quercetin concentration (mg/g of quercetin). Significant differences (P < 0.05) were indicated by different letters (a, b, c, d) and were compared with the control within the same group.

Table 4. Antibacterial activity of *Portulacaria afra* extracts against *Escherichia coli*, *Staphylococcus aureus*, and *Streptomyces griseus* under 10/15°C with water deficit

Microorganisms	Zone of inhibition (millimetres) of extracts																			
	Methanol						Hexane						Water (60°C)						Ethyl Acetate	
	C	48	96	144	C	48	C	48	96	144	C	48	C	48	96	144	C	48	96	144
Leaves	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Streptomyces griseus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stems	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Streptomyces griseus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Roots	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Streptomyces griseus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

C, Control sample extract; 48, 48hr-treatment extract; 96, 96hr-treatment extract; 144, 144hr-treatment extracts. Values are presented as mean \pm Standard Deviation, n=3

Table 5. Antibacterial activity of *Portulacaria afra* extracts against *Escherichia coli*, *Staphylococcus aureus*, and *Streptomyces griseus* under 0/5°C with water deficit

Microorganisms	Zone of inhibition (millimetres) of extracts														
	Methanol			Hexane			Water (60°C)			Ethyl Acetate					
	C	48	96	C	48	96	C	48	96	C	48	96	C	48	96
Leaves	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	16±0.6	0	0	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	18±0.6	0	0	0	0	0	0	0	14±0.6	0	0
<i>Streptomyces griseus</i>	0	0	0	0	18±0.6	0	0	0	0	0	0	0	0	0	0
Stems	0	0	0	0	13±0.6	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	13±0.6	0	0	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	13±0.6	0	0	0	0	0	0	0	0	0	0
<i>Streptomyces griseus</i>	0	0	0	0	13±0.6	0	0	0	0	0	0	0	0	0	0
Roots	0	0	0	0	15±0.6	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	17±0.6	0	0	0	0	24±0.6	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	17±0.6	0	0	0	0	0	0	0	0	0	0
<i>Streptomyces griseus</i>	0	0	0	0	18±0.6	0	0	0	0	0	0	0	0	0	0

C, Control sample extract; 48, 48hr-treatment extract; 96, 96hr-treatment extract; 144, 144hr-treatment extract. Values are presented as mean ± Standard Deviation, n=3.

Table 6. Antibacterial activity of *Portulacaria afra* extracts against *Escherichia coli*, *Staphylococcus aureus*, and *Streptomyces griseus* under 30/40°C with water deficit

Microorganisms	Zone of inhibition (millimetres) of extracts															
	Treatment period (hours)															
	Methanol			Hexane			Water (60°C)			Ethyl Acetate						
	C	48	96	144	C	48	96	144	C	48	96	144	C	48	96	144
Leaves	0	0	0	0	0	16±0.6	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	0	18±0.6	0	0	0	0	0	0	0	14±0.6	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	0	18±0.6	0	0	0	0	0	0	0	0	0	0
Stems	0	0	0	0	0	13±0.6	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	11±0.1	13±0.6	0	12±0.1	0	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	13±0.6	0	0	0	0	0	0	0	0	0	11±0.1	0
Roots	0	0	0	0	15±0.6	0	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	17±0.6	0	0	0	24±0.6	13±0.1	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	18±0.6	0	0	0	0	0	0	0	0	0	0	0
<i>Streptomyces griseus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

C, Control sample extract; 48, 48hr-treatment extract; 96, 96hr-treatment extract; 144, 144hr-treatment extracts. Values are presented as mean ± Standard Deviation, n=3.

Table 7. Antibacterial activity of *Portulacaria afra* extracts against *Escherichia coli*, *Staphylococcus aureus*, and *Streptomyces griseus* under 35/45°C with water deficit

Microorganisms	Zone of inhibition (millimetres) of extracts															
	Treatment period (hours)															
	Methanol			Hexane			Water (60°C)			Ethyl Acetate						
	C	48	96	144	C	48	96	144	C	48	96	144	C	48	96	144
Leaves																
<i>Escherichia coli</i>	0	0	0	10±0.1	16±0.6	0	0	0	0	0	0	0	0	0	10±0.2	11±0.3
<i>Staphylococcus aureus</i>	0	0	0	0	18±0.6	0	0	0	0	0	0	0	14±0.6	0	0	0
<i>Streptomyces griseus</i>	0	0	0	0	18±0.6	0	0	0	0	0	0	0	0	0	0	0
Stems																
<i>Escherichia coli</i>	0	14±0.1	0	0	13±0.6	0	0	0	0	0	0	0	0	0	12±0.1	12±0.3
<i>Staphylococcus aureus</i>	0	0	0	0	13±0.6	0	0	0	0	0	0	0	0	0	0	0
<i>Streptomyces griseus</i>	0	0	0	0	13±0.6	0	0	0	0	0	0	0	0	0	0	0
Roots																
<i>Escherichia coli</i>	0	21±0.1	0	0	15±0.6	0	0	0	0	0	0	0	0	9±0.1	11±0.2	10±0.1
<i>Staphylococcus aureus</i>	0	0	0	0	17±0.6	0	0	0	24±0.6	0	0	0	0	0	0	0
<i>Streptomyces griseus</i>	0	0	0	0	18±0.6	0	0	0	0	0	0	0	0	0	0	0

C, Control sample extract; 48, 48hr-treatment extract; 96, 96hr-treatment extract; 144, 144hr-treatment extracts. Values are presented as mean ± Standard Deviation, n=3

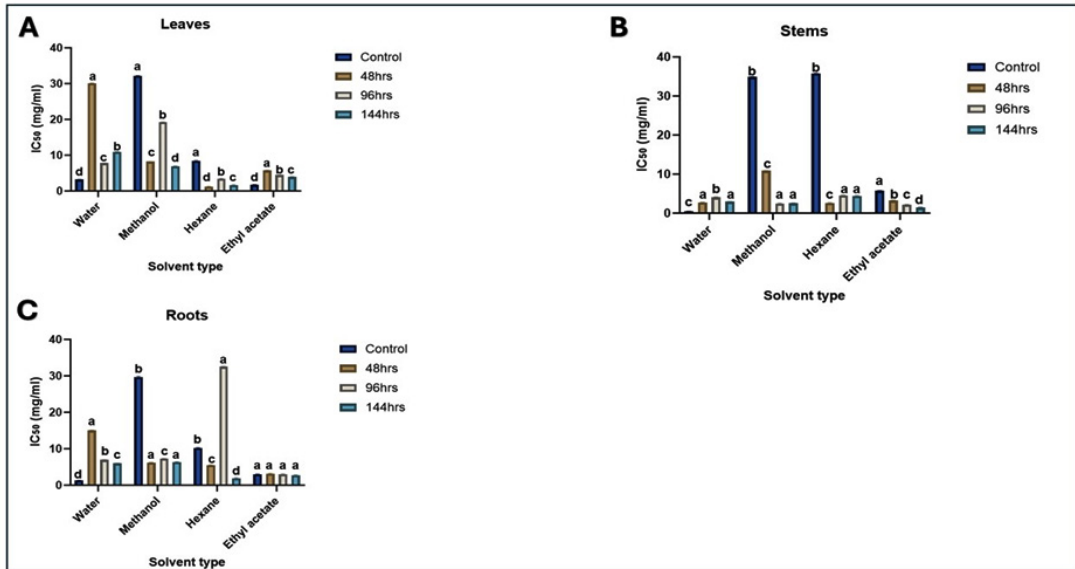


Fig. 10. Graphical representation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity in *Portulacaria afra* plant parts under 10/15°C and water deficit. IC_{50} values were denoted as (Mean±SE, mg/ml), n=3, Error bars=standard error values (SE). Statistically significant differences ($P < 0.05$) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey’s post-hoc test).

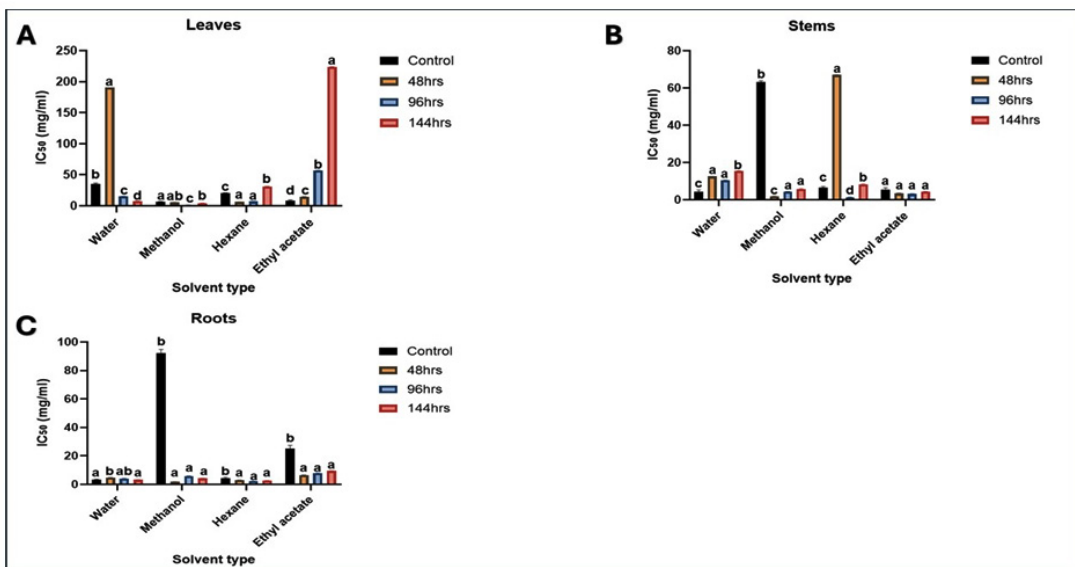


Fig. 11. Graphical representation of Metal chelating antioxidant activity in *Portulacaria afra* plant parts under 10/15°C and water deficit. IC_{50} values were denoted as (Mean±SE, mg/ml), n=3, Error bars=standard error values (SE). Statistically significant differences ($P < 0.05$) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey’s post-hoc test).

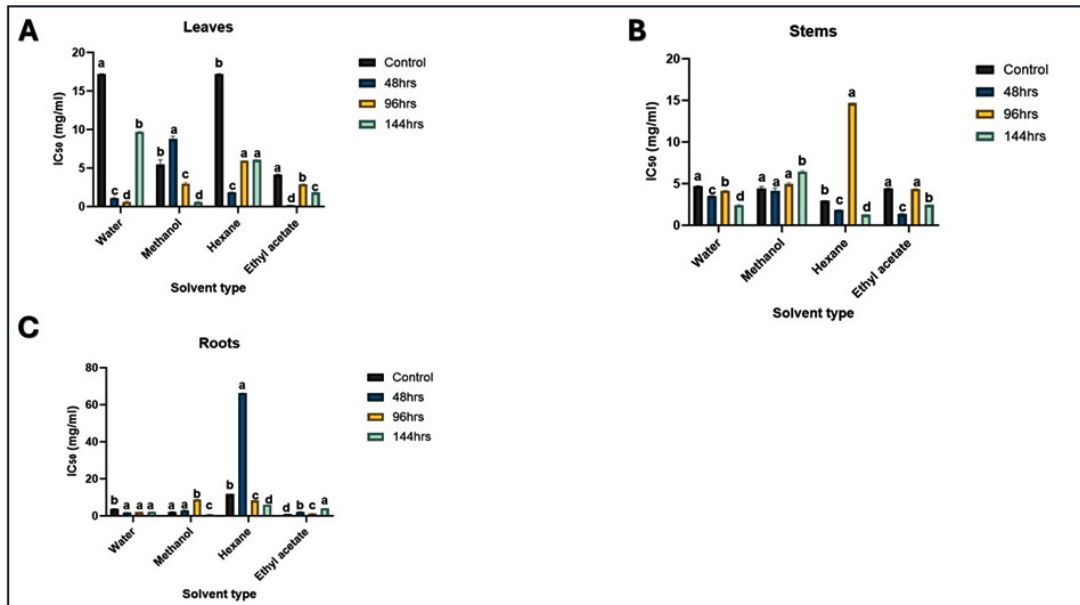


Fig. 12. Graphical representation of Hydrogen peroxide antioxidant activity in *Portulacaria afra* plant parts under 10/15°C and water deficit. IC₅₀ values were denoted as (Mean±SE, mg/ml), n=3, Error bars=standard error values (SE). Statistically significant differences (P < 0.05) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey’s post-hoc test).

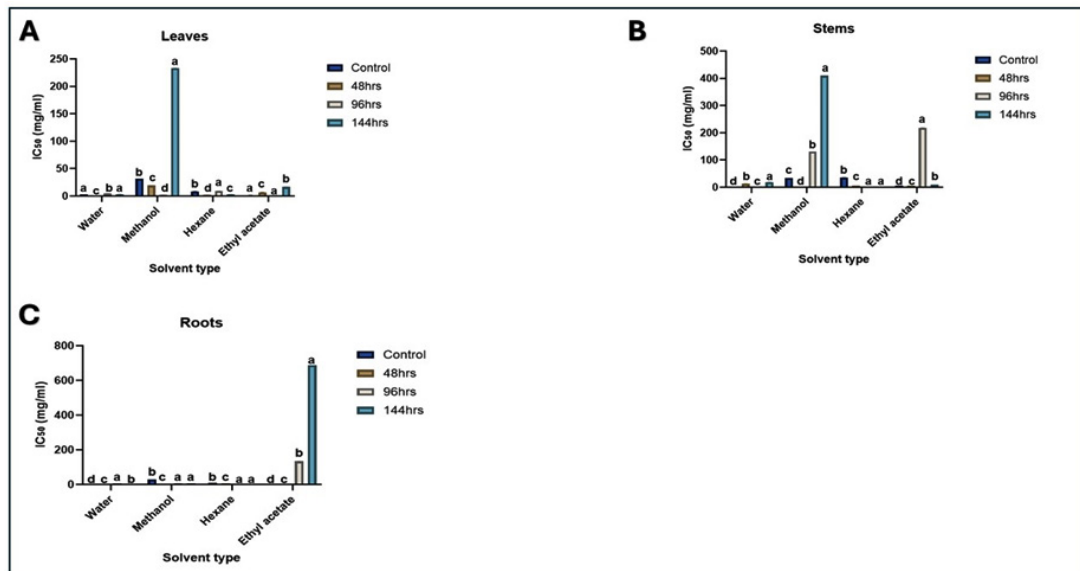


Fig. 13. Graphical representation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity in *Portulacaria afra* plant parts under 0/5°C and water deficit. IC₅₀ values were denoted as (Mean±SE, mg/ml), n=3, Error bars=standard error values (SE). Statistically significant differences (P < 0.05) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey’s post-hoc test).

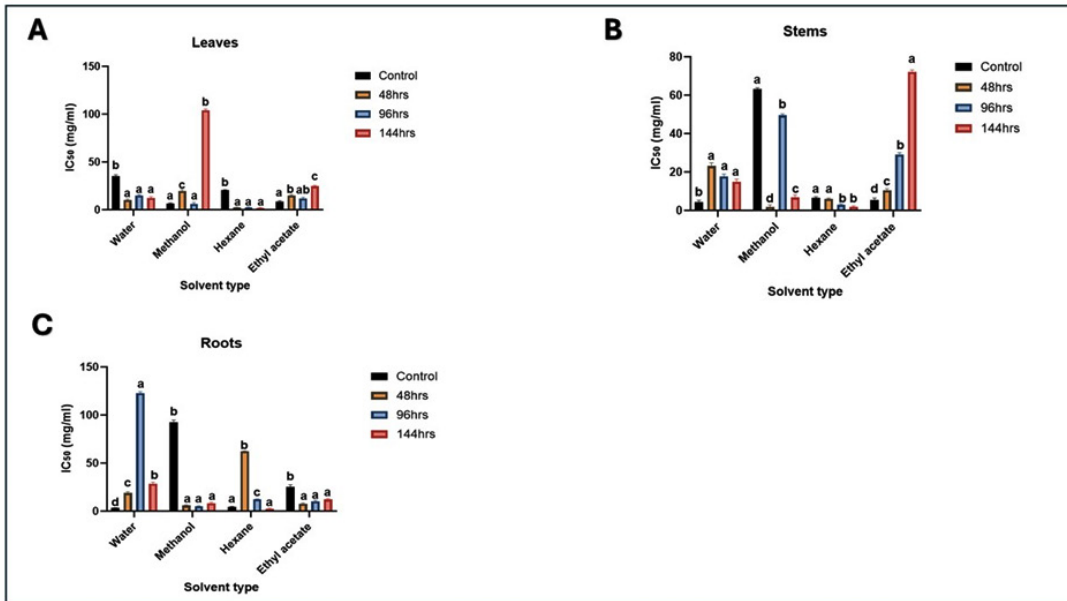


Fig. 14. Graphical representation of Metal chelating antioxidant activity in *Portulacaria afra* plant parts under 0/5°C and water deficit. IC₅₀ values were denoted as (Mean±SE, mg/ml), n=3, Error bars=standard error values (SE). Statistically significant differences (P < 0.05) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey’s post-hoc test).

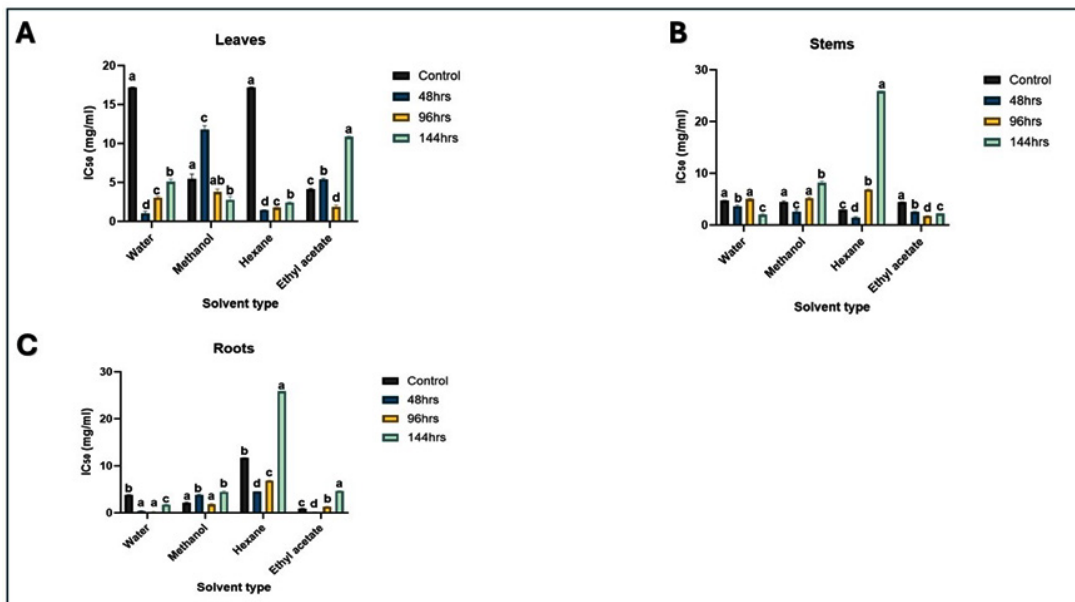


Fig. 15. Graphical representation of Hydrogen peroxide antioxidant activity in *Portulacaria afra* plant parts under 0/5°C and water deficit. IC₅₀ values were denoted as (Mean±SE, mg/ml), n=3, Error bars=standard error values (SE). Statistically significant differences (P < 0.05) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey’s post-hoc test).

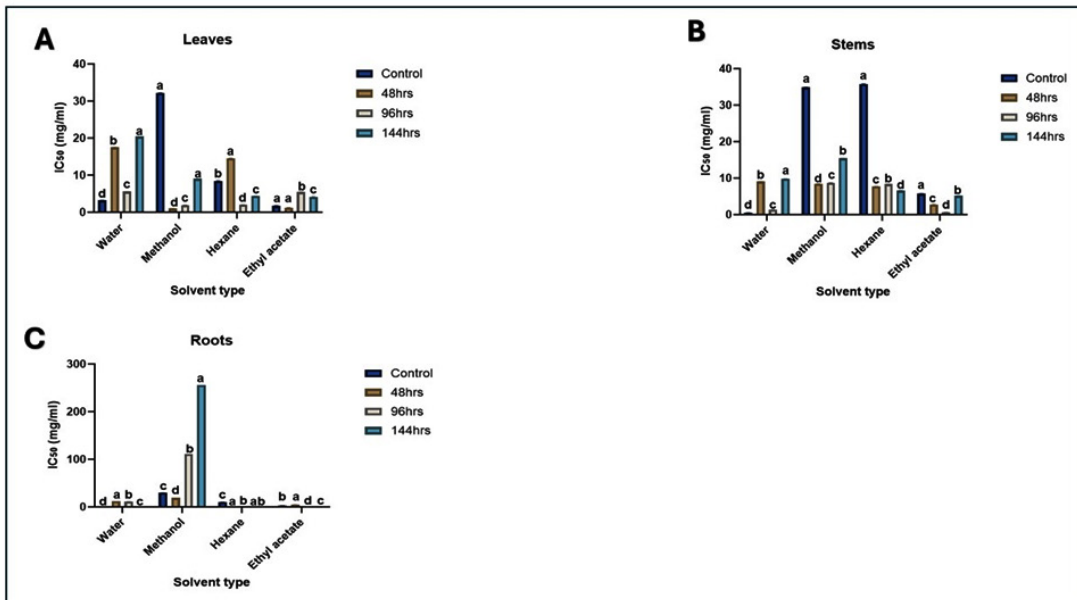


Fig. 16. Graphical representation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity in *Portulacaria afra* plant parts under 30/40°C and water deficit. IC₅₀ values were denoted as (Mean±SE, mg/ml), n=3, Error bars=standard error values (SE). Statistically significant differences (P < 0.05) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey’s post-hoc test).

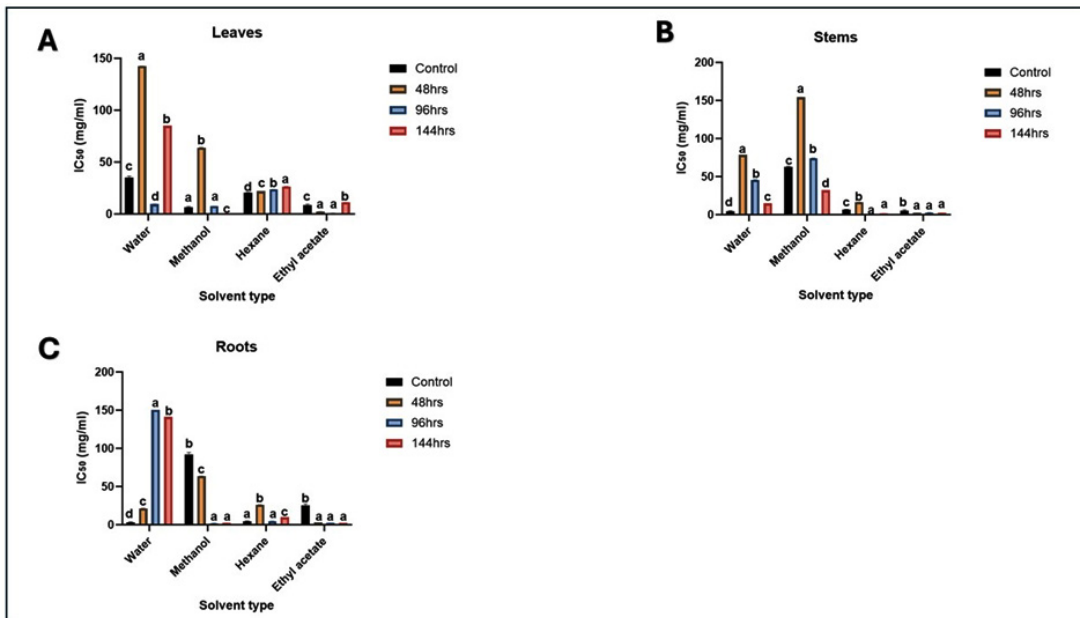


Fig. 17. Graphical representation of Metal chelating antioxidant activity in *Portulacaria afra* plant parts under 30/40°C and water deficit. IC₅₀ values were denoted as (Mean±SE, mg/ml), n=3, Error bars=standard error values (SE). Statistically significant differences (P < 0.05) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey’s post-hoc test)

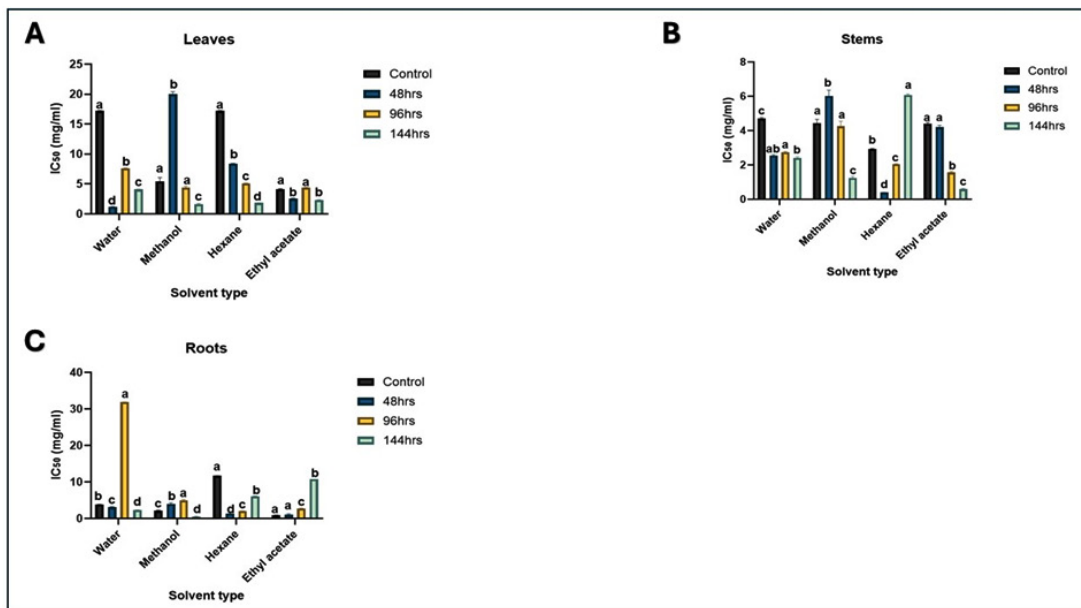


Fig. 18. Graphical representation of Hydrogen peroxide antioxidant activity in *Portulacaria afra* plant parts under 30/40°C and water deficit. IC_{50} values were denoted as (Mean \pm SE, mg/ml), $n=3$, Error bars=standard error values (SE). Statistically significant differences ($P < 0.05$) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey's post-hoc test)

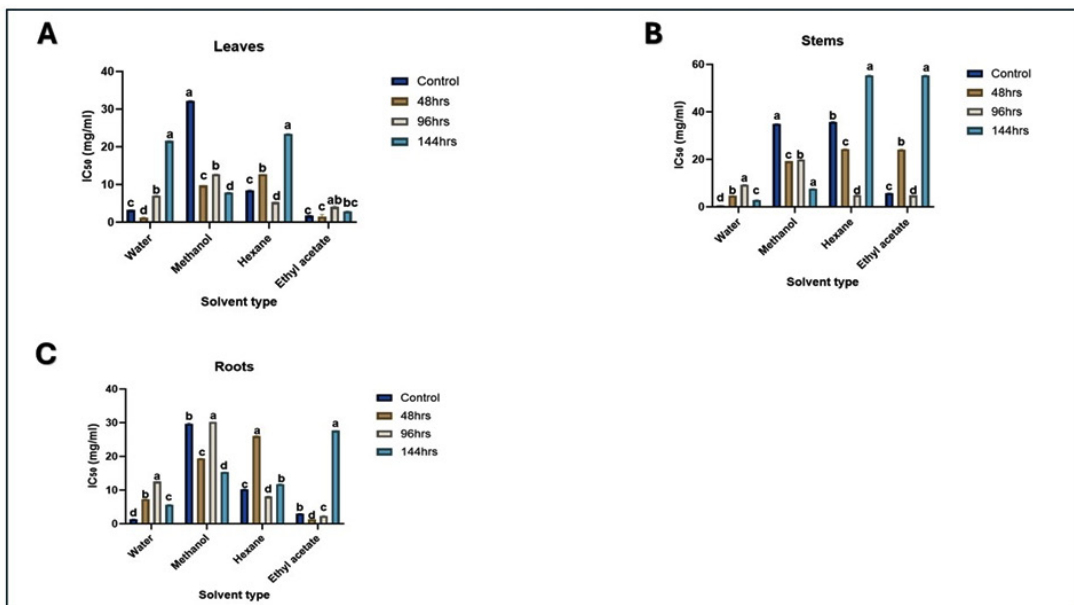


Fig. 19. Graphical representation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity in *Portulacaria afra* plant parts under 35/45°C and water deficit. IC_{50} values were denoted as (Mean \pm SE, mg/ml), $n=3$, Error bars=standard error values (SE). Statistically significant differences ($P < 0.05$) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey's post-hoc test)

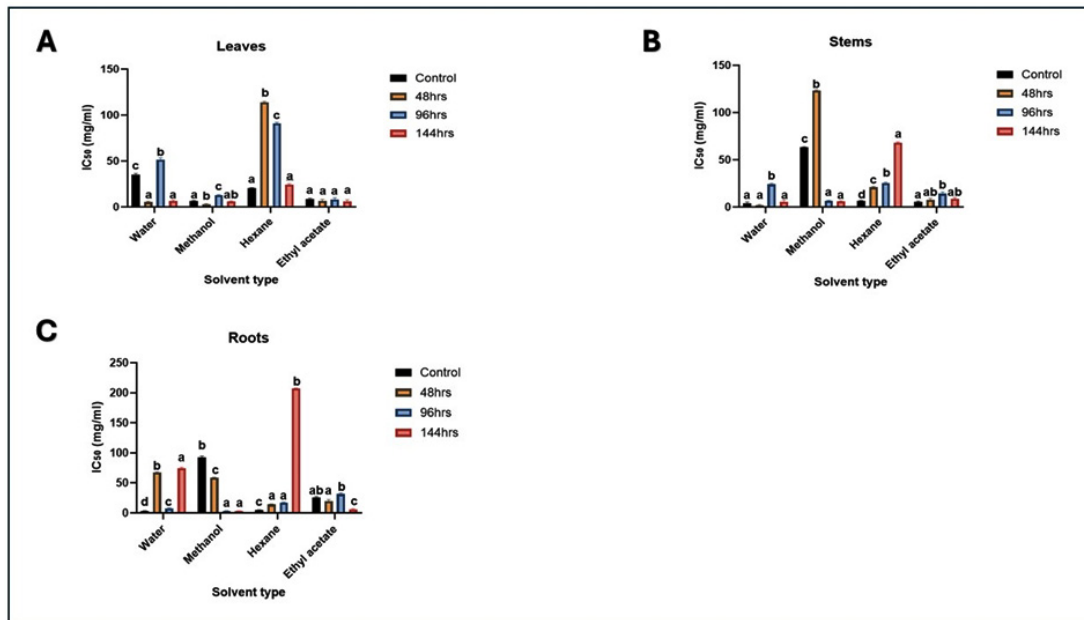


Fig. 20. Graphical representation of Metal chelating antioxidant activity in *Portulacaria afra* plant parts under 35/45°C and water deficit. IC₅₀ values were denoted as (Mean±SE, mg/ml), n=3, Error bars=standard error values (SE). Statistically significant differences (P < 0.05) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey’s post-hoc test).

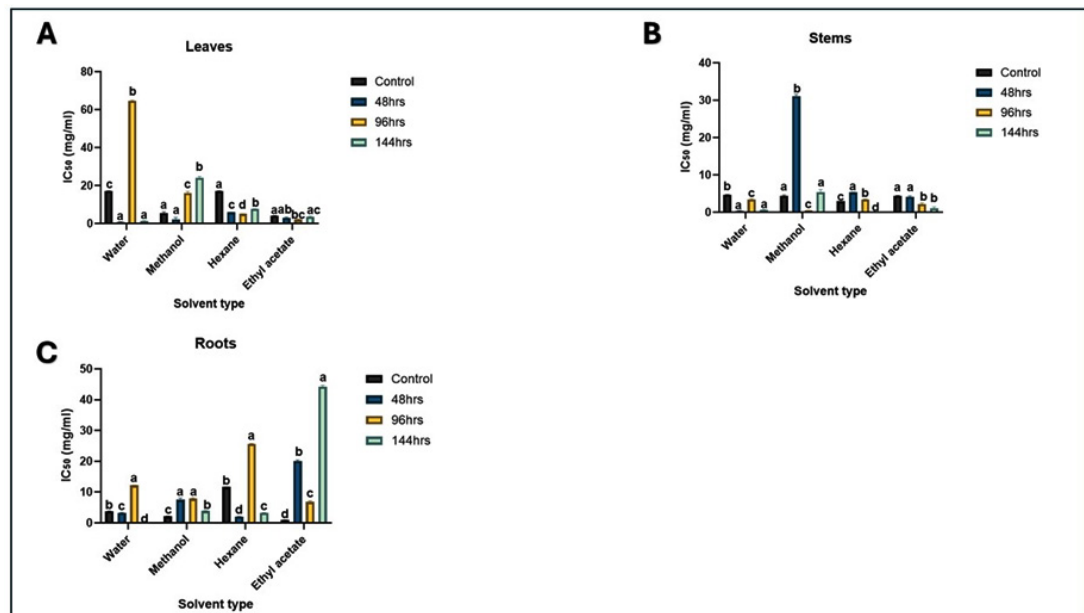


Fig. 21. Graphical representation of Hydrogen peroxide antioxidant activity in *Portulacaria afra* plant parts under 35/45°C and water deficit. IC₅₀ values were denoted as (Mean±SE, mg/ml), n=3, Error bars=standard error values (SE). Statistically significant differences (P < 0.05) were indicated by different letters (a, b, c, d) and were compared with the control within the same column (Tukey’s post-hoc test).

growth³⁰. The strong presence of tannins observed across all solvents in the leaf extracts could be responsible for anti-inflammatory and curative properties of *P. afra*, thus validating its suitability and potency for the treatment of piles, burns and some venereal diseases.

In the pharmaceutical industry, volatile oils are commonly utilized flavouring agents and are known particularly for their antibacterial and carminative attributes³¹. Interestingly, in this study, *n*-hexane, and ethyl acetate extracts across all plant parts exposed to hot (35/45°C) & cold (0/5°C) (Table 3) temperatures with water deficit showed the highest presence of volatile oil concentration, when compared to the other solvents. The concentrations ranged from low to high, the observed outcome is not completely in accordance with earlier research, indicating that warmer seasons or hotter temperatures present better concentrations of volatile oil and essential oils³². In contrast to the above reports, studies have shown how low temperature enhances the production of several groups of volatile terpenoids in essential oil glands on the leaf surface³³. Similar observation was reported for the increase in monoterpenes in sage leaves (*Salvia officinalis* L.) and peppermint essential oil (*Mentha piperita* L.) mostly under low temperatures³³. The increase observed in the volatile oil concentration across the different temperature settings in this study, could suggest the response of this plant species to the physical and chemical stress impacted simultaneously by the combined effect of both extreme temperatures and water deficit than with individual stress. Thus, resulting to the production of terpenoids, which are bioactive compounds that confer several biological activities³². Conversely, volatile oils were produced in low to high quantity in all plant parts under the temperatures [hot (30/40°C); & cold (10/15°C)] (Table 2), while methanolic stem extracts detected a strong presence of volatile oils in both treatments.

Phenols are the most prevalent class of phytoconstituents that perform protective roles by shielding plants from pathogens and antagonistic abiotic factors³⁴. Methanolic extracts showed a high presence of phenols in all plant parts under the temperatures [hot (30/40°C); & cold (10/15°C)] (Table 2), with water deficit when compared with the control samples. Meanwhile, the concentrations

of terpenoids and steroids, also showed an increase in comparison with the control sample extracts across all plant parts under both temperatures with water deficit.

Coumarins present in all plant parts of *Portulacaria afra*, which is commonly used in cancer treatment, renal cell carcinoma and blood diseases has caught the attention of many researchers for its photochemotherapy and therapeutic application in cancer³⁵. Results as shown in the heat map (Table 3) suggest that the production of coumarins is best under hot (35/45°C) & cold (0/5°C) temperatures with water deficit. The methanolic and aqueous extracts of all plant parts, showed a significant increase in comparison to the control in both hot (35/45°C) and cold (0/5°C) temperatures with water deficit (Table 3). Plant carbohydrates are important biochemical indicators of temperature acclimation and cold tolerance development³³. Therefore, the carbohydrate concentration across all plant extracts, compared to the control samples showed an increasing trend only in the methanolic extracts. This observation shifted from absent to considerable amounts in both hot (35/45°C) & cold (0/5°C) temperatures with water deficit (Table 3). While in the methanolic stem extracts exposed to temperatures [hot (30/40°C); & cold (10/15°C)] temperatures with water deficit (Table 2), an increasing pattern was also noted.

Studies have shown that water stress may amplify the accumulation of phytochemicals³⁶, contrary to the belief of the negative impact of severe water deficit. This has been extensively researched to establish its effects on the secondary metabolite profile, frequently leading to increasing output in most cases, which indicates higher quality³⁷. The qualitative phytochemical data from this study, however, is attributed to the simultaneous impact of both extreme hot and cold temperatures with water deficit stress.

A broad class of polyphenols with the chemical structure benzoyl-pyrone are called flavonoids. They are prevalent everywhere in plants, produced by the phenylpropanoid pathway and perform a wide range of pharmacological actions³⁸. It is believed that the organoleptic properties of fresh fruits, fruit juices, and wine are also significantly influenced by phenolic chemicals, including tannins and flavonoids. Therefore, their

potential impact on human health will primarily depend on the environmental variables that regulate their amounts and quality of accumulation in plants.

It was observed that hot temperatures (35/45°C) with water deficit increase the accumulation of total phenolics and total flavonoids in *Portulacaria afra* (Figures 8 and 9), in comparison with cold temperatures (0/5°C) and water deficit. Al-Huqail¹ reported similar higher accumulation of total phenolics content (TPC) in plants under stress, especially in basil plants (*Ocimum basilicum L.*) treated with high temperature, as well as an increase in total phenolics content (TPC) and total flavonoids content (TFC) concentrations in response to water stress and a highly significant increase in flavonoids under high climate temperature condition. The increase in the contents of the chemical compounds analysed, agrees with data in the literatures indicating that this is a response to the generation of reactive oxygen species. Reactive oxygen species (ROS) are produced by plants in response to environmental stress factors such as shade, excessive salt levels, extreme temperatures, drought, or water deficit, which when present in excess can lead to oxidative stress. Moreover, a vast variety of substances, such as arylpyrones and styrylpyrones, stibenenes, tannins, coumarins, flavonoids, lignins, and lignans, are included in the category of phenolics and they are produced in response to abiotic stress⁷. Consequently, they protect the plants and increase their tolerance against various abiotic and biotic stresses⁷. Phenolic compounds, due to their redox attributes, act as antioxidants, effectively neutralizing singlet oxygen, displaying notable pharmacological significance. They have demonstrated antioxidative, anticarcinogenic, antibacterial, and anti-inflammatory effects in previous findings¹, which supports the traditional therapeutic use of *P. afra*. Additionally, exogenous factors are responsible for the biosynthesis and accumulation of phenolic compounds while endogenous factors, developmental stage, and tissue differentiation³⁹ are responsible for the site of synthesis in plants. As a result, variation in the climatic, biotic, and environmental factors experienced during seasons can be used to explain the observed differences across seasons. Hence, the content of phenolic compounds and their temperature-dependent fluctuations as seen in this

study, suggest that there is a link between their distinct functions in each unique plant parts, and the stimuli causing their synthesis.

Lately, there has been a significant rise in antibiotic resistance, creating a growing therapeutic challenge. Using medicinal plant materials is one way to lessen antibiotic resistance⁴⁰. Plants create a wide range of chemicals to defend themselves against different diseases. Plant extracts with targets locations different from those used by antibiotics are anticipated to be effective against microorganisms with drug resistance⁴¹.

The antimicrobial activity results of *P. afra* extracts showed that the antibacterial activity of the extracts is subject to temperature with water deficit (Table 4 – 6). The inhibitory activity observed in the methanolic root and stem extracts (Table 7) against *Escherichia coli*, are similar to the report of previous research demonstrating maximum antibacterial activity against *Escherichia coli* by the leaves of *Amaranthus spinosus L.* during the summer month May-June⁴². The antibacterial activity observed could be correlated with the maximum building up of bioactive compounds in the methanolic stem and root of *P. afra* as a response to the concurrent effect of hot temperatures (35/45°C) with water deficit. Conversely, the other extracts across the plant parts were not active against *Staphylococcus aureus* and *Streptomyces griseus*. A similar trend was observed in *Harpephyllum caffrum* bark extracts where the highest inhibitory activity was detected with plant material collected during summer (December) with increasing antibacterial activity against gram-negative bacteria whereas it declined against gram-positive bacteria⁴³. Meanwhile in hot temperatures (30/40°C) with water deficit (Table 6), no positive antibacterial activity was observed against *Escherichia coli* across all the plant extracts. However, intermediate inhibitory activities, ranging from 11-13mm were observed against gram-positive *Streptomyces griseus* and *Staphylococcus aureus*. Here, the different fractions of the extracts appeared to be more effective against the gram-positive microorganisms. These results can be explained by studies that have proven that gram-negative bacteria have an outer membrane permeability barrier, which makes them more resistant to antimicrobial treatments than gram-positive bacteria⁴⁴. This might also explain the

inhibitory effect seen against *S. aureus* and *S. griseus* to the extracts and non-effectiveness of the tested fractions against *E. coli*.

The slight inhibitory effect of 11mm observed only in the *n*-hexane and ethyl acetate root extracts against gram-positive *Streptomyces griseus* and *Staphylococcus aureus* under cold temperatures (10/15°C) with water deficit (Table 4), is partly in line with findings from Ncube⁴⁵, where the best antibacterial activity was observed in winter season from dichloromethane bulb extracts against *S. aureus*. The observed variation in efficacy to inhibit microorganism growth may be as a function of the distinct cell wall layers characteristic of each microorganism⁴⁶. The outcomes of this study might be linked to various factors, including the chemical composition of distinct solvents, temperature variations, specific plant parts used, microbial proliferation, and the extent of exposure of the test microorganisms⁴⁶.

Antioxidants inhibit and delay oxidation process, by transferring electrons to free radicals to limit fluctuations and to prevent further reaction⁴⁷. The antioxidant activity and medicinal power of plants are significantly dependent on the secondary metabolites present in them, which in turn results to a reduction of oxidative stress by absorbing and neutralising free radicals⁴⁸. Depending on the amount of antioxidant molecules present in plants, which are greatly influenced by their growth environment, plants have various antioxidant properties⁴⁹.

Natural antioxidants attract attention in research, and food production, for their therapeutic properties and defence against oxidative stress-induced diseases⁵⁰. In fact, they are most beneficial for use since they are mostly free of negative side effects in comparison to the synthetic ones⁴⁹.

Antioxidant potential of *Portulacaria afra* leaves, stems, and roots extracts were investigated through a 2, 2 diphenylpicrylhydrazyl (DPPH) free radical assay, hydrogen peroxide scavenging (H₂O₂) and metal chelating activity assay.

The variations observed in Figure 10 – 21 in this study could potentially be because of the dissimilarities in the concentration or potency of active compounds in each part of *P. afra* under these stress conditions⁵¹. In addition, different antioxidant enzymes have temperature sensitivity, and they can only function at specific

temperatures⁵². Their actions also vary according to the crop varieties, their growth phases, and the growing season's tolerance or susceptibility to temperature⁵³. Moreover, the availability of various precursors that the plant needs to produce the active components can vary depending on the temperature and time of the year⁵⁴.

Thus, the results from this study are similar to Basson¹³, where accumulation of phytochemicals and biological activities in *P. afra* were found to either remain constant or increased after exposure to elevated CO₂. Consequently, these results suggest that the responses in the biological activities in the plant parts under concurrent hot and cold temperatures with water deficit condition serve as an adaptive and protective mechanism of *P. afra* against the concurrent stressors exposed to in this study.

CONCLUSION

In conclusion, the phytochemical content, and biological activities in *Portulacaria afra* were significantly influenced by extreme temperature simulations and water deficit. The observed variability in plant responses provided significant insights into the diverse reactions of various plant parts to distinct environmental stimuli and extraction solvents. These include the modulation of secondary metabolites, alterations in antibacterial properties, and adjustments in antioxidant activities, highlighting fluctuations in response to the combined abiotic factors. It is evident that abiotic factors in combination may influence the biological activities of *P. afra*, which shows the adaptation and resilience of the plant reflected in its ability to still provide therapeutic treatment against targeted diseases. Consequently, optimum temperatures and water deficit conditions are recommended for peak concentrations of phytochemicals and biological activities in *P. afra*. Although the results provide valuable insights, one limitation of this study is the use of one plant species, which may affect the generalizability of the findings for combined temperature and water deficit stress on plants. This is because plant responses are species dependent. More diverse plant species would be necessary to further confirm the trends observed both in this study and previous research in order to strengthen the overall validity of the

conclusions. Further studies are still recommended for the characterization of the bioactive compounds in these extracts for *in vivo* studies.

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Conflict of interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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