Effect of concurrent extreme temperatures and water deficit on the phytochemistry, antimicrobial and antioxidant activities of Portulacaria afra using four extraction solvents

Oluwafunbi Christianah Adeleye (2415662@students.wits.ac.za)
University of the Witwatersrand Johannesburg https://orcid.org/0000-0003-1486-4961
Ida Masana Risenga
Wits University: University of the Witwatersrand Johannesburg

Research Article

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Abstract

Background

The phyto-medicinal and ethnopharmacological significance of *Portulacaria afra* has been documented by a few studies, with high presence of coumarins known for anti-cancer properties. This indigenous South African plant, recognized for its succulent nature and medicinal properties, remains relatively under-researched. Furthermore, abiotic factors, seasonal changes, plant part and harvest period affect the phytochemical composition and secondary metabolites accumulation of medicinal plants. As of the present, there remains an absence of published data pertaining to the impact of environmental stress factors on crucial biological activities of *P. afra*. Therefore, this study aimed to investigate, for the first time, the phytochemical composition, antimicrobial and antioxidant activities of *P. afra*'s plant parts, using three antioxidant assays, with four extraction solvents of different polarities under concurrent extreme temperatures (hot & cold) and water deficit condition.

Results

The present study examined the simultaneous impact of varying temperatures [(mid-range high (30/40ºC); and mid-range low (10/15ºC), extreme high (35/45ºC); and extreme low (0/5ºC)] together with water deficit, on the antimicrobial, antioxidant properties, in addition to the extraction of phytochemical compounds from the methanol, ethyl acetate, *n*-hexane, and aqueous (60˚C) extracts of *P. afra* The determination of combined abiotic stress-induced acclimation's impact on the qualitative and quantitative composition of certain phytochemicals was carried out. The assessment of antioxidant potential within leaf, stem, and root extracts was conducted employing the metal chelating activity assay, hydrogen peroxide scavenging (H_2O_2), and the 2,2-diphenylpicrylhydrazyl (DPPH) free radical assay for the respective plant parts.

Qualitative phytochemical investigation of extracts of all the selected plant parts, compared to the control samples, showed an increasing trend with the stronger presence of coumarins, volatile oil, tannins, glycosides, flavonoids, phenols, quinones, terpenoids, saponins, steroids, phytosteroids, as well as carbohydrates while test for amino acids was negative. The qualitative phytochemical analysis clearly revealed that the concurrent cold temperatures [(10/15ºC and 0/5ºC)] and water deficit condition, especially after a 144-hour treatment period, had the strongest phytochemical presence across all plant parts. In contrast to the control group, methanol, and aqueous extracts from all plant sections in both concurrent extreme temperatures with water deficit settings showed the most presence of phytochemicals.

In comparison to the control group, notable increases in both total phenolic (TPCs) and total flavonoid contents (TFCs) were evident across various plant parts, with varying concentrations depending on the extraction solvent, temperature, and treatment period. In the comparative analysis of plant components, the result showed that the extracts derived from various plant parts exhibited maximal accumulation of TPCs and TFCs during the period of concurrent extreme high temperatures (35/45ºC) with water deficit conditions. This was observed in the ethyl acetate leaf and methanolic leaf extracts, after a 96-hour treatment period (9962.29±35.63 and 8598.59±14.28 mg/g, respectively).

In this study, the antimicrobial efficacy of *P. afra's* plant parts were investigated by implementing the agar-well diffusion technique. The assessment focused on evaluating the inhibitory effects against three distinct microorganisms, namely *Staphylococcus aureus, Escherichia coli,* and *Streptomyces griseus*. Experiments revealed that the plant extracts under the extreme hot temperatures (35/45ºC) with water deficit condition demonstrated more potent inhibitory effect against gram-negative *E. coli* compared to the gram-positive microorganisms. The highest level of inhibitory effect of 21mm was recorded against gram-negative *E. coli* from the methanolic root extracts after a 48hr-treatment period. However, the inhibitory activities mainly observed in the mid-hot temperatures (30/40ºC) were intermediate, ranging from 11-13mm against gram-positive *Streptomyces griseus* and *Staphylococcus aureus.* Observation from this investigation showed that plant extracts under the hot temperatures [mid-range high (30/40ºC); and extreme high (35/45ºC)] with water deficit condition showed better activity against all the microorganisms.

The antioxidant activity data showed that the most significant DPPH and metal chelating antioxidant activity was found under the mid-range hot temperatures (30/40ºC) 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 and 0.40±0.078 mg/ml respectively), in comparison with IC_{50} values (3.00±0.092 and 6.66±0.629 mg/ml) for control samples of ethyl acetate root and methanolic leaf extracts respectively. However, *n*-hexane stem extract under concurrent extreme hot temperatures (35/45ºC) and water deficit condition was the most potent for hydrogen peroxide scavenging activity (0.14±0.048 mg/ml) after a 144-hour treatment period, when compared with the control sample with IC_{50} values (2.94±0.017 mg/ml).

Conclusion

This investigation indicates how environmental factors in combination can influence the antimicrobial, antioxidant properties and the phytochemical profile of *P. afra* parts. It is evident that abiotic factors in combination impact the biosynthesis of secondary metabolites, potentially resulting in enhanced yields of phytomedicine and the production of phytocompounds such as volatile oils, coumarins, tannins, glycosides and flavonoids which are known to be lead phytocompounds for treating ailments such as respiratory diseases, cancer, ulcers, central nervous system, and cardiac ailments. Ultimately, this report agrees with a complicated and elaborate response system that varies for each plant parts, species of plant, abiotic factor, treatment/harvest period and extraction solvent. The exploration and investigation of combined abiotic stress applications for the purposeful synthesis of phytocompounds, aimed at targeted phytopharmaceutical production and the examination of associated biological properties, warrant further comprehensive research.

Background

In natural environments, most plant species undergo significant stress. These stressors can be categorized into two primary classifications: biotic and abiotic factors. (Al-Huqail et al., 2020). The biotic components encompass organisms across diverse taxa and their intricate interactions within the environment, encompassing predator-prey dynamics and interrelationships among species (Onyekachi et al., 2019). 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 availability. Their collective impact helps to shape the dynamics and sustainability of ecosystems (Mohuiddin, 2019). They significantly influence plant growth, development, and their productivity by disrupting cellular and developmental processes (Ramasar et al., 2022). In regions characterized by arid or semiarid climates, the prevalent abiotic factors, such as elevated temperatures and water scarcity, exert notable impacts on agricultural productivity, leading to substantial reductions in crop yields (Mahajan et al., 2020).

Various abiotic factors and their adverse effect on phytocompounds has been extensively documented in earlier studies. Given their widespread therapeutic application and utilization as primary resources for drug synthesis, understanding the relationship between abiotic stressors and secondary metabolite production in these plants holds significant scientific importance (Mahajan et al., 2020). 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 activities (Teffo et al., 2021).

The influence of temperature, a significant abiotic factor, on medicinal plants is profound. Elevated temperatures induce heat stress, while reduced temperatures result in cold stress. The substantial impact of high temperatures extends to the composition of phytocompounds (Van der walt *et al.*, 2022). Elevated temperatures typically correlate with heightened biosynthesis of secondary metabolites in plants, thereby enhancing the production of secondary metabolites across various plant species (Li et al., 2020). Conversely, high temperatures inducing stress were observed to result in reduced levels of secondary metabolites in plants, from previous research (Gupta et al., 2019).

Plants subjected to heat stress primarily experience oxidative stress due to the heightened emission of reactive oxygen species (ROS) (Al-huqail et al., 2020). Nevertheless, plants possess intricate and highly structured mechanisms for adaptation and tolerance (Ramasar et al., 2022). In adverse environmental conditions, plants exhibit resilience against diverse abiotic stresses through the activation of adaptive mechanisms. 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 like phenolics (Al-huqail et al., 2020).

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 metabolites (Onyekachi et al., 2019). Earlier research has demonstrated the profound impact of water deficit or drought stress on the composition of secondary metabolites in herbal plants, leading to heightened concentrations of these compounds. An instance is the increased proportion of flavonoids and phenolics exhibited in plants as a response to the stress-induced by water deficit (Albergaria et al., 2020).

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 come (Suzuki et al., 2014). Though a lot of research has shed light on the impacts of a single abiotic stress condition, like heat or drought, on plants, many of these findings cannot be utilized to predict the effect of concurrently occurring abiotic stresses (Zandalinas et al., 2018).

Earlier research findings have reported that plant responses to stresses caused by combined antagonistic abiotic factors, are phenomenal compared to when exposed to single ones. These results have in turn helped to identify the need to study abiotic stress combinations within medicinal plant species (Jaleel et al., 2007).

Globally, Africa is mostly impacted with the adverse effect of climate change caused by anthropogenic activities and little capacity for adaptation (Kapwata et al., 2022). Observed patterns of trends in the African annual-average near-surface temperatures over the last five decades have shown a rapid rise (Vargas Zeppetello et al., 2022). In the subtropics and central tropical Africa, temperatures rise more than double the global rate at which temperature rises (Kapwata et al., 2022).

In South Africa there has been increasing temperatures over the last 40 years, the current heat wave average frequency and temperature is (35°C), while the cold wave/front's average frequency and temperature is (0°C) (Mbokodo et al., 2020). By the year 2039, heightened heat waves are anticipated, particularly within the eastern and northern territories of South Africa, as per predictive models (Kapwata et al., 2022). Several studies and report from the Intergovernmental Panel on Climate Change (IPCC) revealed that southern Africa is a climate change 'hot spot' (Van Der Walt *et al.*, 2022).

Plant chemicals, known as phytochemicals, are generated through primary or secondary metabolism (Agidew, 2022). Their engagement in biological activities encompasses protection of plants from herbivory and antagonistic environmental factors (Idamokoro & Afolayan, 2020). Consuming phytochemically rich, natural plant-based foods yields significant health benefits. They perform several pharmacological activities in the human body by preventing lethal diseases and heart disease conditions (Ayoade et al., 2019). Plants synthesize natural compounds, which are the primary and secondary metabolites (Idamokoro & Afolayan, 2020). Secondary metabolites mediate in the hostile ecological interactions between plants and their habitat; However, they do not significantly contribute to the plants' growth, development, or reproduction (Albergaria et al., 2020). Their antibiotic, antifungal and antiviral properties help to ward off pathogens from attacking the plants (Ahmed et al., 2020).

The taxonomic classification of *Portulacaria afra* falls within the family *Portulacaceae* or alternatively more recently categorized under *Didiereaceae.* According to ethnobotanical knowledge, *P. afra* leaves and leaf juice is renowned for its curative properties against skin diseases and oral infections (De wet et al., 2013).

Phytochemical studies on *P. afra* tissue extracts revealed a strong presence of various phytochemicals (Tabassum et al., 2022). Also, we 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 extract (Adeleye & Risenga, 2022). The absence of comprehensive scientific documentation regarding the impact of environmental factors on *P. afra* is a notable limitation. This prompted an investigation to determine the impact of concurrent extreme temperatures (hot & cold) with water deficit on the phytochemistry, antimicrobial and, antioxidant activities in *P. afra*.

Recent studies have shown that plant responses to stresses caused by combined antagonistic abiotic factors, are phenomenal compared to when exposed to single ones. This present study aims to investigate the effect of concurrent extreme temperatures and water deficit on the production of phytochemicals using qualitative and quantitative phytochemical tests and to evaluate the antimicrobial and antioxidant properties in *P. afra*'s plant parts using three antioxidant assays. The stresses were applied under both hot and cold temperature treatments, concurrently with water deficit, to give the opportunity to detect the exposure duration and temperature-dependent effects. The outcome will help to determine the influence of these stressors on changes in content of the main classes of secondary metabolites, antimicrobial and antioxidant activities in *P. afra's* plant parts, and to help determine the best season to grow and harvest the plant. Moreover, it will be useful both for understanding the mechanisms of defence against adverse conditions and for approaches to the targeted synthesis of valuable secondary metabolites. Additionally, the results will help to make better predictions of potential impacts on the secondary metabolites' accumulation, productivity and phytopharmacological activities of succulent medicinal plants under climate change. Also, to enhance the accuracy of correlative distribution models.

Methods

In accordance with standard laboratory protocols, analytical-grade chemicals and solvents were employed throughout the experimental procedures. The specific substances, namely gallic acid, Folin-Ciocalteau 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 numbers1162-658.

In this study, the Conviron specifications were meticulously detailed: the model N0- PGW 40, bearing the serial N0- 170028, was manufactured in China (280033R01). 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 *P. afra* were cultivated at the University of the Witwatersrand in Johannesburg for propagation.

Plant cultivation

Green cuttings with non-lignified stems were carefully chosen from a pathogen-free parent plant of *P. afra*. Utilizing sterile secateurs, cuttings measuring 40–45 cm in length and 4 cm in diameter were meticulously collected and subsequently relocated to the greenhouse for transplantation (Duarte et al., 2019).

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. This soil was chosen because it has superior drainage, offers moist conditions, and is devoid of pathogens that can harm the cuttings before they establish roots. Cuttings were carefully placed within the partially filled potting mix soil, gently compacted, and subsequently topped up to capacity with additional potting mix soil. (Ficko & Naeth, 2021).

The plants underwent a three-month acclimatization period and root system development in the greenhouse facilities within the premises of University of the Witwatersrand in Johannesburg. This duration allowed for the emergence of fresh foliage, signifying successful growth adaptation (Poorter et al., 2012). The plant growth was observed and sustained by administering 500 ml of water every 48 hours due to their low water requirement (Liu et al., 2019).

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

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 (2019) predictions, IPCC (2018), and Mbokodo et al (2020) 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. The varying temperature settings used are as follows: control (25/27ºC); mid-range high (30/40ºC);

and mid-range low (10/15ºC), extreme high (35/45ºC); and extreme low (0/5ºC). Each plant was placed in the controlled climate simulation chamber (Conviron chambers, PGW40) with the high and low temperatures set concurrently with water deficit while other parameters like CO_2 (400 ppm), humidity (60%), light (160 nits/level 1) pH, salinity, were kept at ambient (Poorter et al., 2012). The lightning was programmed to operate for a duration of 12 hours daily, spanning from 6 am to 6 pm. Control temperature ranges were based on records from the South African Weather Service (2020).

In this study, the experimental group of plants underwent exposure to specific treatments without receiving water for a duration of 144 hours (equivalent to 6 days), while the control samples were maintained at an ambient temperature of 25°C and were watered every second day with 500ml of water (Liu et al., 2019). In adherence to South African weather service 2020 records, the experimental controls maintained a maximum nighttime temperature of 25°C (from 7pm to 5am) and a maximum daytime temperature of 27°C (from 5am to 7pm). In this study, plants subjected to treatments labelled as A, B, C, and D were deprived of water. Specifically, treatments A and B involved subjecting samples to cold temperatures with maximum night-time temperatures of 0 and 10ºC and maximum daytime temperatures of 5 and 15ºC, while treatments C and D subjected samples to elevated temperatures with maximum night-time temperatures of 30 and 40°C and maximum daytime temperatures of 35 and 45°C.

5 plants were harvested for all plant parts episodically 3 times for up to 6 days (144hrs), where plants were harvested every 48hrs (48, 96, 144) (Table 1). Samples were subjected to oven drying at 40°C for a period ranging between 2 to 3 days post-harvest. (Poorter et al., 2012). This procedure was repeated three times for each of the treatments. Where, n = 45 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

Plant materials 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 material was finely ground into a crude powder employing an electric grinder. This powdered form was then encapsulated within foil and secured in an airtight container. Subsequently, it was stored in a dark cupboard at room temperature until required for subsequent testing procedures (Labar et al., 2019).

Extracts Preparation

The crude plant extract was produced through sequential extraction utilizing: 80% methanol, *n*-hexane, ethyl acetate, and 100% distilled water, boiled at 60°C. Each extraction involved placing 3g of powdered plant material into individual containers, with the addition of 30 ml of the respective solvent (Khanyile et al., 2021). The methanol, *n*-hexane, ethyl acetate mostly involved agitation by shaking on a shaker, the water extract underwent preparation using a sonicator at a specific temperature (60°C) for a duration of 45 minutes. The agitation of all extracts lasted 48 hours, except for the water extracts (Pakade et al., 2013). The water extracts underwent centrifugation aimed at reducing their viscosity, followed by filtration through filter paper into vials (Basson et al., 2022). The residual supernatant was removed, and subsequently, the vials were enveloped in foil and refrigerated until the commencement of the tests.

Preliminary Phytochemical Screening Tests

The chemical constituents within the leaf, stem, and root extracts underwent evaluation through visual observation for alterations in colour or precipitation with the adoption and few alterations to the procedure described by Harbone (1998), Roghini and Vijayalakshmi (2018). A comprehensive screening was conducted to test for the presence of quinones, coumarins, phenols, carbohydrates, terpenoids, flavonoids, glycosides, steroids, saponins, amino acids, phytosteroids, and volatile oils, and the resulting data were documented.

Evaluation of Total Phenolics and Total Flavonoids Contents

Folin-Ciocalteu's colorimetric procedure was used in analysing the total phenolics and flavonoids content of *P. afra's* extracts in this study. Standard solutions of gallic acid at varying concentrations were employed to construct a calibration curve. The concentrations of total phenolic content within the extracts were extrapolated from this curve, denoting the findings as gallic acid equivalents per gram (mg GAE/g) of the extract's dry weight. Likewise, the total flavonoid contents of the extracts from different parts of the plant were assessed using a quercetin-based standard curve, with the results expressed as quercetin per gram (mg RE/g) of dry weight. All measurements were conducted in triplicate, and data was presented as mean ± standard error. $P < 0.05$ was used to determine the statistical significance of the findings.

Antimicrobial activity assay

The antimicrobial inhibitory activity of *P. afra* extracts were assessed by following the agar-well diffusion procedure described by Amabye and Tadesse (2016) with a few modifications (Teffo et al., 2022).

Agar-well diffusion method

In this study, Petri dishes were first sterilized and autoclaved prior to the addition of two types of nutrient agar-medium: Mueller-Hinton agar and Baird Parker agar. Once the agar solidified, sterile cotton swabs were employed to uniformly inoculate the surfaces of the agar with specific test microorganisms. Baird Parker agar plates were inoculated with *Staphylococcus. aureus*, whereas Mueller-Hinton agar plates received inoculations of *Escherichia coli* and *Streptomyces griseus*. Six wells were created in the inoculated media using a 6mm sterile borer. Subsequently, 100µl of plant extracts were introduced into five of these wells, while the sixth well received 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 experiments were conducted in triplicate, and the data were analysed using Microsoft Excel to express the results

as mean ± standard deviation. $P < 0.05$ was used to determine the statistical significance of the findings and Tukey test HSD in RStudio was used in highlighting the specific differences observed between treatments.

in vitro Antioxidant Activity Assessment

DPPH scavenging activity assay

A 2, 2 diphenyl picryhydrazyl (DPPH) free radical assay by Braca et al. (2002) was used with some modifications (Basson et al., 2022). The preparation of plant extracts from 3 grams of dried and powdered leaves, stems, and roots of *P. afra* involved their immersion in each of the solvents used in this study. 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 quantities, ranging from 10 to 50 µL, was mixed with 700 µL of the working solution and 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 their 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 Dinis et al. (1994) with few modifications (Adusei et al., 2019). 2 ml of extracts was combined with 0.25 ml of a 250 mM iron (II) chloride solution and was observed upon addition of 0.25 ml of ferrozine. The resulting mixture was vigorously shaken and left undisturbed at room temperature for a duration of 10 minutes. Following this, the mixture's absorbance was quantified at a wavelength of 562 nm. Methanol was employed as the blank, while a control group lacking the extracts was used. The percentage of metal chelation was determined with the prescribed formula:

Metal chelation (%) = {(A0 – A1)/ A0} × 100

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

Hydrogen peroxide scavenging (H_2O_2) assay

The hydrogen peroxide scavenging activity was determined by adapting the methods of Ruch et al. (1989) and Bouabid et al. 2020. 1 ml of extracts was combined with 3.4 ml of phosphate buffer (50 mM, pH 7.4). Following this, 600 µL of 40 mM hydrogen peroxide (30%) was introduced into the solution. The resulting mixture was allowed to stand at room temperature for a duration of 40 minutes. Subsequently, the absorbance of the solution was determined using a spectrophotometer set at a wavelength of 230 nm. The hydrogen peroxide scavenging activity was quantified using the provided formula:

Scavenged H_2O_2 (%) = [(Absorbance of control – Absorbance of sample)/Absorbance of control] x 1

Statistical Analysis

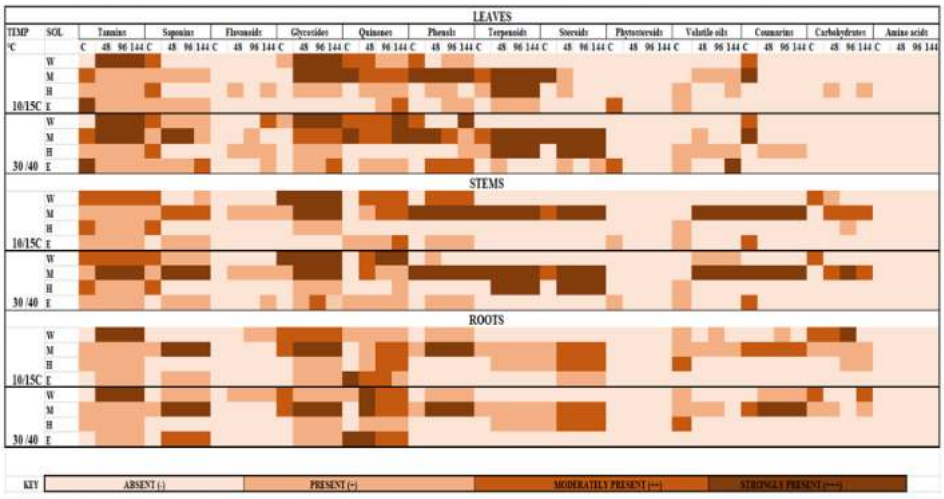
The concentration values were plotted against the % inhibition values in Microsoft Excel to derive a trend line equation facilitating the calculation of the IC50. A one-way analysis of variance (ANOVA) was employed to ascertain significant differences. All experiments were conducted thrice, and data were presented as mean ± standard error (SE) for n = 3. $P < 0.05$ was used to determine the statistical significance of the findings and Tukey test HSD in RStudio was used to ascertain where the significance lies.

Results

Qualitative screening of phytochemicals

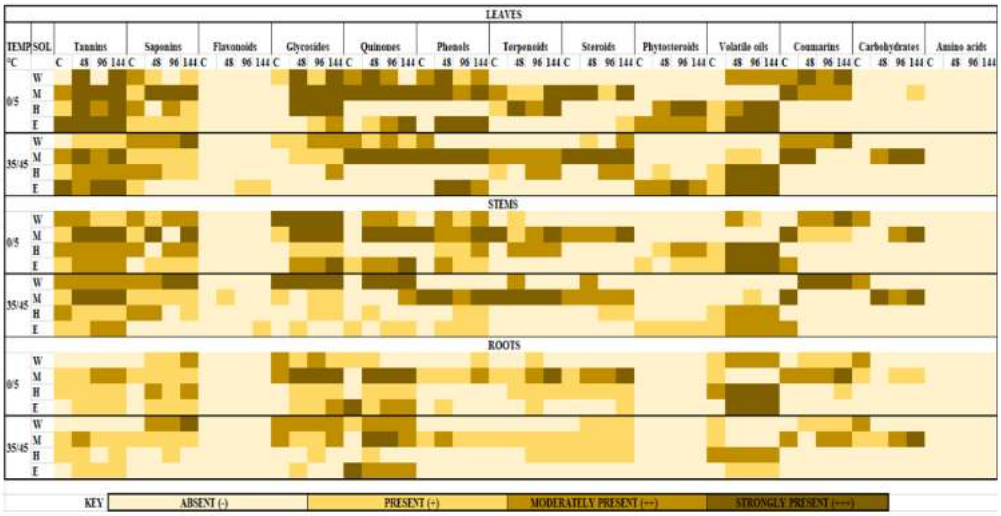
The aqueous (60°C), methanol, *n-hexane*, and ethyl acetate whole plant extracts of *P. afra* were screened for their phytochemical constituents under concurrent extreme temperatures (hot & cold) and water deficit condition. For the qualitative phytochemical screening, the leaf, stem, and root extracts were evaluated for the presence of saponins, flavonoids, glycosides, quinones, phenols, terpenoids, steroids, phytosteroids, volatile oil, carbohydrate, amino acids and coumarins. In general, result of phytochemicals in the plant parts varied in the extracts prepared from the different solvents mentioned above, under both concurrent cold and 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 phytochemicals in the leaf, stem, and root of *P. afra*, when plant parts were subjected under concurrent mid-range 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 extreme cold & hot temperatures (0/5°C and 35/45°C) with water deficit conditions.

Table 2Heat map for the phytochemical tests of *P. afra* samples under 10/15°C and 30/40°C



C, control samples; W, hot water extract (60°C) extract; M, methanolic extract; H, *n*-hexane extract; E, ethyl acetate extract

Table 3: Heat map for the phytochemical tests of P. afra samples under 0/5°C and 35/45°C



C, control samples; W, hot water extract (60°C) extract; M, methanolic extract; H, *n*-hexane extract; E, ethyl acetate extract

Quantitative analysis of phytochemical

The quantitative analyses were performed to determine the TPCs and TFCs within the leaf, stem, and root extracts of *P. afra*. Quantitative results for the total flavonoids content (TFCs) in (Tables 4 and 5) and total phenolics contents (TPCs) in (Tables 6 and 7) respectively, show the variations in the phytoconstituents across all the plant parts extracts with the different solvents and treatment periods, under concurrent cold temperatures [mid-range low (10/15°C); and extreme low (0/5°C)] with water deficit condition.

Tables 8 and 9 show data for total flavonoids content (TFCs) and total phenolics content (TPCs) in (Tables 10 and 11). The results here present the order of fluctuations in these chemical compounds across all the plant parts extracts with the different solvents and treatment periods, under concurrent hot temperatures [mid-range high (30/40°C); and extreme high (35/45°C)] with water deficit condition.

Table 4
Total flavonoid content in *P. afra* extracts under 10/15°C using four solvents

P. afra parts	TFCs (mg/g)			
	Solvents			
Treatment periods	Methanol	Hexane	Water (60°C)	Ethyl Acetate
LEAVES	150.47 ± 1.17^{d}	141.34 ± 0^{d}	592.70 ± 49.99^{c}	108.60 ± 5.13^{d}
Control				
48hrs	2659.84 ± 3.12^{c}	3522.03 ± 3.12^{a}	7529.09 ± 3.12^{a}	1149.82 ± 2.04^{a}
96hrs	3861.25 ± 3.12^{a}	2555.01 ± 6.23^{c}	358.3 ± 3.53^{d}	208.72 ± 6.56^{c}
144hrs	3036.75 ± 4.08^{b}	3067.37 ± 5.13^{b}	2681.04 ± 6.56^{b}	1360.66 ± 5.13^{b}
STEMS	107.42 ± 2.04^{d}	134.51 ± 1.18^{d}	585.63 ± 48.29^{d}	114.49 ± 2.04^{d}
Control				
48hrs	939.04 ± 1.10^{c}	7358.3 ± 5.40^{a}	1188.69 ± 2.04^{c}	801.18 ± 18.96^{b}
96hrs	2710.48 ± 4.25^{b}	1812.96 ± 2.36^{c}	4222.85 ± 3.12^{a}	298.23 ± 5.40^{c}
144hrs	4371.26 ± 4.25^{a}	1974.32 ± 3.12^{b}	3997.88 ± 6.12^{b}	1816.49 ± 4.25a
ROOTS	359.01 ± 8.16^{d}	104.59 ± 8.16^{d}	901.30 ± 149.87^{a}	154.53 ± 2.36^{d}
Control				
48hrs	1771.73 ± 4.08^{c}	2518.49 ± 3.12^{a}	743.46 ± 5.40^{a}	1492.58 ± 17.79^{b}
96hrs	4166.31 ± 2.36^{a}	2090.93 ± 5.13^{c}	693.99 ± 4.08^{a}	1280.57 ± 2.04^{c}
144hrs	1829.45 ± 1.18^{b}	2326.5 ± 4.08^{b}	3148.65 ± 7.17^{b}	2492.58 ± 2.04^{a}

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 varying letters within the same column for the respective ^{a}Values.

Table 5
Total flavonoid content in *P. afra* extracts under 0/5°C using four solvents

P. afra parts	TFCs (mg/g)			
	Solvents			
Treatment periods	Methanol	Hexane	Water (60°C)	Ethyl Acetate
LEAVES	150.47 ± 1.17^{d}	141.34 ± 0^{c}	592.70 ± 49.99^{a}	108.60 ± 5.13^{c}
Control				
48hrs	951.94 ± 4.08^{b}	373.62 ± 17.11^{a}	307.66 ± 58.29^{b}	781.15 ± 5.89^{b}
96hrs	447.82 ± 3.12^{c}	618.61 ± 6.23^{b}	406.83 ± 56.54^{ab}	854.18 ± 1.18^{a}
144hrs	1216.96 ± 5.4^{a}	325.32 ± 22.47^{a}	206.6 ± 62.48^{b}	840.05 ± 6.56^{a}
STEMS	107.42 ± 2.04^{d}	134.51 ± 1.18^{a}	585.63 ± 48.29^{a}	114.49 ± 2.04^{c}
Control				
48hrs	1371.26 ± 10.47^{a}	271.14 ± 2.36^{c}	1027.33 ± 254.56^{a}	765.84 ± 4.25^{b}
96hrs	1067.37 ± 3.12^{c}	193.4 ± 4.25^{a}	716.37 ± 124.50^{a}	625.68 ± 6.23^{a}
144hrs	1222.85 ± 9.64^{b}	622.14 ± 29.59^{b}	693.99 ± 61.74^{a}	622.14 ± 2.36^{a}
ROOTS	359.01 ± 8.16^{d}	104.59 ± 8.16^{a}	901.30 ± 149.87^{a}	154.53 ± 2.36^{c}
Control				
48hrs	1046.17 ± 4.71^{a}	101.53 ± 1.18^{a}	737.57 ± 47.16^{a}	749.35 ± 6.23^{a}
96hrs	406.6 ± 4.25^{c}	140.4 ± 6.23^{b}	112.13 ± 5.13^{b}	315.9 ± 2.04^{b}
144hrs	623.32 ± 6.12^{b}	100.36 ± 0.01^{a}	180.45 ± 42.91^{b}	752.89 ± 2.36^{a}

All values were presented mean ± SE, n = 3, and were quantified in terms of quercetin concentration (mg/g of quercetin). Significant differences ($p < 0.05$) were indicated by varying letters within the same column for the respective [a]Values.

Table 6
Total phenolic content in *P. afra* extracts under 10/15°C using four solvents

***P. afra* parts**	**TPCs (mg/g)**			
	Solvents			
Treatment periods	**Methanol**	**Hexane**	**Water (60°C)**	**Ethyl Acetate**
LEAVES	6073.20 ± 4.10[b]	1237.04 ± 13.47[c]	1038.18 ± 1.17[d]	3008.08 ± 81.65[c]
Control				
48hrs	6730.91 ± 2.33[a]	3843.1 ± 6.73[a]	8106.13 ± 5.39[c]	2442.42 ± 11.66[d]
96hrs	4973.33 ± 2.33[c]	3812.12 ± 34.99[a]	8348.55 ± 14.25[b]	3708.42 ± 17.82[b]
144hrs	4813.06 ± 5.39[d]	4987.88 ± 11.66[b]	9517.51 ± 8.91[a]	8327.95 ± 17.82[a]
STEMS	9521.01 ± 216.94[b]	1755.55 ± 69.98[c]	1820.67 ± 25.08[d]	1472.73 ± 11.66[d]
Control				
48hrs	5844.92 ± 1.78[a]	8262.63 ± 116.64[a]	9541.08 ± 3.37[b]	2873.4 ± 17.82[c]
96hrs	5535.15 ± 1.17[a]	8531.99 ± 178.17[a]	8073.06 ± 8.91[c]	4119.19 ± 11.66[b]
144hrs	5725.05 ± 1.17[a]	6646.46 ± 116.64[b]	9921.55 ± 8.91[a]	4482.83 ± 11.66[a]
ROOTS	1567.47 ± 29.16[d]	1351.52 ± 11.66[c]	3331.31 ± 58.32[d]	2361.62 ± 11.66[d]
Control				
48hrs	6900.4 ± 10.10[a]	4296.97 ± 34.99[a]	3897.78 ± 4.04[c]	6568.35 ± 35.63[a]
96hrs	6324.65 ± 5.34[b]	4983.84 ± 20.2[b]	5602.83 ± 10.69[b]	4992.59 ± 35.63[b]
144hrs	5363.03 ± 3.50[c]	4317.17 ± 20.2[a]	8980.61 ± 30.5[a]	4723.23 ± 23.33[c]

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 varying letters within the same column for the respective [a]Values.

Table 7
Total phenolic content in *P. afra* extracts under 0/5°C using four solvents

P. afra parts	TPCs (mg/g)			
	Solvents			
Treatment periods	Methanol	Hexane	Water (60ºC)	Ethyl Acetate
LEAVES	6073.20 ± 4.10^{a}	1237.04 ± 13.47^{d}	1038.18 ± 1.17^{d}	3008.08 ± 81.65^{a}
Control				
48hrs	2287.34 ± 1.35^{b}	8599.33 ± 67.34^{a}	9055.22 ± 6.73^{a}	1216.84 ± 17.82^{d}
96hrs	2036.84 ± 0.67^{d}	7723.91 ± 67.34^{b}	5701.68 ± 17.82^{c}	1620.88 ± 6.73^{c}
144hrs	2237.51 ± 0.67^{c}	6175.08 ± 67.34^{c}	6550.17 ± 6.73^{b}	2637.71 ± 17.82^{b}
STEMS	9521.01 ± 216.94^{b}	1755.55 ± 69.98^{a}	1820.67 ± 25.08^{c}	1472.73 ± 11.66^{c}
Control				
48hrs	2477.91 ± 0.67^{a}	4032.32 ± 93.31^{b}	8098.99 ± 11.66^{a}	1661.28 ± 6.73^{b}
96hrs	2304.85 ± 2.33^{a}	2308.42 ± 71.27^{ab}	1158.72 ± 0.67^{d}	2051.85 ± 6.73^{a}
144hrs	2376.23 ± 0.67^{a}	3709.09 ± 763.77^{b}	6907.07 ± 23.33^{b}	1398.65 ± 13.47^{d}
ROOTS	1567.47 ± 29.16^{c}	1351.52 ± 11.66^{a}	3331.31 ± 58.32^{d}	2361.62 ± 11.66^{a}
Control				
48hrs	2184.98 ± 0.67^{a}	5621.55 ± 723.27^{bc}	7122.56 ± 6.73^{a}	2152.86 ± 6.73^{b}
96hrs	2160.07 ± 0.67^{a}	3278.11 ± 117.41^{ab}	4247.14 ± 17.82^{c}	1028.28 ± 23.33^{d}
144hrs	2525.72 ± 0.67^{b}	6348.82 ± 1096.47^{c}	6186.53 ± 6.73^{b}	1513.13 ± 11.66^{c}

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 varying letters within the same column for the respective [a]Values.

Table 8
Total flavonoid content in *P. afra* extracts under 30/40°C using four solvents

P. afra parts	TFCs (mg/g)			
	Solvents			
Treatment periods	Methanol	Hexane	Water (60ºC)	Ethyl Acetate
LEAVES	150.47 ± 1.17^{d}	141.34 ± 0^{d}	592.70 ± 49.99^{d}	108.60 ± 5.13^{d}
Control				
48hrs	3815.31 ± 1.18^{b}	2191.05 ± 6.23^{c}	3897.76 ± 3.12^{b}	4486.69 ± 8.24^{a}
96hrs	4007.30 ± 3.12^{a}	4969.61 ± 4.08^{b}	5593.88 ± 3.12^{a}	2726.97 ± 6.23^{c}
144hrs	2195.76 ± 2.04^{c}	6784.69 ± 6.56^{a}	3424.26 ± 4.25^{c}	4378.32 ± 4.25^{b}
STEMS	107.42 ± 2.04^{d}	134.51 ± 1.18^{d}	585.63 ± 48.29^{d}	114.49 ± 2.04^{d}
Control				
48hrs	2942.52 ± 5.13^{b}	2645.7 ± 3.12^{c}	1971.97 ± 3.12^{c}	1760.62 ± 1.81^{c}
96hrs	2359.48 ± 3.12^{c}	3848.29 ± 3.12^{b}	3107.42 ± 4.08^{b}	7715.19 ± 2.04^{a}
144hrs	3861.25 ± 3.12^{a}	4411.31 ± 4.08^{a}	4035.57 ± 4.25^{a}	6769.38 ± 1.178^{b}
ROOTS	359.01 ± 8.16^{d}	104.59 ± 8.16^{d}	901.30 ± 149.87^{d}	154.53 ± 2.36^{d}
Control				
48hrs	2668.08 ± 3.11^{a}	2564.43 ± 4.25^{c}	1550.29 ± 3.12^{c}	4993.17 ± 5.13^{a}
96hrs	1403.06 ± 4.25^{c}	3719.91 ± 3.12^{a}	2980.21 ± 100.71^{a}	3615.08 ± 3.12^{b}
144hrs	2538.52 ± 5.40^{b}	3633.92 ± 4.08^{b}	2277.03 ± 2.04^{b}	1452.22 ± 1.10^{c}

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 varying letters within the same column for the respective [a]Values.

Table 9
Total flavonoid content in *P. afra* extracts under 35/45°C using four solvents

P. afra parts	TFCs (mg/g)			
	Solvents			
Treatment periods	Methanol	Hexane	Water (60°C)	Ethyl Acetate
LEAVES	150.47 ± 1.17[d]	141.34 ± 0[d]	592.70 ± 49.99[c]	108.60 ± 5.13[c]
Control				
48hrs	4598.59 ± 4.08[b]	1869.49 ± 7.16[c]	1053.24 ± 11.24[b]	4123.91 ± 3.12[a]
96hrs	8598.59 ± 14.28[a]	4110.95 ± 67.32[a]	457.24 ± 2.04[d]	4110.95 ± 67.32[a]
144hrs	2093.29 ± 4.08[c]	3538.52 ± 42.84[b]	1835.34 ± 28.56[a]	1910.72 ± 3.12[b]
STEMS	107.42 ± 2.04[d]	134.51 ± 1.18[c]	585.63 ± 48.29[a]	114.49 ± 2.04[c]
Control				
48hrs	4039.1 ± 56.11[a]	2739.93 ± 20.40[a]	984.92 ± 1.178[c]	1332.39 ± 5.13[a]
96hrs	713.12 ± 4.33[c]	4933.1 ± 27.55[b]	6314.72 ± 109.15[b]	1333.57 ± 10.20[a]
144hrs	3572.67 ± 54.07[b]	2789.4 ± 16.32[a]	505.54 ± 3.12[a]	869.49 ± 1.178[b]
ROOTS	359.01 ± 8.16[d]	104.59 ± 8.16[d]	901.30 ± 149.87[a]	154.53 ± 2.36[d]
Control				
48hrs	6188.69 ± 2.04[a]	5284.1 ± 79.56[b]	1139.22 ± 14.28[a]	5428.98 ± 26.52[a]
96hrs	1096.82 ± 3.53[c]	6814.13 ± 2.04[a]	1226.38 ± 15.31[a]	2823.56 ± 31.63[c]
144hrs	1337.10 ± 16.32[b]	4666.90 ± 116.31[c]	4511.43 ± 150.00[b]	5284.1 ± 10.20[b]

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 varying letters within the same column for the respective [a]Values.

Table 10
Total phenolic content in *P. afra* extracts under 30/40°C using four solvents

P. afra parts	TPCs (mg/g)			
	Solvents			
Treatment periods	Methanol	Hexane	Water (60ºC)	Ethyl Acetate
LEAVES	6073.20 ± 4.10^{b}	1237.04 ± 13.47^{d}	1038.18 ± 1.17^{d}	3008.08 ± 81.65^{d}
Control				
48hrs	8590.84 ± 3.56^{a}	5675.42 ± 71.27^{c}	7460.47 ± 7.13^{b}	8224.65 ± 27.99^{a}
96hrs	5565.93 ± 4.86^{c}	6698.99 ± 93.31^{b}	8820.74 ± 9.71^{a}	5778.86 ± 23.48^{b}
144hrs	4578.72 ± 3.56^{d}	7668.69 ± 46.65^{a}	5047 ± 7.13^{c}	5504.11 ± 14.25^{c}
STEMS	9521.01 ± 216.94^{b}	1755.55 ± 69.98^{d}	1820.67 ± 25.08^{d}	1472.73 ± 11.66^{d}
Control				
48hrs	5890.03 ± 8.44^{a}	5242.42 ± 58.32^{b}	8444.04 ± 2.33^{a}	9715.76 ± 19.83^{a}
96hrs	5805.86 ± 1.16^{a}	4299.66 ± 89.08^{c}	4080.20 ± 5.35^{c}	8731.25 ± 30.29^{b}
144hrs	5805.86 ± 1.17^{a}	5882.15 ± 89.08^{a}	5451.45 ± 3.56^{b}	7574.14 ± 86.41^{c}
ROOTS	1567.47 ± 29.16^{d}	1351.52 ± 11.66^{c}	3331.31 ± 58.32^{c}	2361.62 ± 11.66^{d}
Control				
48hrs	8243.84 ± 5.35^{a}	6420.88 ± 89.08^{a}	3735.22 ± 7.13^{b}	6231.65 ± 35.63^{b}
96hrs	4476.16 ± 8.81^{c}	4468.01 ± 89.08^{b}	5011.99 ± 7.13^{a}	8494.28 ± 48.56^{a}
144hrs	7985.25 ± 7.28^{b}	6690.24 ± 89.08^{a}	2778.99 ± 4.67^{d}	5167.68 ± 61.72^{c}

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 varying letters within the same column for the respective ^{a}Values.

Table 11
Total phenolic content in *P. afra* extracts under 35/45°C using four solvents

P. afra parts	TPCs (mg/g)			
	Solvents			
Treatment periods	Methanol	Hexane	Water (60ºC)	Ethyl Acetate
LEAVES	6073.20 ± 4.10^{d}	1237.04 ± 13.47^{d}	1038.18 ± 1.17^{d}	3008.08 ± 81.65^{c}
Control				
48hrs	9405.25 ± 4.67^{c}	3008.75 ± 71.27^{c}	3284.18 ± 242.8^{c}	8301.01 ± 11.66^{b}
96hrs	9591.11 ± 8.08^{a}	5810.1 ± 46.65^{a}	4072.05 ± 6.73^{b}	9962.29 ± 35.63^{a}
144hrs	9523.77 ± 5.39^{b}	4651.85 ± 26.94^{b}	7432.32 ± 23.33^{a}	2449.16 ± 6.73^{d}
STEMS	9521.01 ± 216.94^{b}	1755.55 ± 69.98^{d}	1820.67 ± 25.08^{d}	1472.73 ± 11.66^{c}
Control				
48hrs	5443.38 ± 3.56^{a}	4724.58 ± 53.87^{c}	5426.6 ± 8.91^{c}	3028.28 ± 11.66^{a}
96hrs	1745.05 ± 4.67^{b}	6645.12 ± 26.94^{a}	7258.25 ± 3.37^{a}	1620.88 ± 6.73^{b}
144hrs	5930.91 ± 2.33^{a}	5783.17 ± 117.41^{b}	6564.65 ± 5.83^{b}	2994.61 ± 17.82^{a}
ROOTS	1567.47 ± 29.16^{c}	1351.52 ± 11.66^{c}	3331.31 ± 58.32^{d}	2361.62 ± 11.66^{c}
Control				
48hrs	7540.81 ± 4.04^{b}	6452.53 ± 40.40^{b}	6428.96 ± 24.28^{a}	5540.74 ± 117.41^{a}
96hrs	6664.04 ± 2.02^{a}	8270.7 ± 106.90^{a}	5425.59 ± 6.73^{b}	9311.79 ± 53.87^{b}
144hrs	6605.06 ± 6.46^{a}	8149.49 ± 40.40^{a}	5035.02 ± 6.73^{c}	5055.89 ± 269.36^{a}

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 varying letters within the same column for the respective [a]Values.

Antimicrobial activity assay

The antimicrobial inhibitory activity of *P. afra* extracts were assessed with the agar well diffusion assay. Gram-negative *E. coli*, gram-positive *S. aureus* and gram-positive *S. griseus* were the test microorganisms used in this study. Observed variation in the inhibitory effect of the extracts against the tested microorganisms at different treatment periods are presented in Table 12–15. Our findings indicate that the antimicrobial activity of the extracts is subject to the plant part and temperature with water deficit conditions.

Table 12: Antimicrobial activity of *P. afra* extracts under 10/15°C with water deficit conditions

	Zone of inhibition (mm) of extracts	Treatment period (hours)															
	Microorganisms	Methanol				Hexane				Water (60°C)				Ethyl Acetate			
		C	48	96	144	C	48	96	144	C	48	96	144	C	48	96	144
Leaves	*E. coli*	-	-	-	-	16±0.6	-	-	-	-	-	-	-	-	-	-	-
	S. aureus	-	-	-	-	18±0.6	-	-	-	-	-	-	-	14±0.6	-	-	-
	S. griseus	-	-	-	-	18±0.6	-	-	-	-	-	-	-	-	-	-	-
Stems	*E. coli*	-	-	-	-	13±0.6	-	-	-	-	-	-	-	-	-	-	-
	S. aureus	-	-	-	-	13±0.6	-	-	-	-	-	-	-	-	-	-	-
	S. griseus	-	-	-	-	13±0.6	-	-	-	-	-	-	-	-	-	-	-
Roots	*E. coli*	-	-	-	-	15±0.6	-	-	-	-	-	-	-	-	-	-	-
	S. aureus	-	-	-	-	17±0.6	-	-	-	24±0.6	-	-	-	-	-	11±0.3	-
	S. griseus	-	-	-	-	18±0.6	11±0.1	-	-	-	-	-	-	-	-	-	-

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

Table 13: Antimicrobial activity of *P. afra* extracts under 0/5°C with water deficit conditions

	Zone of inhibition (mm) of extracts	Treatment period (hours)															
	Microorganisms	Methanol				Hexane				Water (60°C)				Ethyl Acetate			
		C	48	96	144	C	48	96	144	C	48	96	144	C	48	96	144
Leaves	*E. coli*	-	-	-	-	16±0.6	-	-	-	-	-	-	-	-	-	-	-
	S. aureus	-	-	-	-	18±0.6	-	-	-	-	-	-	-	14±0.6	-	-	-
	S. griseus	-	-	-	-	18±0.6	-	-	-	-	-	-	-	-	-	-	-
Stems	*E. coli*	-	-	-	-	13±0.6	-	-	-	-	-	-	-	-	-	-	-
	S. aureus	-	-	-	-	13±0.6	-	-	-	-	-	-	-	-	-	-	-
	S. griseus	-	-	-	-	13±0.6	-	-	-	-	-	-	-	-	-	-	-
Roots	*E. coli*	-	-	-	-	15±0.6	-	-	-	-	-	-	-	-	-	-	-
	S. aureus	-	-	-	-	17±0.6	-	-	-	24±0.6	-	-	-	-	-	-	-
	S. griseus	-	-	-	-	18±0.6	-	-	-	-	-	-	-	-	-	-	-

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

Table 14: Antimicrobial activity of *P. afra* extracts under 30/40°C with water deficit conditions

	Zone of inhibition (mm) of extracts	Treatment period (hours)																
	Microorganisms	Methanol				Hexane				Water (60ºC)				Ethyl Acetate				
		C	48	96	144	C	48	96	144	C	48	96	144	C	48	96	144	
Leaves	*E. coli*	-	-	-	-	16±0.6	-	-	-	-	-	-	-	-	-	-	-	
	S. aureus	-				18±0.6				-				14±0.6				
	S. griseus	-				18±0.6				-				-				
Stems	*E. coli*	-	-	-	-	13±0.6	-	-	-	-	-	-	-	-	-	-	-	
	S. aureus	-			11±0.1	13±0.6		12±0.1		-				-				
	S. griseus	-				13±0.6				-				-		11±0.1		
Roots	*E. coli*	-	-	-	-	15±0.6	-	-	-	-	-	-	-	-	-	-	-	
	S. aureus	-				17±0.6				24±0.6	13±0.1			-				
	S. griseus	-				18±0.6				-				-				

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

Table 15: Antimicrobial activity of *P. afr*a extracts under 35/45°C with water deficit conditions

	Zone of inhibition (mm) of extracts	Treatment period (hours)															
	Microorganisms	Methanol				Hexane				Water (60ºC)				Ethyl Acetate			
		C	48	96	144	C	48	96	144	C	48	96	144	C	48	96	144
Leaves	*E. coli*	-	-	-	10±0.1	16±0.6	-	-	-	-	-	-	-	-	-	10±0.2	11±0.
	S. aureus	-	-	-	-	18±0.6	-	-	-	-	-	-	-	14±0.6	-	-	-
	S. griseus	-	-	-	-	18±0.6	-	-	-	-	-	-	-	-	-	-	-
Stems	*E. coli*	-	14±0.1	-	-	13±0.6	-	-	-	-	-	-	-	-	-	12±0.1	12±0.
	S. aureus	-	-	-	-	13±0.6	-	-	-	-	-	-	-	-	-	-	-
	S. griseus	-	-	-	-	13±0.6	-	-	-	-	-	-	-	-	-	-	-
Roots	*E. coli*	-	21±0.1	-	-	15±0.6	-	-	-	-	-	-	-	-	9±0.1	11±0.2	10±0.
	S. aureus	-				17±0.6				24±0.6				-			
	S. griseus	-				18±0.6				-				-			

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

Antioxidant Activity

The in vitro antioxidant potential of all the extracts was determined by a 2, 2 diphenylpicryhydrazyl (DPPH) free radical assay, hydrogen peroxide scavenging (H_2O_2), and metal chelating activity assay. Figures 3-14 experimentally reveal the various changes observed in the antioxidant activities of *P. afra* plant parts' extracts with different solvents and treatment periods, under hot and cold temperatures [mid-range high (30/40ºC); and mid-range low (10/15ºC), extreme high (35/45ºC); and extreme low (0/5ºC)] concurrently with water deficit conditions.

Discussion

The medicinal plant components are categorised according to the synthesis, accumulation, and distribution patterns of phytochemicals. They are the root, stem, leaf, flower, fruits, and seed (Belkheir et al., 2016). In specific organs, tissues, and cells, distinct regulatory pathways and transport routes facilitate the synthesis of diverse secondary metabolites across various regions of medicinal plants. This phenomenon arises due to the complexity and diversity inherent in these secondary metabolites. (Belkheir et al., 2016).

The qualitative screening for phytochemicals in selected plant parts of *P. afra* under concurrent cold and hot temperatures with water deficit are presented in Table 2. Extraction solvents are important in the extraction of potential antioxidant compounds, and are determined by variations in chemical characteristics, polarities, and solubility of these compounds (Abubakar & Haque, 2020). Hence, the solvents options for extraction of phytochemicals in this study were chosen based about the polarity of the solute, for better accuracy in dissolving the solute (Abubakar & Haque, 2020).

In this study, multiple solvents were selected for the extraction process. The results indicated a fluctuation in presence of phytochemicals in the leaf, stem, and root of *P. afra*, when plant parts were subjected to the various temperature settings with water deficit. When compared to the control, it is evident in this investigation that the methanol extracts, across all the parts in both hot and cold conditions displayed the most presence of phytochemicals. This was however, followed by the aqueous extracts. Previous findings have documented that methanol and water targets 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 volatile (Roopashree & Naik, 2019).

In this study, tannins were found in every treatment in our investigation, across all plant sections and solvents. Tannins displayed a strong presence across all solvents in the leaf extracts under extreme cold temperatures (0/5°C) with water deficit condition, with increasing trends when compared to the control samples. In contrast, it showed a slightly increasing trend under concurrent extreme hot temperatures (35/45°C) with water deficit condition especially as the temperature dropped to the mid-ranges (30/40°C) but not across all solvents. Tannins is a generic name for a complex large biomolecule of polyphenol nature with abundance of hydroxyls and other suitable groups such as carboxyl to form strong complexes with various macromolecules (Das et al., 2020). They have anti-inflammatory and curative properties used to treat piles, burns and some venereal diseases (Boroushaki et al., 2016). They also function as insecticides and regulate plant growth (Nandagoapalan and Marimuthu, 2016). The strong presence of tannins observed across all solvents in the leaf extracts, may potentially enhance anti-inflammatory and curative properties of *P. afra*, thus making it more suitable and potent for the treatment of piles, burns and some venereal diseases.

A broad class of polyphenol chemicals 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 actions (Agidew, 2022). The flavonoids content across all plant parts in the mid-range hot (30/40°C) and cold (10/15ºC) temperatures, showed a slight increase, however, there was a moderate appearance in the aqueous leaf extract under the mid-range hot temperatures (30/40°C) after a 144-hour treatment period. Under the concurrent extreme hot temperatures (35/45ºC) with water deficit condition, there was a slight increase in the ethyl acetate leaf extracts after a 96-144-hour treatment periods, when compared to the control. In addition, the same trend was observed in the ethyl acetate and methanolic stem extracts under the same temperature stress (35/45ºC) while they were absent in all plant parts and across all solvents under the concurrent extreme cold (0/5ºC) with water deficit treatments. 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.

Saponins have curative properties due to the triterpenoids or steroid they possess (Jagtap et al., 2022). The methanolic leaf extracts out of all four solvents demonstrated a drastic change, indicating a strong presence of saponins under concurrent extreme cold temperatures (0/5ºC) with water deficit condition. However, in mid-range hot (30/40ºC) and cold (10/15ºC) temperatures, it was found in the methanol, ethyl acetate, and water extracts of all plant sections. Conversely, under concurrent extreme hot temperatures (35/45ºC) with water deficit condition, aqueous extracts across all plant parts displayed the most significant presence of saponins ranging from low to a high. This study suggests that the production of saponins in *P. afra* increases under concurrent extreme high and low temperatures with water deficit condition in comparison with the control groups. Furthermore, the concentration of glycosides in all plant parts across the different solvents indicated an increasing trend when compared to the control, which ranged from absent to a strongly present appearance under concurrent extreme cold temperatures (0/5ºC) with water deficit condition. While in the mid-range hot (30/40ºC) and cold temperatures (10/15ºC), a similar trend was observed apart from the ethyl acetate leaf and stem extracts under the cold treatment. Methanol and aqueous extracts exhibited a strong presence of glycosides in all plant parts.

In the pharmaceutical industry, volatile oils serve as commonly utilized flavouring agents and are leveraged for their medicinal properties, particularly for their antibacterial and carminative attributes

(Irshad et al., 2020). Interestingly, in this study, *n*-hexane, and ethyl acetate extracts across all plant parts under concurrent extreme hot (35/45ºC) and cold (0/5ºC) temperatures with water deficit showed the most significant increasing trend 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 oils (Soni et al., 2015). In contrast to the above reports, research findings according to Hussain et al. (2008) showed that essential oils from the aerial parts of basil *(Ocimum basilicum)* had the most amounts of essential oil in winter and minimum in summer. There are several reports available on how low temperature enhances the production of several groups of volatile terpenoids in essential oil glands on the leaf surface. 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 temperatures (Olennikov et al., 2017). The elevation shown in the volatile oil concentration across the varying temperature conditions 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 with water deficit than with individual stress. Thus, resulting to the production of terpenoids, which is a bioactive compound that confers several biological activities, and serve as a protective agent for essential oils (Soni et al., 2015). Conversely, volatile oils were produced in low to high quantity in all plant parts under the mid-range temperatures [hot (30/40ºC); and cold (10/15ºC)], while methanolic stem extracts detected a strong presence of volatile oils in both treatments. Ethyl acetate leaf extracts showed the strongest presence of volatile after a 144-hour hot compared to the control group.

The quinones present in both aqueous and methanolic leaf extracts remained relatively constant with the control sample extracts, presenting a moderate to a high presence in both extreme hot (35/45ºC) and cold (0/5ºC) temperatures with water deficit condition. However, the methanolic, ethyl acetate and aqueous stem and root extracts appeared to be favoured under the concurrent extreme cold temperatures (0/5ºC) with water deficit conditions, with a dramatic increase in the quinones concentration when compared with the control sample extracts.

Phenols are the most prevalent class of phytoconstituents that perform protective roles by shielding plants from pathogens and antagonistic abiotic factors (Takó et al., 2020). Methanolic extracts showed a high presence of phenols in all plant parts under the mid-range temperatures [hot (30/40ºC); and cold (10/15ºC)] with water deficit condition 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 deficits condition.

Coumarins present in all plant parts of *P. afra*, which is commonly used in cancer treatment, renal cell carcinoma and blood diseases has caught the attention of many researchers due to the photochemotherapy and therapeutic application in cancer (Lončar et al., 2022). The methanolic and aqueous extracts of all plant parts, showed a significant increase in comparison to the control in both concurrent extreme hot (35/45ºC) and cold (0/5ºC) temperatures with water deficit condition. In general, results as shown in the heat map (Tables 2 and 3) suggest that the production of coumarins is best under the extreme hot (35/45ºC) and cold (0/5ºC) temperatures with water deficit.

Plant carbohydrates are important biochemical indicators of temperature acclimation and cold tolerance development (Olennikov et al., 2017). Therefore, the carbohydrate concentration across all plant parts and solvents, in comparison with the control samples showed an increasing trend only in the methanolic extracts. This dramatic improvement shifted from absent to high amounts in both concurrent extreme hot (35/45ºC) and cold (0/5ºC) temperatures with water deficit condition. While in the methanolic stem extracts under the mid-range temperatures [hot (30/40ºC); and cold (10/15ºC)] temperatures with water deficit condition, an increasing pattern was also noted.

Studies have shown that water stress may amplify the accumulation of phytochemicals (Yeshi et al., 2022), contrary to the belief of the negative impact of severe water deficit (Yeshi et al., 2022),). 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 quality (Mahajan et al., 2020). The qualitative phytochemical data from this study, however, might be the result of the simultaneous impact of both extreme hot and cold temperatures with water deficit stress.

Studies have shown that seasons have an impact on chemical composition, even if each plant's makeup and accumulation of secondary metabolites varies with seasons (Li et al., 2020). While seasons and variation in temperatures are the primary cause of fluctuations in secondary metabolites, water also has a significant impact on these differences (Yeshi et al., 2022).

Quantitative results for the total flavonoids (TFCs) content in (Tables 4 and 5) and total phenolics contents (TPCs) in (Tables 6 and 7) respectively, show the variations in the phytoconstituents across all the plant parts extracts with the different solvents and treatment periods, under concurrent cold temperatures [mid-range low (10/15ºC); and extreme low (0/5ºC)] with water deficit condition. The highest TPCs and TFCs in all the plant parts extracts in comparison with the control samples were found in the mid-range cold temperatures (10/15ºC) with water deficit settings. This was recorded in the aqueous stem extracts after a 144-hour treatment period and aqueous leaf extracts after a 48-hour treatment period (9921.55 ± 8.91 and 7529.09 ± 3.12 mg/g respectively) with control samples values for TPCs (1820.67 ± 25.08 mg/g) and TFCs values (592.70 ± 49.99 mg/g) in the aqueous stem and leaf extracts respectively.

In contrast, results of phytoconstituents in (Tables 8 and 9) for total flavonoids content (TFCs) and total phenolics content (TPCs) in (Tables 10 and 11), present the order of fluctuations in these chemical compounds across all the plant parts extracts with the different solvents and treatment periods, under concurrent hot temperatures [mid-range high (30/40ºC); and extreme high (35/45ºC)] with water deficit condition. Here, the highest amount of TPCs and total TFCs in all the extracts, when compared to the control samples were noticed under the extreme hot temperatures (35/45ºC) in ethyl acetate leaf and methanolic leaf extracts after a 96-hour treatment period (9962.29 ± 35.63 and 8598.59 ± 14.28 mg/g respectively) with control sample values for TPCs (3008.08 ± 81.65 mg/g) and TFCs values (150.47 ± 1.17 mg/g) in the ethyl acetate leaf and methanolic leaf extracts respectively.

The data reported in this study indicates that concurrent extreme hot temperatures (35/45ºC) with water deficit condition appear to cause an accumulation of total phenolics and total flavonoids in *P. afra*, in comparison with the concurrent cold temperatures with water deficit condition. Similarly, Al-Huqail et al. (2020) reported higher accumulation of TPCs in plants under stress, especially in basil plants (*Ocimum basilicum L.*) treated with high temperature. He also observed an increase in TPCs and TFCs 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. Moreover, a vast variety of substances, such as arylpyrones and styrylpyrones, stibenes, tannins, coumarins, flavonoids, lignins, and lignans, are included in the category of phenolics and they are produced in response to abiotic stress (Albergaria et al., 2020). Consequently, they protect the plants and increase their tolerance against various abiotic and biotic stresses (Prinsloo & Nogemane, 2018). 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 findings (Al-Huqail et al., 2020). Additionally, exogenous factors are responsible for the biosynthesis and accumulation of phenolic compounds while endogenous factors, developmental stage, and tissue differentiation (Chowdhary et al., 2021) 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 some phenolic compounds and their temperature-dependent fluctuations as seen in this study, suggest that there is a link between their different 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. The utilization of antibiotic resistance inhibitors of plants origin is one way to lessen antibiotic resistance (Álvarez-Martínez et al., 2020). 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 resistance (Anand et al., 2019).

The antimicrobial activity results of *P. afra* extracts with at varying treatment periods as presented in Table 12–15 show that the antimicrobial activity of the extracts was found to be subject to temperature with water deficit condition. In the extreme hot temperatures (35/45ºC) with water deficit condition, the

highest level of inhibitory effect of 21mm against gram-negative *E. coli* when compared with the control samples was recorded from the methanolic root extracts after a 48hr-treatment period. This was also followed by a considerable effect of 14mm against *E. coli* from the methanolic stem extracts. Furthermore, the activities mainly observed in the ethyl acetate extracts were intermediate, ranging from 9-12mm, with an increasing trend when compared with the control samples (Table 15). These activities mainly observed in the ethyl acetate extracts, suggest the presence of phytochemicals that are responsible for inhibition in them. These results are in accordance with previous research demonstrating maximum antibacterial activity against *E. coli* by the leaves of *Amaranthus spinosus L*. during the summer month May-June (Mitra, 2014). 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 the extreme hot temperatures (35/45ºC) with water deficit condition. 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 bacteria (Buwa & Staden, 2007). Meanwhile in the mid-range hot temperatures (30/40ºC) with water deficit condition, there were no zones of inhibition recorded for *E. coli* across all the plant part extracts. However, the inhibitory activities mainly observed were intermediate, ranging from 11-13mm against gram-positive *Streptomyces griseus* and *Staphylococcus aureus*. Here, the different fractions produced from 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 bacteria (Zouari-Bouassida et al., 2018). This might explain the inhibitory effect seen against *S. aureus* and *S. griseus* to the extracts and non-effectiveness of the tested fractions against *E. coli*.

A slight inhibitory effect of 11mm was both 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 and a decline in activity when compared with the control samples, under mid-range cold temperatures (10/15ºC) with water deficit condition respectively. This result is partly in line with findings from Ncube et al., (2011), where the best antibacterial activity was observed in winter season from dichloromethane bulb extracts against *S. aureus.* However, there were no zones of inhibition across all the plant part extracts under the extreme cold temperatures (0/5ºC) with water deficit condition. From this findings, the examined plant extracts under the hot temperatures [mid-range high (30/40ºC); and extreme high (35/45ºC)] with water deficit condition showed better activity against all the microorganisms. The observed variation in extract efficacy inhibiting microorganism growth may be as a function of the distinct cell wall layers characteristic of each microorganism (Cengiz et al., 2023). 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 microorganisms (Cengiz et al., 2023).

Plants vary in their bioactive phytochemical content based on intrinsic characteristics including the age of the plant, plant part, portions used, as well as extrinsic factors like the geographical climate, temperature, the nature of the soil and season (van Wyk Sartor & Prinsloo 2020).

Additionally, to the inherent morphological, physiological, and biochemical differences between leaf, stem and root, the observed differences in the activity between the plant parts of *P. afra* could also be explained by dynamics in the content and presence of these bioactive compounds, which could change in parallel with the presence or absence of the stimuli and result in changing antibacterial properties.

Antioxidants inhibit and delay oxidation process, by transferring electrons to free radicals to limit fluctuations to prevent further reaction (Pisoschi et al., 2021). Natural antioxidants have captured the interests of various fields, especially in research, food production and among end users, due to their high therapeutic properties and protection against lethal diseases brought about by oxidative stress (Idamokoro & Afolayon, 2020). In fact, they are most beneficial for use since they are free of side effects in comparison to the synthetic ones (Akbari et al., 2022).

The antioxidant activity and medicinal power of plants is significantly dependent on the secondary metabolites found in them, which in turn results to a reduction or prevention of oxidative stress by absorbing and neutralising free radicals (Pisoschi et al., 2021). Depending on the amount of antioxidant molecules present in the plant, which is greatly influenced by its growth environment, plants have various antioxidant properties (Akbari et al., 2022).

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. Antioxidant potential of *P. afra* leaf, stem and root extracts were investigated through a 2, 2 diphenylpicryhydrazyl (DPPH) free radical assay, hydrogen peroxide scavenging (H_2O_2) and metal chelating activity assay. The DPPH free radical is quick and is primarily for the assessment of antioxidant activity against the DPPH radicals (Kim et al., 2022). Through a reduction in the maximum absorption of DPPH, this technique evaluates the antioxidant activity of substances that can function as hydrogen donors and decolorize DPPH-free radicals from purple to yellow. Secondary metabolites are primarily responsible for medicinal plants' capacity to scavenge free radicals (Kim et al., 2022). When hydrogen peroxide turns into hydroxyl and reactive radical metal complexes, it becomes generally damaging (Jomova et al., 2023). Metal chelating is a technique used for detecting certain metal radicals, and by interacting with and stabilizing the metal free radicals, metal chelating prevents oxidative stress (Rahali et al., 2017).

Plant extracts inhibit oxidation by interacting with free radicals, chelating free catalytic metals, and serving as scavengers of oxygen by-products. Earlier research has also revealed the ways metal chelating activity is utilized in the estimation of the antioxidant capacity of plant extracts (Gulcin & Alwasel 2022).

Figures 3–14 experimentally reveal the various changes observed in the antioxidant activities of *P. afra* extracts with different solvents and treatment periods, under hot and cold temperatures concurrently with water deficit conditions, when compared with the control samples. Under concurrent cold temperatures [mid-range low (10/15ºC); and extreme low (0/5ºC)] with water deficit condition, a significant increasing trend of antioxidant capacity was observed across all plant parts' extracts when compared to the control samples. Interestingly, both ethyl acetate root extracts under extreme cold temperatures (0/5ºC) and leaf extracts under mid-range cold temperatures (10/15ºC) showed the highest hydrogen peroxide scavenging activity (0.16 ± 0.029 and 0.16 ± 0.039 mg/ml respectively), amongst the plant's parts after a 48-hour treatment period, with IC_{50} values (0.89 ± 0.160 and 4.13 ± 0.098 mg/ml) for control samples of ethyl acetate root and leaf extracts respectively. *n*-hexane root extracts under extreme cold temperatures (0/5ºC) showed the highest antioxidant activity (0.73 ±

0.119 mg/ml) against DPPH radical, after a 144-hour treatment period with IC_{50} for control sample (10.23 ± 0.156 mg/ml). Similarly, *n*-hexane stem extracts under mid-range cold temperatures (10/15ºC) displayed the strongest metal chelating ability (1.26 ± 0.157 mg/ml) after a 96-hour treatment period with IC_{50} for control sample (6.65 ± 0.600 mg/ml).

Likewise, under concurrent hot temperatures [mid-range high (30/40ºC); and extreme high (35/45ºC)] with water deficit conditions, a drastic increase in antioxidant activity was displayed in some of the extracts in comparison with the control samples. The most significant hydrogen peroxide scavenging activity was seen in the *n*-hexane stem extracts (0.14 ± 0.048 mg/ml) under extreme hot temperatures (35/45ºC) after a 144-hour treatment period, in comparison with other plant part extracts and with IC_{50} for control sample (2.94 ± 0.017 mg/ml). Ethyl acetate root extracts under the mid-range hot temperatures (30/40ºC) showed the highest antioxidant activity (0.26 ± 0.065 mg/ml) after a 96-hour treatment period, against DPPH radical with IC_{50} (3.00 ± 0.092 mg/ml) for the control. While the methanolic leaf extracts under mid-range hot temperatures (30/40ºC) displayed the strongest metal chelating ability (0.40 ± 0.078 mg/ml) after a 144-hour treatment period, with IC_{50} value for control (6.66 ± 0.629 mg/ml).

Considering all these, the overall highest DPPH and metal-chelating antioxidant activity under both cold and hot temperatures with water deficit conditions was found under the mid-range hot temperatures (30/40ºC) 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 and 0.40 ± 0.078 mg/ml respectively).

However, *n*-hexane stem extracts under concurrent extreme hot temperatures (35/45ºC) with water deficit conditions showed the overall strongest hydrogen peroxide scavenging activity (0.14 ± 0.048 mg/ml) after a144-hour treatment period.

From our observation, data available here also indicate that antioxidant activities of certain plant parts extracts are further enhanced under the concurrent extreme cold temperature (0/5ºC) with water deficit conditions.

The variation in this investigation could potentially be because of the dissimilarities in the concentration or potency of active compounds in the various plant parts under these stress conditions (Toscano et al., 2019). In addition, different antioxidant enzymes have temperature sensitivity, and they can only function at specific temperatures (Estrada-Cárdenas et al., 2021). Their actions also vary according to the crop varieties, their growth phases, and the growing season's tolerance or susceptibility to temperature (Ramazan et al., 2021). 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 year (Hasanuzzaman et al., 2013).

Thus, the results from this study suggest that the antioxidant activities in the plant sections 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.

Conclusions

In conclusion, the content of pharmacologically active secondary metabolites in *P. afra* is significantly influenced by environmental conditions, particularly in combination, according to the temperature with water deficit simulations from our findings. In this study, the observed variability in plant responses provides significant insights into the diverse reactions of various plant organs to distinct environmental stimuli. This includes the modulation of secondary metabolites, alterations in antimicrobial properties, and adjustments in antioxidant activities, showcasing nuanced fluctuations in response to the combined environmental factors. We could also infer that in addition to extraction of samples using solvent with different polarities, plant part, procedure, the state of sample and treatment period also matters in phytochemical extraction and accumulation. It is evident that abiotic factors in combination may influence secondary metabolites' accumulation, which could enhance the production of phytochemicals and phytomedicine's output.

Further studies are still recommended to accurately characterize the bioactive compounds in these extracts for the exploitation of this plant in various pharmaceutical fields and the promotion of the desired biological activities.

Abbreviations

P.afra
Portulacaria afra
IPCC
Intergovernmental Panel on Climate Change (IPCC)
DMSO
Dimethyl Sulfoxide
DPPH
2, 2 Diphenylpicryhydrazyl
ANOVA
A one-way analysis of variance
TFCs
Total flavonoid contents
TPCs
Total Phenolic contents,

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are in this published article and are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there are no conflicts of interest.

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Authors' contributions

OC collected, analysed, and interpreted the data for this study. OC wrote the manuscript, while IM supervised the work and was a major contributor in editing the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Portulacaria afra **leaves and stems**

Figure 2

Portulacaria afra roots

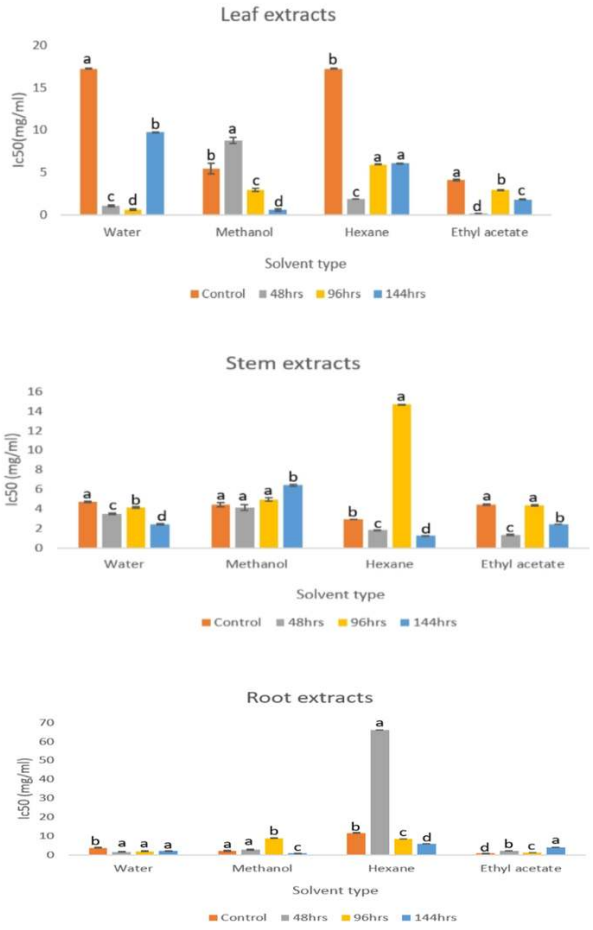

Figure 3

Graphical representation of (H O) antioxidant activity in *P. afra* plant parts under 10/15°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

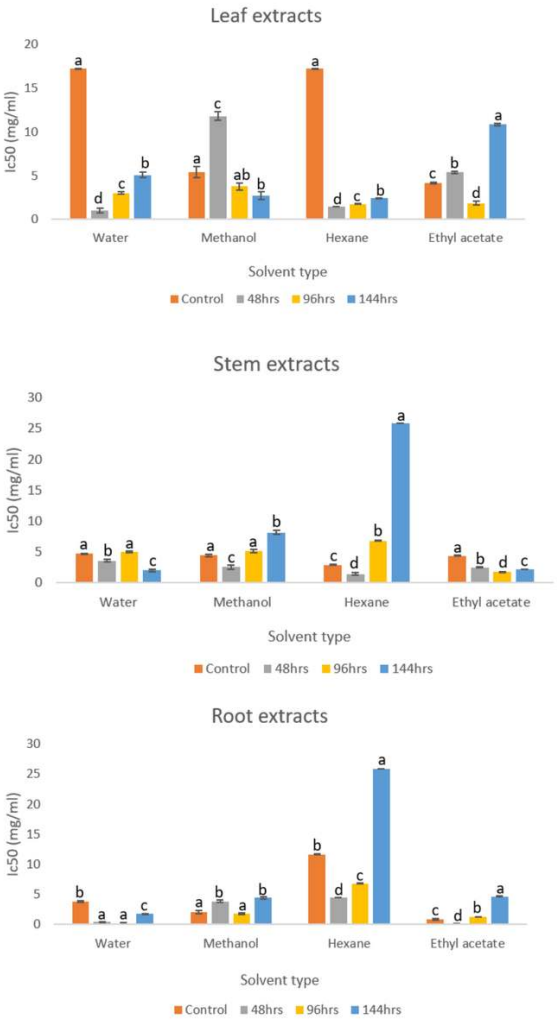

Figure 4

Graphical representation of (H O) antioxidant activity in *P. afra* plant parts under 0/5°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

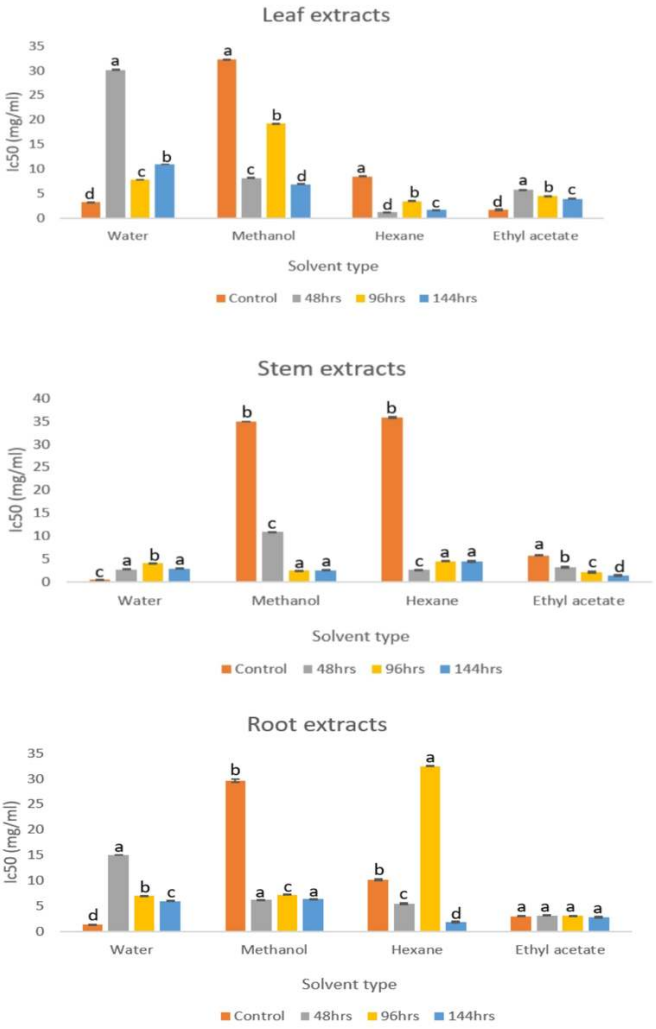

Figure 5

Graphical representation of DPPH antioxidant activity in *P. afra* plant parts under 10/15°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$). Error bars=standard error values (SE).

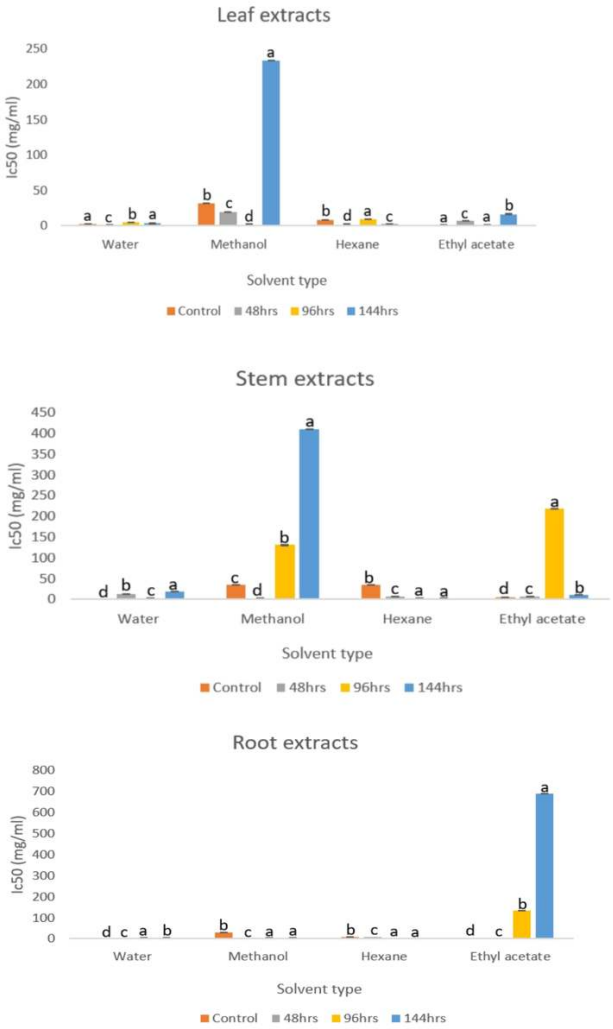

Figure 6

Graphical representation of DPPH antioxidant activity in *P. afra* plant parts under 0/5°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

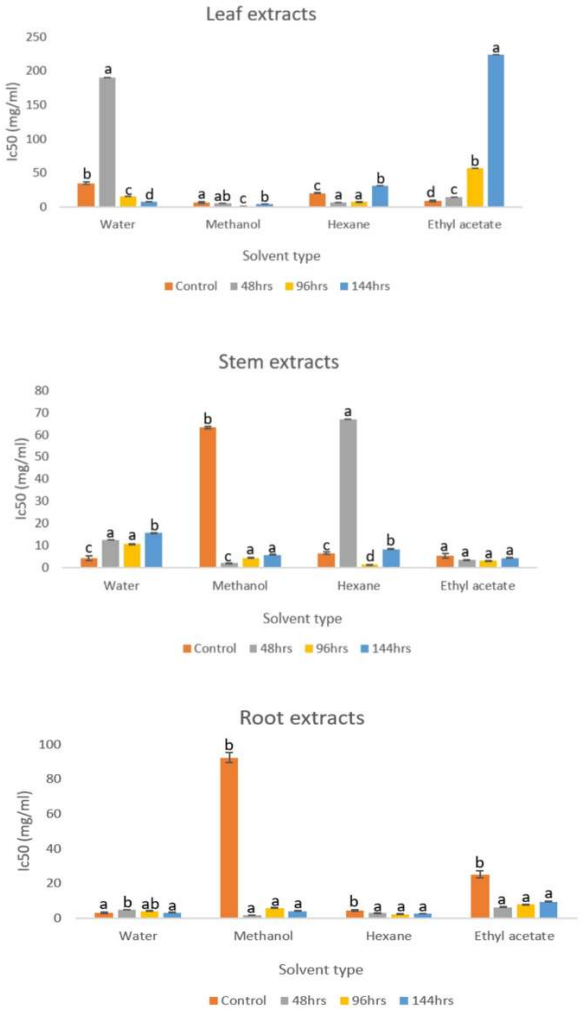

Figure 7

Graphical representation of metal chelating antioxidant activity in *P. afra* plant parts under 10/15°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

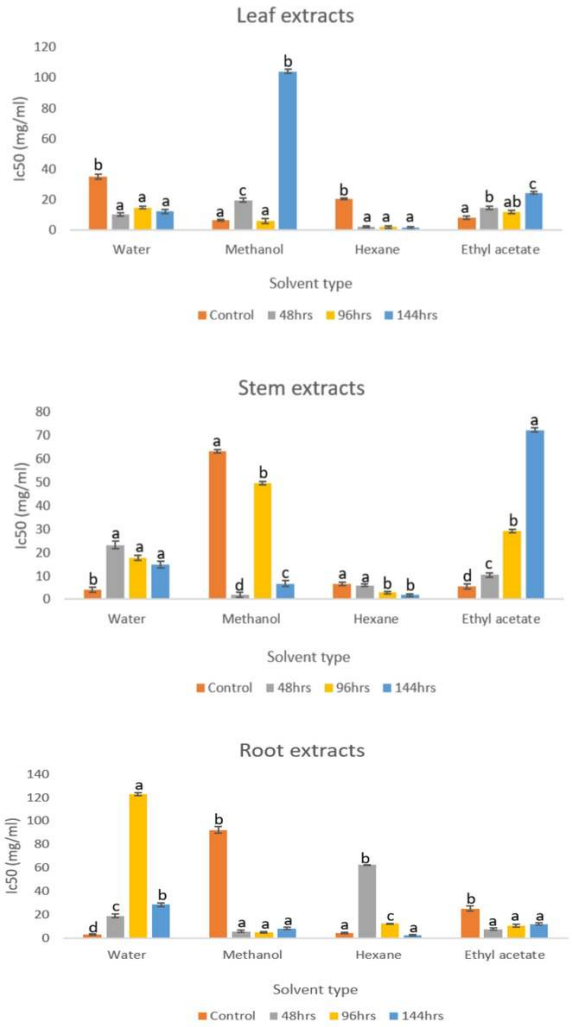

Figure 8

Graphical representation of metal chelating antioxidant activity in *P. afra* plant parts under 0/5°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

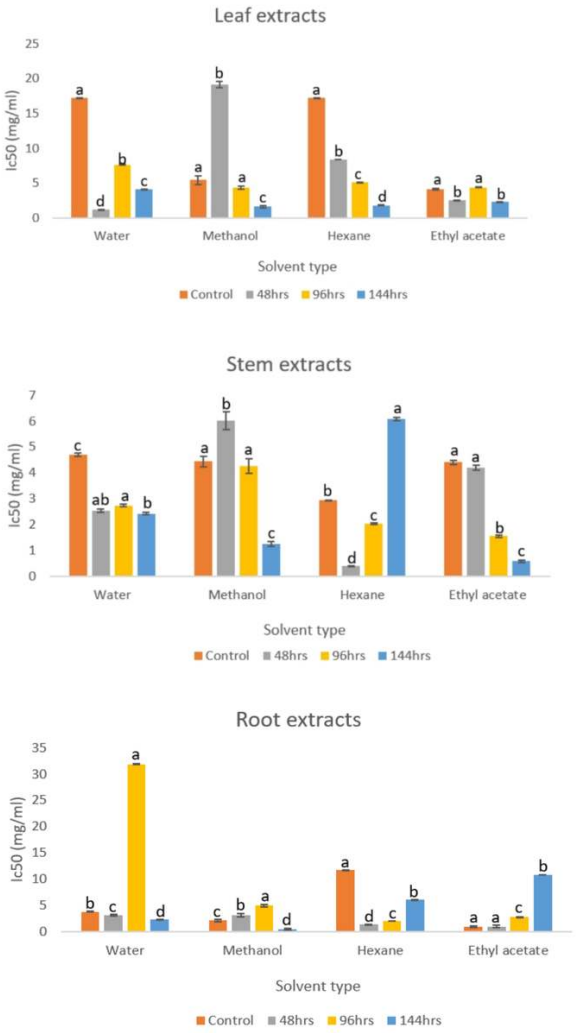

Figure 9

Graphical representation of (H O) antioxidant activity in *P. afra* plant parts under 30/40°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

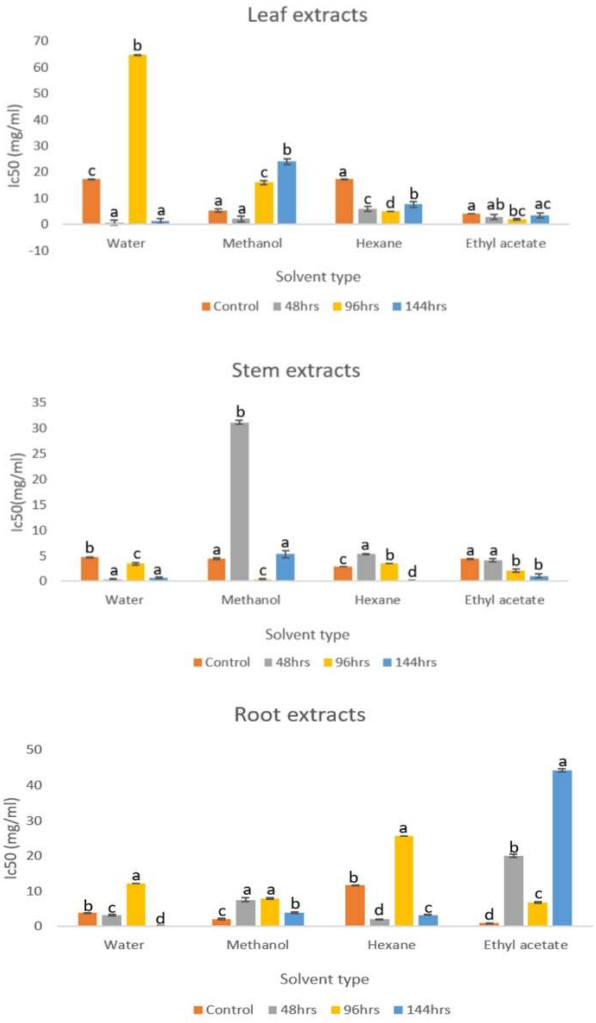

Figure 10

Graphical representation of (H O) antioxidant activity in *P. afra* plant parts under 35/45°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

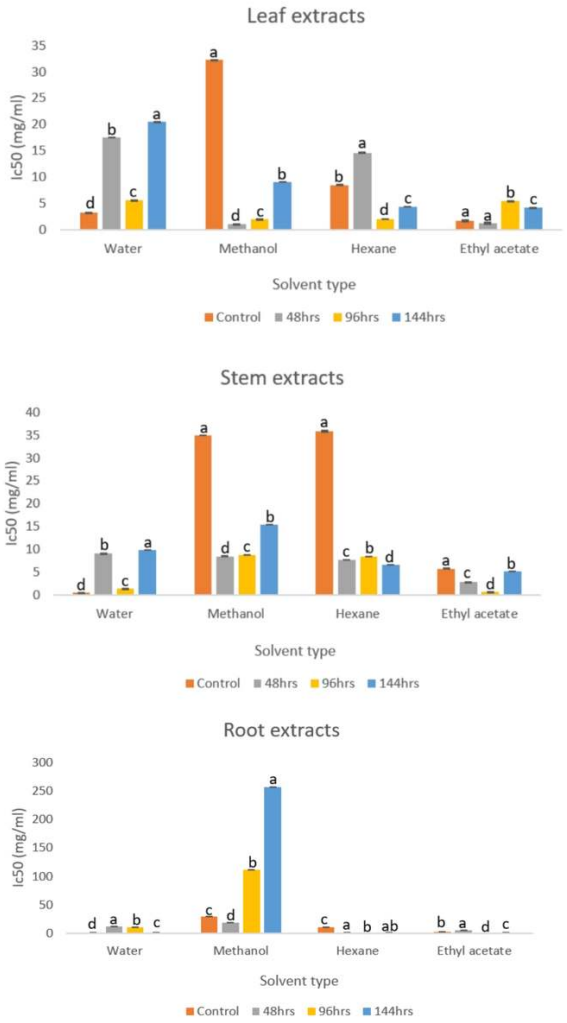

Figure 11

Graphical representation of DPPH antioxidant activity in *P. afra* plant parts under 30/40°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

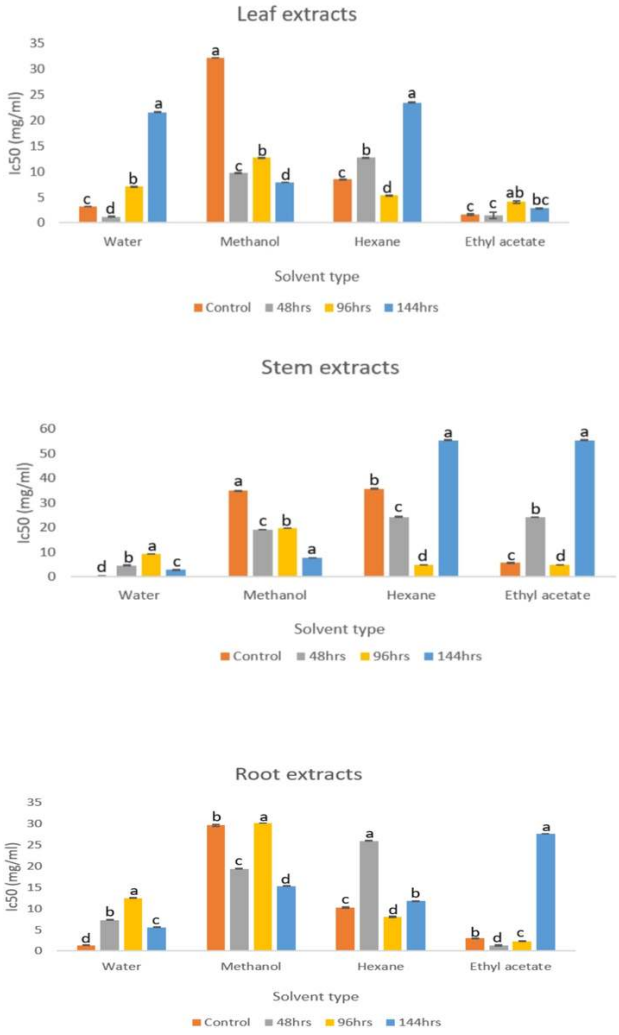

Figure 12

Graphical representation of DPPH antioxidant activity in *P. afra* plant parts under 35/45°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

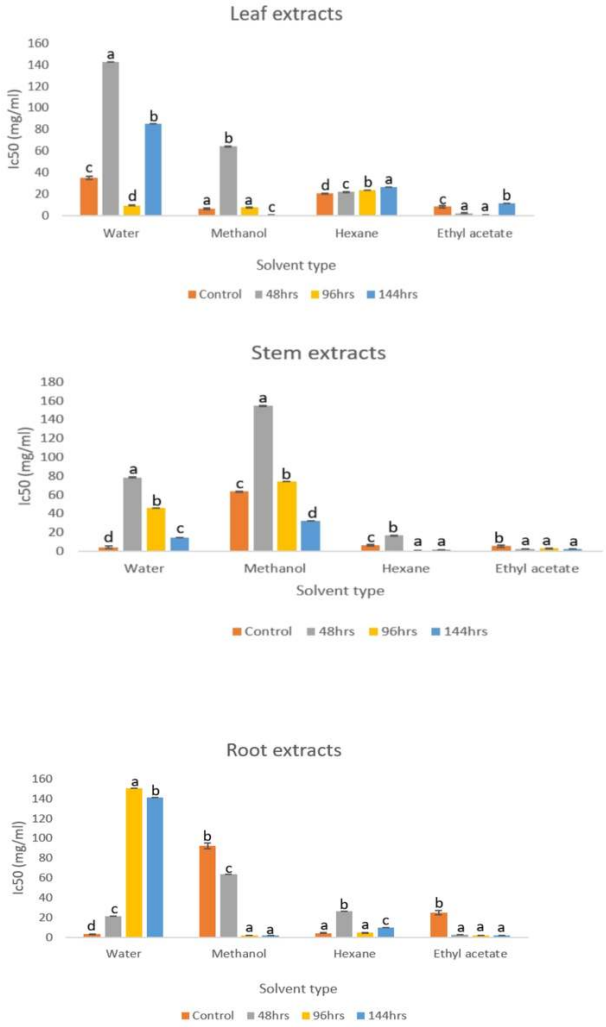

Figure 13

Graphical representation of metal chelating antioxidant activity in *P. afra* plant parts under 30/40°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

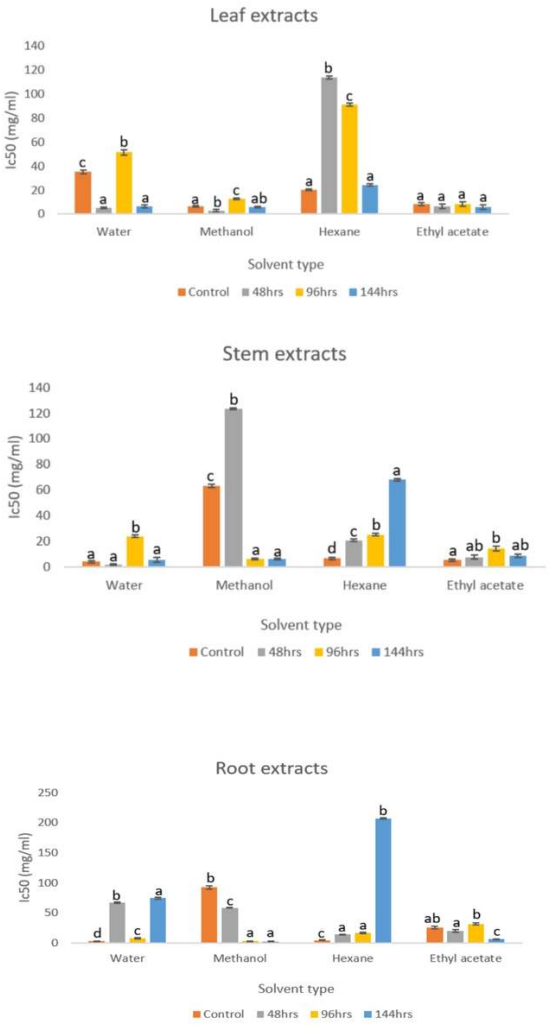

Figure 14

Graphical representation of metal chelating antioxidant activity in *P. afra* plant parts under 35/45°C with water deficit conditions. IC_{50} values were denoted as (±SE, mg/ml), n=3, and [a]Values with different alphabets were significantly ($p < 0.05$) different. Error bars=standard error values (SE).

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