Science Forum (Journal of Pure and Applied Sciences)

Journal Homepage: https://atbuscienceforum.com.ng/index.php/jpas

Effects of Different Watering Regimes on the Phytochemical Components and Physiological Parameters of Leaf Methanolic Extracts of *Aloe Vera* and *Cassia Tora*

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Abstract

This study investigates the influence of varying watering regimes on the phytochemical composition and physiological responses of *Aloe vera* and *Cassia tora* leaf extracts prepared using methanol. The primary objective was to evaluate how different water stress levels affect the production of bioactive compounds and physiological parameters within these medicinal plants. Four distinct watering treatments were applied: daily watering (control), and extended intervals of 2, 4, and 8 days between watering cycles. Standard phytochemical and physiological assays were employed to detect and quantify the presence of key secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids. Both qualitative and quantitative analyses were conducted to characterize the chemical profiles of the methanolic leaf extracts under each watering condition. The qualitative screening identified major phytochemical groups, while quantitative analysis measured their relative concentrations in response to water availability. Furthermore, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted to identify specific phytoconstituents present in the extracts of *A. vera* and *C. tora*. The findings reveal that water stress significantly influences both the concentration and diversity of phytochemicals in the plants studied, with potential implications for their medicinal efficacy and adaptive responses under drought conditions.

Keywords
Phytochemicals, Watering regimes, Extracts, Bioactive compounds, A. vera, C. tora, GC-MS.

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INTRODUCTION

Phytochemicals are natural bioactive compounds found in plants, including the medicinal plants, fruits, vegetables, flowers, leaves, roots, and fibers, and they act as a defense system against diseases or more accurately protect the plants against diseases (Krishnaiah *et al.*, 2009). Some of the important bioactive phytochemicals' constituents are the glycosides, alkaloids, flavonoids, tannins, steroids, terpenoids, essential oils and Phenolic compounds (Edeoga *et al.*, 2005). Species of Cassia are sources of polyphenols, anthraquinone derivatives (Bahorun *et al.*,2005; Ayo, 2010), flavonoids and polysaccharides. These biologically active chemical substances are known as secondary metabolites in medicinal plants, form the foundations of modern prescription drugs (Sofowora 1993).

Physiological parameters in plants include photosynthetic rate, stomatal conductance, transpiration rate, leaf water potential, chlorophyll content and relative water content. The efficiency of photosynthesis is influenced by light intensity, carbon dioxide concentration and temperature, recent studies highlighted various strategies plant use to optimise photosynthesis under changing the environmental conditions (Long *et al.*, 2021). Stomata are microscopic pores on leaves that regulate gas exchange, stomatal conductance affects photosynthesis and transpiration rate. Recent findings have shown how environmental stressors can alter stomatal behaviour, imparting overall plant performance (Mencuccini *et al.*, 2020).

Aloe barbadensis Miller, commonly referred to as *Aloe vera*, is one of more than 400 species of *Aloe* belonging to family Liliaceae that originated in South Africa, but have been indigenous to dry subtropical and tropical climates, including the southern USA (Reynolds and Dweck 1999). Recently, only a few species of Aloe have been considered for commercial importance, of which *Aloe vera* is considered the most potent and, thereby, the most popular plant in the research field. (Eshun and Qian, 2004). *Aloe vera* has been used in folk medicine for over 2000 years, and has 21 remained an important component in the traditional medicine of many contemporary cultures, such as China, India, the West Indies, and Japan. *Aloe vera* is a succulent plant. Succulents are xerophytes, which are adapted to living in areas of low water availability and are characterized by possessing a large water storage tissue.

Cassia tora (Leguminosae) is a wild crop and grows in most parts of India as a weed. According to Ayurveda the leaves and seeds are acrid (Ahmad *et al.*, 1988) laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardiotonic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorder. Chemical component of *Cassia tora* are anthraquinones, chrysophanol, emodin, obtusifolin, obtusin, chryso-obtulin, aurantio-obtusin, and their glycosides, Naphthopyrones, rubrofusarin, norrubrofusarin, rubrofusaring and entiobioside. *Cassia* is a large genus of around 500 species of flowering plants in the family Leguminosae (Lohda *et al.*, 2010).

Medicinal plants can be considered as any plant species that contains secondary metabolites that can be used for therapeutic purposes or can be used as precursors for the synthesis of new drugs. The importance of medicinal plant in drug development is known to us and humans have used them for different diseases from the beginning of human history.

MATERIALS AND METHODS

Experimental Site and Source of Plant Material

The experiment was conducted in the Biology Laboratory (11^0 42'20" N 9^0 22'13" E) of Biological Sciences department of Federal University Dutse, Jigawa State. The plant materials used in this study *Aloe vera* (L.) and *Cassia tora* (L.), were carefully sourced to ensure their authenticity and quality. Aloe vera seedlings were procured from a well-established horticultural garden specializing in the cultivation of *Aloe vera* plants around Dutse area, as recommended by Smith *et al*. (2020). These plants were chosen for their maturity and absence of visible diseases or pests. The collection process adhered to ethical and sustainable harvesting practices. *Cassia tora* (L.) seeds were acquired from the Botanical Garden of the Department of Biological Sciences, Federal University Dutse, where it is commonly found as weed.

Watering Regimes and Treatment Applications

The experiment involved the application of different watering regimes to evaluate their effects on both *Cassia tora* (L.) and *Aloe vera* (L.) plants. The water application intervals involved 2-day, 4- day and 8-day interval including control, with daily water application. The control group received daily watering to maintain consistent soil moisture levels. Each plant was provided with a predetermined volume of water daily, ensuring that the soil remained consistently moist but not waterlogged (Smith *et al.*, 2018).

Collection and Preparation of Plant Materials

Collected plant materials (leaves) were subjected to thorough cleaning to ensure their suitability for extraction. The plant materials were washed under running tap water to remove any surface contaminants or impurities. After washing, the materials were rinsed in distilled water to further eliminate any residual impurities. The cleaned plant materials were airdried in a shaded area at room temperature for weeks. This drying period was crucial to reduce moisture content, ensuring the materials were suitable for the extraction process. The dried plant parts were ground into a fine powder using a home grinder. The weight of the resulting ground powder was measured accurately for subsequent extraction steps.

Preparation of Leaf Extracts

The extraction of phytochemicals from the ground plant material was performed using a standard Soxhlet extraction method, as recommended in the literature (Sunil *et al.*, 2017; Elgorashi and Van, 2004). A total of 500 grams of the processed plant materials were used for each extraction. Methanol was chosen as the extraction solvent due to its efficiency in extracting a wide range of phytochemicals. The 500 grams of plant material was placed in a Soxhlet apparatus. The plant material was subjected to Soxhlet extraction using 1000 ml of methanol as the solvent. Extraction was

carried out at a temperature range of 55 - 88°C for a period of 24 hours. Following Soxhlet extraction, the crude extract was concentrated using a rotary evaporator at 40°C to remove excess solvent. This step aimed to obtain a more concentrated extract. The concentrated crude extract was subjected to freeze drying, which involved removing the remaining solvent under low-temperature conditions. This process ensured the complete removal of the solvent while preserving the integrity of the extracted phytochemicals. The resulting dry extract was stored at a temperature of 4°C to maintain its stability and quality for further use in subsequent analyses.

Gas-Chromatography and Mass Spectrometry

Methanolic extracts from *C. tora* and *A. vera* were sent to the Multi User laboratory of the Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria. GC-MS machine from Agilent Technologies (5977A MSD) was used for the phytochemical analysis.

QUALITATIVE PHYTOCHEMICAL SCREENING

Carbohydrates - Molisch's Test: To detect carbohydrates, a few milligrams of each extract were dissolved in distilled water, and a few drops of 10% alcoholic a-naphthol solution were added, followed by the addition of concentrated sulfuric acid. The appearance of a violet ring at the interface indicated the presence of carbohydrates (Khandelwal, 2005).

Saponins - Froth Test: A small amount of each extract was vigorously shaken in a test tube with distilled water. The formation of a persistent froth indicated the presence of saponins (Trease & Evans, 1989).

Tannins - $FeCl_3$ Test: A few milligrams of each extract were mixed with a few drops of 10% ferric chloride (FeCl3) solution. The formation of a blue-black or greenish-black colour indicated the presence of tannins (Khandelwal, 2005).

Flavonoids - Alkaline Test: A portion of each extract was treated with a few drops of 10% sodium hydroxide (NaOH) solution. The appearance of an intense yellow colour indicated the presence of flavonoids (Trease & Evans, 1989).

Cardiac Glycosides - Kellar-Killiani Test: A small amount of each extract was dissolved in glacial acetic acid, and a few drops of 1% ferric chloride (FeCl3) solution were added. Concentrated sulfuric acid was carefully added along the sides of the test tube. A brown ring at the interface indicated the presence of cardiac glycosides (Kokate, 1994).

Anthraquinones - Borntrager's Test: Each extract was mixed with dilute hydrochloric acid (HCl) and shaken. Afterward, an equal volume of chloroform was added, and the presence of a reddish-pink color in the chloroform layer indicated the presence of anthraquinones (Trease & Evans, 1989).

Steroids - Lieberman Burchard Test: A small amount of each extract was dissolved in chloroform, and a few drops of acetic anhydride were added, followed by concentrated sulfuric acid. The formation of a

greenish-blue color indicated the presence of steroids (Kokate, 1994).

Terpenoids - Salkowski's Test: A portion of each extract was mixed with chloroform and concentrated sulfuric acid was added carefully along the sides of the test tube. The appearance of a red-brown color in the chloroform layer indicated the presence of terpenoids (Kokate, 1994).

Alkaloids - Dragendorff's Test: Each extract was dissolved in dilute hydrochloric acid (HCl) and then treated with a few drops of Dragendorff's reagent. The formation of an orange or reddish-brown precipitate indicated the presence of alkaloids (Trease & Evans, 1989).

Wagner's Test and Meyer's Test for Alkaloids: Each extract was subjected to Wagner's and Meyer's tests separately by adding a few drops of Wagner's and Meyer's reagents, respectively. The formation of a brown or reddish-brown precipitate confirmed the presence of alkaloids (Kokate, 1994).

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Saponins

The presence and quantity of saponins were determined using the froth test (Trease & Evans, 1989). A portion of each extract was vigorously shaken in a test tube with distilled water. The height of the froth was measured, and the percentage of saponins was calculated by comparing it to a standard saponin solution

Flavonoids

The presence and quantity of flavonoids were assessed using the aluminium chloride colorimetric method (Chang *et al.*, 2002). Each extract was mixed with aluminium chloride reagent, and the absorbance was measured at 415 nm. Quercetin was used as the standard, and the percentage of flavonoids was calculated.

Tannins

The presence and quantity of tannins were determined using the ferric chloride ($FeCl_3$) test (Khandelwal, 2005). A few milligrams of each extract were mixed with a few drops of 10% $FeCl_3$ solution. The absorbance was measured at 510 nm, and the percentage of tannins was calculated.

Alkaloids

The presence and quantity of alkaloids were assessed using the acid-base titration method (Harborne, 1973). Each extract was dissolved in hydrochloric acid (HCl) and titrated with sodium hydroxide (NaOH) solution. The percentage of alkaloids was calculated based on the volume of NaOH used.

Cardiac Glycosides

The presence and quantity of cardiac glycosides were determined using the Kellar-Killiani test (Kokate, 1994). The absorbance of each extract was measured at 520 nm, and the percentage of cardiac glycosides was calculated.

Experimental Design and Statistical Analysis

Completely Randomized Block Design (CRBD) was used in the watering regime experiments. The antibacterial effects were experimented under completely randomized block design. Two-way Anova was used to analyze the data obtained from the physiological parameters.

Least significance difference was computed where there is significance, Tukey-Kramer test was used as post-hoc test. IBM-SPSS Statistical Package version 20 was used for the statistic.

RESULTS AND DISCUSSION:

Effects of Different Watering regimes on the Physiological Parameters of *C. tora* and *A. vera*

The results in **Table 1** revealed that both *Cassia tora* and *Aloe vera* exhibit varying degrees of physiological response to different Watering regimes. *Aloe vera* displayed greater tolerance to prolonged water stress, maintaining higher RWC and photosynthetic activity compared to *Cassia tora*. *Cassia tora,* on the other hand, exhibited more significant declines in these physiological parameters under prolonged water stress conditions. These findings provide valuable insights into the adaptive strategies of these two plant species under water stress, with *Aloe vera* showing a higher degree of resilience.

Relative Water Content (RWC) is a critical indicator of plant hydration and water status. In the control group, both *C. tora* and *A. vera* exhibited relatively high RWC values, indicative of well-hydrated conditions. This aligns with Jones (2007), that have shown that healthy plants typically maintain high RWC values when water is readily available. However, as the watering intervals increased, RWC decreased for both plant species, consistent with the expected response to water stress (Chaves *et al.*, 2003). *C. tora* experienced a more pronounced decrease in RWC compared to *A. vera* under prolonged water stress conditions, suggesting that *A. vera* may possess better water retention mechanisms.

The Stomatal Conductance (SC) reflects the rate of gas exchange and water loss through stomata. The decrease in SC observed as irrigation intervals lengthened is in line with the typical response of plants to reduce water loss under water-stressed conditions (Flexas *et al.*, 2004). Both *C. tora* and *A. vera* exhibited reductions in SC, but *A. vera* consistently maintained higher SC values, even under prolonged water stress. This may indicate that *A. vera* has better control over stomatal closure to limit water loss, a trait often associated with drought tolerance (Farooq *et al.*, 2012).

However, Photosynthetic Rate (PR) is a key parameter for assessing a plant's ability to carry out photosynthesis, which can be compromised under water stress. The decline in PR observed in both species as Watering regimes increased is consistent with the reduction in stomatal conductance, which restricts CO_2 uptake (Flexas *et al.*, 2004). Notably, *A. vera* exhibited a higher and more stable PR compared to *C. tora* under all Watering regimes. This suggests that *A. vera* may have mechanisms to maintain photosynthetic activity, even under moderate water stress, which could contribute to its resilience (Chaves *et al.*, 2009).

Leaf Water Potential (LWP) reflects the plant's ability to extract water from the soil. The more negative LWP values observed as irrigation intervals lengthened are indicative of increased water stress in both species (Turner, 1988). *A. vera* consistently maintained less negative LWP values compared to *C. tora*,

indicating that it may have mechanisms to access water from deeper soil layers or possess greater water-holding capacity in its tissues (Flexas *et al.*, 2010).

In plants, Chlorophyll content is essential for photosynthesis, and reductions can indicate impaired photosynthetic capacity under stress (Kalaji *et al.*, 2017). Both *C. tora* and *A. vera* exhibited decreases in CC under prolonged water stress. However, *A. vera* retained higher CC levels, suggesting better chlorophyll preservation and potentially more efficient light-harvesting mechanisms (Guidi *et al.*, 2011). The results demonstrate that both *C. tora* and *A. vera* exhibit physiological responses consistent with water stress. However, *A. vera* appears to be more resilient to prolonged water stress, maintaining higher RWC, SC, PR, less negative LWP, and higher CC compared to *C. tora*.

Table 1: Effects of Different Watering Regimes on the Physiological Parameters of *C. tora* and *A. vera* Plants

Watering regimes	Parameters	*Cassia tora*	*Aloe vera*
Control	RWC (%)	74.2 ± 2.1	82.6 ± 1.8
	SC (mol/m²/s)	0.07 ± 0.3	0.08 ± 0.2
	PR (µmol/m²/s)	21.4 ± 1.0	22.1 ± 0.8
	LWP (MPa)	-1.2 ± 0.1	-1.1 ± 0.2
	CC (µg/cm²)	25.5 ± 1.2	27.0 ± 1.0
2-Day	RWC (%)	68.5 ± 2.3	82.8 ± 1.9
	SC (mol/m²/s)	0.06 ± 0.002	0.07 ± 0.003
	PR (µmol/m²/s)	9.8 ± 0.9	20.5 ± 0.7
	LWP (MPa)	-1.4 ± 0.04	-1.3 ± 0.05
	CC (µg/cm²)	23.8 ± 1.1	27.0 ± 0.9
4-Day	RWC (%)	60.3 ± 2.0	70.2 ± 1.7
	SC (mol/m²/s)	0.57 ± 0.02	0.60 ± 0.02
	PR (µmol/m²/s)	7.6 ± 0.8	18.2 ± 0.6
	LWP (MPa)	-1.6 ± 0.03	-1.5 ± 0.04
	CC (µg/cm²)	21.5 ± 1.0	22.5 ± 0.8
8-Day	RWC (%)	59.8 ± 1.9	63.6 ± 1.6
	SC (mol/m²/s)	0.52 ± 0.02	0.55 ± 0.02
	PR (µmol/m²/s)	5.1 ± 0.7	15.8 ± 0.6
	LWP (MPa)	-1.8 ± 0.02	-1.7 ± 0.03
	CC (µg/cm²)	19.2 ± 0.9	20.0 ± 0.7

Key: RWC: Relative Water Content, SC: Stomatal Conductance, PR: Photosynthetic Rate, LWP: Leaf Water Potential, CC: Chlorophyll Content.

Effects of Different Watering Regimes on the Phytochemical Profile of Methanolic extracts from *A. vera* and *C. tora*.

Results in **Table 2 & 3** shows that *Cassia tora*, also known as "Sickle senna" or "Sickle pod," possesses a rich and diverse phytochemical profile, which undergoes notable changes when subjected to water stress. Saponins, glycosides with diverse biological activities, have been identified in *C. tora*. When water stress occurs, this plant exhibits an intriguing response. Research by Kumar and Rani (2016) has demonstrated that saponin concentrations can vary under water stress conditions. These compounds, known for their antimicrobial and anti-inflammatory properties, may be enhanced or modified as a part of the plant's defense mechanism. For the Flavonoids content, which is a class of secondary metabolites, contribute to the coloration of flowers and fruits in plants.

In *C. tora*, flavonoids are abundant, and their presence can influence the response of this plant to water stress. Studies by Basha and Sankaranarayanan (2015) have indicated that flavonoid levels may vary in response to water availability. Flavonoids are known for their antioxidant properties, and their modulation under stress conditions may be a part of the plant's adaptation strategy. However, Tannins, another group of polyphenolic compounds, play roles in plant defense mechanisms against herbivores and pathogens. In *C. tora*, tannins have been reported (Basha & Sankaranarayanan, 2015). Water stress may impact the concentration of tannins, potentially influencing the plant's ability to deter herbivores or pathogens in times of reduced water availability.

Alkaloids, diverse nitrogen-containing compounds, are found in *C. tora* and can display various pharmacological activities. Kumar and Rani (2016) have documented the presence of alkaloids in the plant. The specific influence of water stress on alkaloid concentrations is an area of ongoing research, as these compounds may serve adaptive functions in response to environmental challenges. Cardiac glycosides, compounds with effects on heart function, have been detected in *C. tora*, although their precise roles are not fully understood (Rauf *et al.*, 2019). Water stress may influence the biosynthesis or concentration of cardiac glycosides, potentially impacting the plant's ability to respond to stress-related physiological changes.

Secondly, *A. vera*, celebrated for its therapeutic properties, showcases a phytochemical profile that responds dynamically to water stress. *Aloe vera* contains saponins, which are believed to play a role in the plant's healing properties. Saponins have surfactant properties and may contribute to the plant's capacity to thrive in arid conditions (Hamman, 2008). Water stress may enhance or modulate the production of saponins as part of the plant's survival strategy. Flavonoids in *Aloe vera* are associated with antioxidant and anti-inflammatory effects (Surjushe *et al.*, 2008). Water stress may influence the concentration of flavonoids, potentially augmenting the plant's ability to mitigate oxidative stress and inflammation under challenging environmental conditions.

Tannins, with astringent properties, are present in *Aloe vera* (Eshun & He, 2004). These compounds may contribute to the plant's wound-healing and skin-care properties. Water stress could impact tannin levels, possibly influencing the plant's response to tissue damage and desiccation. While alkaloids are found in *Aloe vera*, their concentrations are typically low (Hamman, 2008). Their specific roles in the plant's response to water stress remain a subject of study. Alkaloids may play subtle yet significant roles in *Aloe vera*'s adaptations to environmental challenges. Cardiac glycosides are present in trace amounts in *Aloe vera* (Eshun & He, 2004). Their therapeutic significance in *Aloe vera* is not well-defined, and the influence of water stress on their concentrations warrants further investigation.

Table 2: Qualitative Phytochemical profile of methanolic extracts from *A. vera* and *C. tora*.

Phytochemicals	Plants	
	C. tora	*A. vera*
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Cardiac Glycosides	+	+
Anthraquinones	-	+
Steroids	+	+
Terpenoids	+	+
Alkaloids	+	+

Key: + =Present; - = Absent.

Table 3: Quantitative Phytochemical Profile of Methanolic extracts from *A. vera* and *C. tora* Affected by Watering Regimes.

Phytochemicals (%)	Plants	
	C. tora	*A. vera*
Saponins	10	7.86
Flavonoids	25	10
Tannins	3.23	2.5
Alkaloids	4.48	16
Cardiac Glycosides	1.23	1.31

Gas-Chromatography Mass Spectrometry Analysis of *C. tora* and *A. vera* Methanolic Extracts.

The results in **Table 4** shows GC-MS analysis of methanolic extracts from water-stressed *Aloe vera* (*A. vera*) has provided a rich dataset of compounds, each offering insights into the plant's response to water scarcity. From the result, 9-Octadecenal, (Z), an unsaturated aldehyde may serve as a signaling molecule or participate in oxidative stress responses. Unsaturated aldehydes have been linked to the regulation of stress-related genes and antioxidant defense mechanisms in plants (Sharma *et al.*, 2012). Long-chain alkenes, like 9-nonadecene, are associated with stress tolerance in plants. They can act as signaling molecules, triggering protective responses (Bertin *et al.*, 2003). The presence of Chlorinated compounds particularly Tetradecane, 1-chloro can be stress markers. They indicate the plant's response to environmental stressors, potentially related to changes in membrane permeability (Kour *et al.*, 2015).

However, Cyclopentane, 1-methyl-3-(1-methylethyl) may be involved in secondary metabolism modulation under stress conditions. Such compounds can contribute to the synthesis of stress-related metabolites (Wasternack and Strnad, 2018). Polyunsaturated compounds like hexadecatriene are known to act as antioxidants. They protect plant cells from oxidative damage induced by stress (Kumar *et al.*, 2013). The presence of aromatic compounds, especially Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl often play roles in plant signaling and defense mechanisms. They can influence the synthesis of secondary metabolites (Gershenzon and Dudareva, 2007). Carbonic acid, decyl prop-1-en-2-yl ester, a compound that may have implications for lipid metabolism and signaling processes. Lipid metabolism can be altered under stress to support energy requirements (Devaiah *et al.*, 2007). Sesquiterpenes like β-bisabolene possess antioxidant and anti-inflammatory properties. They can help mitigate stress-induced damage (Yang *et al.*, 2015). Similarly, synthetic antioxidants like butylated hydroxytoluene can enhance the plant's defense against oxidative stress during water scarcity (Furukawa *et al.*, 2014). The presence of these compounds collectively highlights *Aloe vera*'s multifaceted response to water stress. It likely involves the activation of stress-related genes, adjustments in lipid metabolism, and the production of antioxidants to counteract oxidative damage. The ability of *A. vera* to synthesize these compounds may contribute to its resilience in arid environments, where water scarcity is a prevalent stressor.

From the other hand, the result of gas chromatography-mass spectrometry (GC-MS) analysis in **Table 5a & 5b** of methanolic extracts from water-stressed *Cassia tora* (*C. tora*) presents an array of compounds that provide valuable insights into the plant's response to water scarcity. Butylated hydroxytoluene is a synthetic antioxidant known for its ability to scavenge free radicals and protect cells from oxidative damage (Cotelle *et al.*, 2018). Its presence suggests that *C. tora* responds to water stress by enhancing its antioxidant defense mechanisms. The presence of Pentadecanoic Acid, 14-

methyl-, and Methyl Ester may serve as an energy source during stress, as fatty acids can be utilized as substrates for mitochondrial respiration (Chapman and Ohlrogge, 2012). This could represent a metabolic adaptation to cope with water scarcity.

Unsaturated fatty acid derivatives, such as 9-Octadecenoic Acid (Z)-, Methyl Ester, have potential anti-inflammatory properties (Olefsky and Glass, 2010). Its increased abundance may indicate a response to stress-related inflammation. However, 9,12-Octadecadienoic Acid, Methyl Ester, commonly known as linoleic acid, it is a precursor for various bioactive compounds. Linoleic acid derivatives can have anti-inflammatory effects (Calder, 2015), suggesting a role in stress-induced inflammation mitigation. The methyl Stearate compound, a saturated fatty acid derivative, may contribute to membrane stability under stress conditions (Murphy, 2012), helping to maintain cell integrity.

Phthalates are known for their role in stress signaling. Diethyl Phthalate, as detected in this extract can influence plant responses to abiotic stress (He *et al.*, 2019). Diethyl phthalate might play a part in stress perception and signaling pathways in *C. tora*. Presence of 4,7,10,13,16,19-Docosahexaenoic Acid, Methyl Ester, particularly Docosahexaenoic acid (DHA), which is an omega-3 fatty acid with potential anti-inflammatory and neuroprotective properties (Minnella *et al.*, 2020). Its presence suggests that *C. tora* may activate mechanisms to counteract inflammation and oxidative stress under water stress conditions. Squalene is a precursor for the synthesis of phytosterols, which play vital roles in plant membrane structure and function (Adu-Kwarteng *et al.*, 2018). Increased squalene levels may reflect membrane modifications in response to water stress. Erucic acid is an unsaturated fatty acid that can influence membrane fluidity. Its presence may indicate adjustments in membrane composition to adapt to stress (Murphy, 2012). These identified compounds collectively signify a complex response of *C. tora* to water stress. They suggest potential alterations in antioxidant defenses, energy metabolism, inflammation modulation, and membrane stability.

Table 4: Gas-Chromatography Mass Spectrometry Analysis of *A. vera* Methanolic Extract.

Peak	RT	P A (%)	Name of Compound
1	10.073	1.2957	9-Octadecenal, (Z)-
2	10.6074	1.6937	9-Nonadecene
3	10.7937	1.5152	Tetradecane, 1-chloro-
4	11.8941	0.9819	Cyclopentane, 1-methyl-3-(1-methylethyl)-
5	12.5086	1.877	1, E-8, Z-10-Hexadecatriene
6	12.8804	3.9301	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
7	13.2237	3.3533	Carbonic acid, decyl prop-1-en-2-yl ester
8	13.4831	3.4355	Beta. -Bisabolene
9	13.647	6.6813	Butylated Hydroxytoluene
10	13.8521	1.6	(1S,5S)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl) bicyclo[3.1.0]hexane

11	13.8928	2.1716	Oleic Acid
12	14.8942	1.0116	Ethanol, 2-(3,3-dimethylcyclohexylidene)-, (Z)-
13	15.434	6.2844	Henicos-1-ene
14	15.5952	3.3588	Hexadecane
15	17.8758	0.7166	Heptadecane
16	18.5323	0.9483	Methyl tetradecanoate
17	19.9299	2.4578	5-Eicosene, (E)-
18	20.0631	0.64	Tritetracontane
19	21.7047	0.9773	2-Tetradecanone
20	22.7853	10.703	Pentadecanoic acid, 14-methyl-, methyl ester
21	23.5979	1.046	Phthalic acid, 5-methylhex-2-yl butyl ester
22	24.0466	0.6997	Cycloeicosane
23	26.0589	9.9241	9,12-Octadecadienoic acid, methyl ester
24	26.1845	22.8056	9-Octadecenoic acid, methyl ester
25	26.6706	2.9178	Methyl stearate
26	27.3907	0.9605	E-11-Hexadecenoic acid, ethyl ester
27	27.8179	0.144	Heptadecyl trifluoroacetate
28	29.1085	3.0398	n-Propyl 11-octadecenoate
29	29.5898	0.6309	Octadecanoic acid, propyl ester
30	30.2409	1.235	cis-5,8,11,14,17-Eicosapentaenoic acid
31	37.8518	0.9634	Squalene

Table 5a: Gas-Chromatography Mass Spectrometry Analysis of *C. tora* Methanolic Extract.

Peak	RT	P A (%)	Name of Compound
1	13.6387	0.2004	Butylated Hydroxytoluene
2	15.4266	0.192	5-Octadecene, (E)-
3	18.4259	0.9165	3-Butynylbenzene
4	19.9252	0.3641	1-Octadecene
5	22.7844	5.2233	Pentadecanoic acid, 14-methyl-, methyl ester
6	23.5932	0.6823	Dibutyl phthalate
7	24.0434	0.5886	Trichloroacetic acid, pentadecyl ester
8	24.1261	1.1888	Hexadecanoic acid, ethyl ester
9	26.0593	6.5583	9,12-Octadecadienoic acid, methyl ester
10	26.1911	19.663	9-Octadecenoic acid (Z)-, methyl ester
11	26.6748	5.0828	Methyl stearate
12	27.0888	0.5551	Methyl 8,11,14,17-eicosatetraenoate
13	27.2836	0.5665	Linoleic acid ethyl ester
14	27.393	2.8875	(E)-9-Octadecenoic acid ethyl ester
15	27.5007	0.6396	Ethyl Oleate

16	27.8197	0.4702	1-Docosene
17	27.8931	1.2984	Octadecanoic acid, ethyl ester
18	29.0123	0.2513	Methyl 9,12-heptadecadienoate
19	29.108	3.3506	n-Propyl 11-octadecenoate
20	29.5913	0.9662	Octadecanoic acid, propyl ester
21	29.7735	0.4278	cis-11-Eicosenoic acid
22	30.1177	0.3479	6-Butyl-1,4-cycloheptadiene
23	30.2541	13.5929	cis-5,8,11,14,17-Eicosapentaenoic acid
24	30.6097	0.7136	7,10,13-Hexadecatrienoic acid, methyl ester
25	30.903	0.9233	cis-11-Eicosenoic acid
26	31.0307	0.2023	Oleic Acid
27	31.2839	0.3775	Heptadecyl heptafluorobutyrate
28	31.3444	0.439	Ethanol, 2-(tetradecyloxy)-
29	31.9798	0.8099	Oleic Acid
30	32.4882	0.2459	cis-Vaccenic acid

Table 5b: Gas-Chromatography Mass Spectrometry Analysis of *C. tora* Methanolic Extracts.

Peak	RT	P A (%)	Name of Compound
31	32.7277	0.2116	Oleic Acid
32	33.1772	0.7821	Erucic acid
33	33.316	15.2724	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-
34	33.5667	4.5695	Methyl 6,9,12,15,18-heneicosapentaenoate
35	33.8723	1.705	Diethyl Phthalate
36	34.0849	2.8543	3-Eicosene, (E)-
37	34.296	0.5693	9-Eicosenoic acid, (Z)-
38	34.3682	0.1159	i-Propyl 9-tetradecenoate
39	34.493	1.8519	3-Eicosene, (E)-
40	34.6783	0.2119	13-Octadecenal, (Z)-
41	35.676	0.7313	Oleic Acid
42	35.8114	0.2555	Oleic Acid
43	37.8522	1.3136	Squalene
44	38.4314	-0.1833	2-Methyl-Z, Z-3,13-octadecadienol
45	38.583	0.0134	i-Propyl 9-tetradecenoate

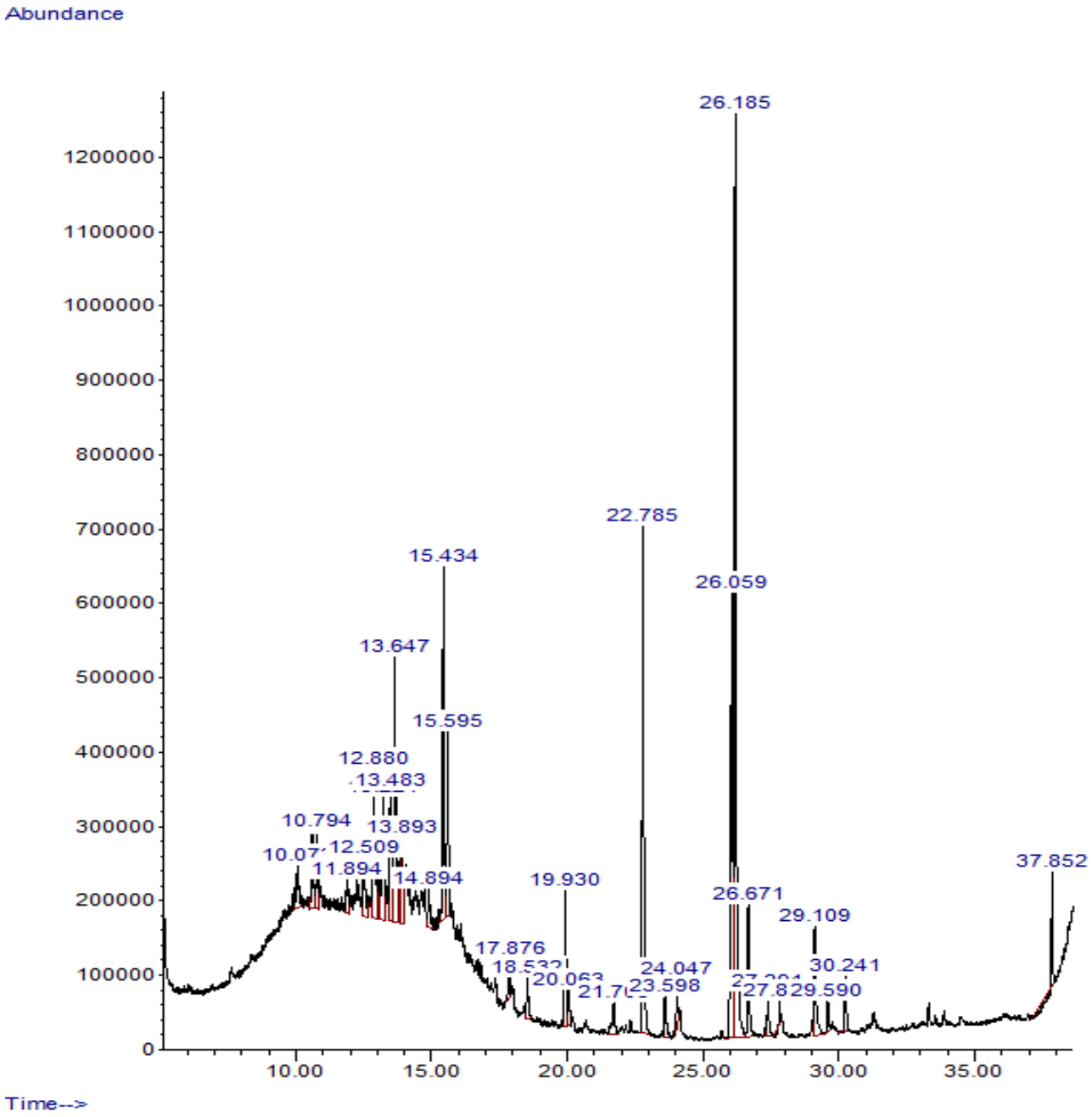

Figure 1: Gas-Chromatography Mass Spectrometry Chromatogram of *A. vera* Methanolic Extract

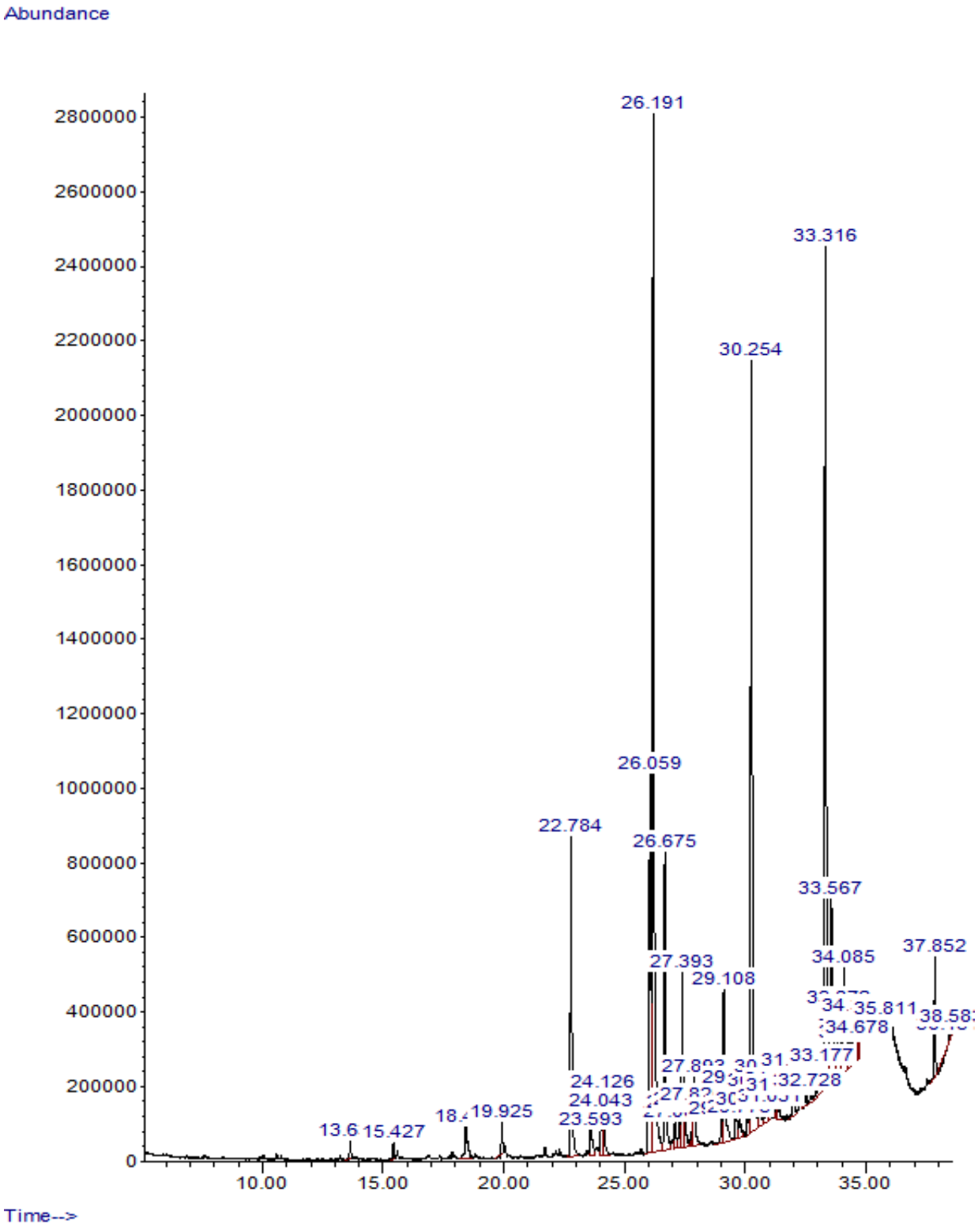

Figure 2: Gas-Chromatography Mass Spectrometry Chromatogram of *C. tora* Methanolic Extract

CONCLUSION

Both *C. tora* and *A. vera* exhibited a common trend of reduced physiological parameters (RWC, SC, PR, LWP, CC) as irrigation intervals increased, indicative of watering regimes. However, *A. vera* consistently displayed a higher degree of resilience compared to *C. tora* under prolonged water stress conditions. *A. vera* maintained higher RWC, SC, PR, and CC and less negative LWP, even under extended irrigation intervals.

Aloe vera possesses inherent mechanisms for efficient water retention, reduced transpiration, and sustained photosynthetic activity in the face of water scarcity. In contrast, *C. tora* exhibited more pronounced reductions in these physiological parameters, particularly under severe watering regime conditions. This indicates a comparatively lower tolerance to prolonged watering regime. While some compounds may increase in concentration to serve protective roles, others may undergo modifications to address physiological changes induced by watering regimes. This dynamic interplay between water stress and phytochemicals underscores the resilience and adaptability of these plants, offering valuable insights for future research in the fields of botany, agriculture, and medicine.

CONFLICT OF INTEREST

The authors have not declared any conflicts of interest.

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