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Analysis of Primary and Secondary Metabolites, Physical Properties, Antioxidant and Antidiabetic Activities, and Chemical Composition of *Rosmarinus officinalis* Essential Oils under Differential Water Stress Conditions

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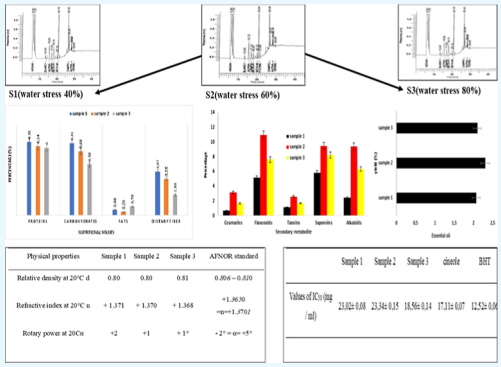
ABSTRACT: This study investigated the effects of varying water stress levels on *Rosmarinus officinalis* essential oils (EO). Three samples (S1, S2, and S3) were cultivated under different stress levels (40, 60, and 80%). Increased water stress led to changes in primary and secondary metabolites, EO contents, and physical properties. Antioxidant activity varied, with S2 exhibiting the highest IC_{50} value. In terms of antidiabetic activity, S2 showed robust α-amylase inhibition, while S3 displayed a commendable influence. For α-galactosidase inhibition, S3 had a moderate effect, and S2 stood out with increased efficacy. Gas chromatography−mass spectrometry analysis revealed stress-induced changes in major compounds. The study enhances the understanding of plant responses to water stress, with potential applications in antioxidant therapy and diabetes management. The findings emphasize the importance of sustainable water management for optimizing the EO quality in its various uses.



1. INTRODUCTION

The intricate interplay of climate dynamics profoundly influences the complex tapestry of weather patterns,[1] weaving intricate narratives for plant diversity and chemical compositions across ecosystems.[2] In the midst of this intricate ballet, human activities, particularly the heightened release of greenhouse gases since the Industrial Revolution,[3] trigger planetary shifts in temperature and rainfall, signaling alarming consequences in the realm of climate transformation.[4] If these patterns persist, the current trajectory of greenhouse gas emissions predicts a significant global temperature rise of 5.3 °C by 2100, posing a severe threat to the world's biodiversity.[5] The current temperature increase, already surpassing historical levels by 1.1 °C,[6] resonates widely across habitats, impacting both flora and fauna,[7] and raising concerns about the future of the planet.[8] Once mere whispers in the ecological narrative, these effects now resound with unprecedented intensity, marking a departure from past epochs.[9] The ethereal conductor positions climate change as a tempo-sensitive force, creating a symphony of reverberations across the canvases of plant biodiversity and molecular harmonies.[10] In this grand symphony, climate change disrupts the rhythmic cadence of crucial life cycle events for plants,[11] affecting processes such as petal unfolding, fruiting, and leaf descent.[12] Elevated temperatures expedite these events,[13] disrupting the delicate interactions between plants and their pollinators, or seed dispersers.[14] This dissonance cascades into broader movements, resonating throughout plant reproduction and the orchestration of ecosystems.[15]

Plants, as verdant conductors, play a pivotal role in various ecological services,[16] including carbon sequestration, water flow modulation,[17] and soil enrichment.[18] The evolving composition of the climate disrupts these processes, causing discord that reverberates across broader ecological narratives and socioeconomic tales.[19] In this evolving sonata, the stories of plants unfold a new chapter, influencing ancient traditions and cultural narratives centered around healing herbs and aromatic essences.[20] Weather, both direct and indirect, shapes the evolving saga of plant growth,[21] threading through biomass and influencing the intricate dance of chemical compositions.[22] In the cosmic audience, humanity's inadvertent actions amplify the crescendo of climate's impact, transposing its effects onto the

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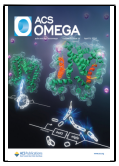

melodies of plant life and the nuances of molecular interactions.[24] In this interplay, the discordant strains of resource exploitation amid climate turbulence amplify the turmoil of our current environmental overture.[25]

The urgency of addressing climate change and mitigating its consequences has become paramount in safeguarding the richness of plant biodiversity, intricate chemical harmonies, and overall ecosystem vitality. This imperative calls for a global coalition to reduce greenhouse gas emissions, adopt sustainable practices, and preserve natural reservoirs. Through collaborative efforts, we can aspire to mitigate the detrimental effects of climate change on plant existence, paving the way for a more sustainable and balanced trajectory for our planet. The essence of this investigation revolves around two primary objectives: providing guidance for adapting to climate change's disruptions and unraveling the mysteries of plants' responses to climatic oscillations, exploring the intricacies of their fundamental processes and secondary metabolisms.

Recent research has closely examined the effect of water stress on the primary and secondary metabolites of the plant *Rosmarinus officinalis*, commonly known as rosemary. These studies have revealed that when plants are subjected to water stress conditions, their metabolism undergoes significant changes. Primary metabolites, such as carbohydrates, proteins, and amino acids, are often affected by water deficiency, as the plant must reorganize its resources to survive under stress conditions.[53−57]

The objective of this study is to assess the impact of water stress on the primary and secondary metabolites, physical properties, and antioxidant and antidiabetic activities of *R. officinalis* essential oils (EO) at various levels of water stress.

2. MATERIALS AND METHODS

2.1. Methodology. The three planting samples were subjected to varying degrees of water stress for a duration of one year. The first sample experienced a water stress level of 40%, the second sample endured a water stress level of 60%, and the third sample encountered a water stress level of 80%.

2.2. Phytochemical Screening. To conduct the phytochemical screening, we employed well-established qualitative analysis methods as referenced in.[18−21] These methods are widely recognized and utilized in the field of phytochemistry for identifying the primary and secondary metabolite families present in plant samples. The screening process enables the determination of the presence or absence of specific compounds or compound groups in the samples.

After identifying the major families of primary and secondary metabolites through qualitative analysis, we performed quantitative assays on the secondary metabolites. Reliable methodologies cited in[22−25] were applied to quantify these metabolites. These established methodologies provide a robust framework for accurately measuring and determining the levels or concentrations of the identified metabolites in plant samples.

2.3. Essential Oil. The plant material utilized in this study consisted of dried leaves that were subjected to shade drying. To extract the EO, approximately 100 g of the dried leaves underwent hydrodistillation using a Clevenger-type apparatus.[26]

2.4. Physical Properties. In our investigation, we assessed the physicochemical attributes and the quantity of EOs obtained from rosemary using a methodology outlined in the European Pharmacopoeia. This approach is a standardized protocol that defines the specific procedures for extracting EOs from rosemary, ensuring consistency and reliability in the results.[27]

We determined the physicochemical characteristics of rosemary EOs extracted according to the protocol described in the European Pharmacopoeia (Ph. Euro, 2014). The physicochemical characteristics sought are

- Density: using a METTLER TOLEDO 30 PX type densimeter.
- Rotational power: using an ATAGO AP300 polarimeter.
- The refractive index: using a NAR-1TLIQUID type refractometer.

2.5. Gas Chromatography−Mass Spectrometry. To compare the chemical compositions of the three samples obtained under distinct climatic conditions, we employed gas chromatography (GC) coupled with mass spectrometry (MS). The analysis was conducted using an Agilent 7890A Series instrument equipped with a multimode injector and a 123-BD11 column (15 m × 320 μm × 0.1 μm).

To facilitate the separation of compounds present in the samples, 4 μL of EOs were injected into the column using a split 1/4 mode. Helium gas was utilized as the carrier gas at a flow rate of 2 mL/min.

The compositions of the extracts and fractions were determined by calculating the percentage of total compounds detected in the sample. This was accomplished through full scan mode analysis within the range of 30−1000 m/z with a gain factor of 5 and electron impact ionization. The ion source and quadrupole temperatures were maintained at 230 and 150 °C, respectively.

For the temperature program, the oven was initially set at 30 °C and gradually increased until reaching a final temperature of 360 °C. This temperature gradient facilitated the separation and detection of different compounds present in the samples, enabling us to analyze and compare their chemical compositions.[29]

2.6. Antioxidant Activity. The antioxidant potential of EO was assessed through the β-carotene bleaching assay, a method that measures its capacity to mitigate the oxidative degradation of β-carotene within a linoleic acid β-carotene emulsion (Taga et al., 1984). To perform the assay, β-carotene (10 mg) was dissolved in 10 mL of chloroform ($CHCl_3$). Then, 0.2 mL of this solution was added to a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was eliminated by evaporating it using a rotary evaporator set at 40 °C for 5 min. Subsequently, distilled water (50 mL) was gradually introduced to the resulting residue under vigorous agitation to form an emulsion. This emulsion was combined with 0.2 mL of EO in a tube. The absorbance of the mixture was promptly measured at 470 nm, and the test emulsion was incubated in a water bath at 50 °C for 5 min. Following incubation, the absorbance was measured again. Butylated hydroxytoluene (BHT) served as a positive control, while in the negative control, the EOs were replaced with an equal volume of ethanol.

The antioxidant activity (%) of the oil was determined by quantifying the extent of β-carotene bleaching, employing a specific formula.

$$\text{inhibition } \% = \left(\frac{A - \text{CT}}{\text{CO} - \text{CT}}\right) \times 100$$

In the study, A and CT symbolize the measured absorbances for the oil and the control samples, respectively, following a 5 min incubation period. CO refers to the absorbance values of the control sample measured at the beginning of the incubation. To determine the concentration of EO that offers 50% antioxidant

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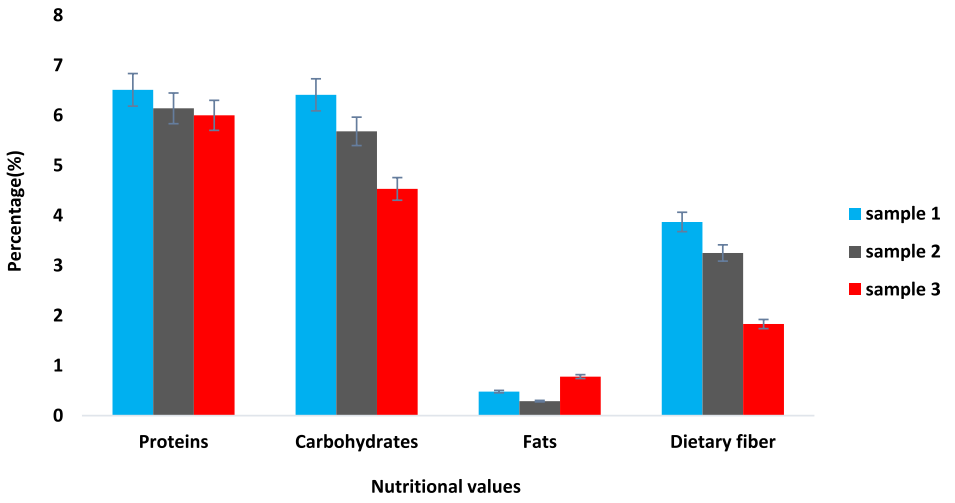

Figure 1. Percentage of nutritional values.

Table 1. Differences in Mineral Composition among Various Samples and Plant Species

elements	sample 1	sample 2	sample 3
Ca	6.57	6.11	4.88
P	2.46	2.1	1.58
K	4.37	4	3.65
Na	1.35	1.21	0.8
Cl	1.83	1.22	0.52
S	2.39	2	0.42
Mg	7.01	6.07	6.03
Fe	6.53	6.4	6.21
Mn	5.85	4.39	2.97
Zn	2.12	1.35	0.22
Pb	1.5	0.67	0.11
Se	1.35	1.01	0.19
Cu	1.2	1.19	0.61
Co	2.59	1.85	0.62

analysis of variance

source des variations	sum of squares	degree of freedom	average square	F	probability	critical value for F
elements	183.5952	13	14.12270769	96.12565774	1.45864×10^{-18}	2.11916569
sample	12.0961	2	6.04805	41.16581586	8.76667×10^{-9}	3.369016359
error	3.8199	26	0.146919231			
total	199.5112	41				

activity (EC_{50}), the researchers plotted the antioxidant percentage against the varying concentrations of the oil.[28]

2.7. Antidiabetic Activity. The primary objective of this study was to investigate the inhibitory effects on the activities of α-amylase and α-glucosidase using varying concentrations of test substances. These enzymes break down starch and *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG), respectively. The α-amylase inhibition test followed the protocol established by Naja et al. (2022). In this procedure, a mixture of the sample (250 μL) and α-amylase enzyme (240 U/mL) in 0.02 M sodium phosphate buffer (pH = 6.9) was incubated at 37 °C for 20 min. After the initial incubation, a 1% starch solution in the same buffer was added, and the reaction continued at 37 °C for 15 min.

The percentage of inhibition was determined using the formula: % inhibition = (AC − ACb) − (AS − ASb)/(AC − ACb) × 100. Here, AC represents the control, ACb is the control blank, AS signifies the sample, and ASb denotes the sample blank. This formula allows for the accurate quantification of inhibition caused by the test substances, providing insights into their effectiveness against α-amylase.

The α-glucosidase inhibition test was carried out with modifications to the method described by Asraoui et al. (2021). A mixture of the extracts and fractions (150 μL) and α-glucosidase enzyme (0.1 U/mL) in 0.1 M sodium phosphate buffer (pH = 6.7) was incubated at 37 °C for 10 min. After preincubation, a substrate solution (1 mM pNPG in 0.1 M sodium phosphate buffer, pH = 6.7) was added, and the reaction continued at 37 °C for 30 min. The reaction was halted by adding 1 M Na_2CO_3, and the absorbance was measured at 405 nm by using a spectrophotometer. To ensure accuracy, all tests were conducted in triplicate at various concentrations, allowing for the determination of IC_{50} values. Acarbose was used as a positive control for comparison.

These assays were designed to provide valuable insights into the potential of *Chenopodium ambrosioides* extracts and fractions as inhibitors of α-amylase and α-glucosidase, with potential applications in the development of natural therapies for diabetes

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Table 2. Portrays the Proportions of Amino Acids under the Influence of Three Distinct

amino acids	sample 1	sample 2	sample 3
aspartic acid	0.35	0.33	0.29
cysteine	0.69	0.46	0.21
glycine	1.54	1.35	1.02
histidine	0.44	0.22	0.18
isoleucine	1.31	1.09	0.42
leucine	2.31	2.11	1.24
lysine	0.29	0.13	0.04
phenylalanine	1.45	1.31	1.11
proline	1.21	1.3	1.03
serine	2.01	1.11	0.2
tyrosine	0.13	0.05	0.03
valine	0.1	0.01	0.01

analysis of variance

source of variations	sum of squares	degree of freedom	average square	*F*	probability	critical value for *F*
elements	183.5952	13	14.12270769	96.12565774	1.45864×10^{-18}	2.11916569
sample	12.0961	2	6.04805	41.16581586	8.76667×10^{-9}	3.369016359
error	3.8199	26	0.146919231			

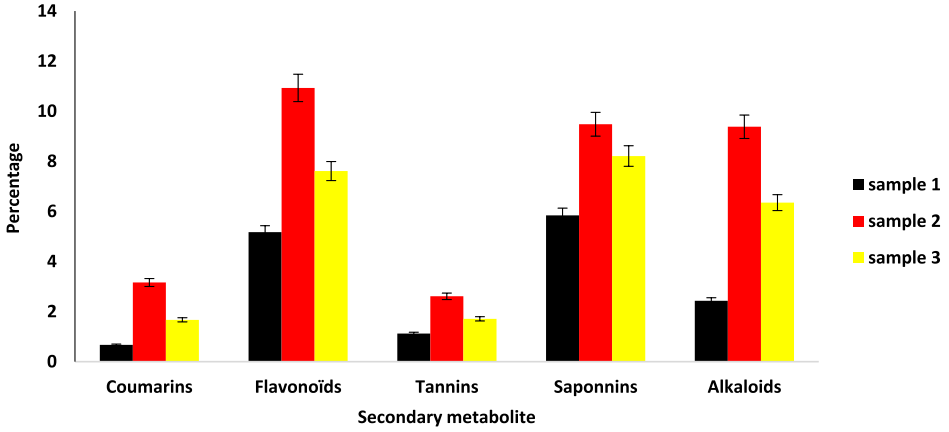

Figure 2. Percentage of secondary metabolite.

management. The outcomes of these tests will assist in evaluating the efficacy of these samples as natural inhibitors for diabetes treatment, contributing to the advancement of natural-based interventions for managing diabetes.[29]

2.8. Statistical Analysis. The data are expressed as the mean ± standard error and underwent statistical analysis using Graph Pad Prism 5 Software (San Diego, CA, USA) and Excel. One-way analysis of variance (ANOVA) was employed for the analysis of multiple-group comparisons (XLSTAT statistical software).

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening. *3.1.1. Nutritional Values.* As evident from Figure 1, the composition of basic nourishments displayed fluctuations across the trio of plants. Broadly, the concentrations of their constituents dwindled progressively from sample 1 through sample 2 to sample 3, aligned with the prevailing climatic conditions marked by heightened temperature and diminished precipitation. Yang et al. and Hessini et al.[30,31] substantiated that scarcity of water leads to diminished biomass production.

3.1.2. Mineral Compositions. Table 1 reveals differences in mineral composition among the various samples and plant species. Predominantly, the highest mineral compound contents include K, Mg, Fe, Mn, and Ca, while the remaining minerals exhibit lower concentrations across all three samples of each plant. These concentrations generally decline under precipitation-induced water stress. This pattern aligns with the findings of Canarini et al.,[32] who demonstrated the direct impact of water scarcity on growth, photosynthetic activity, and the attenuation of water transport to the roots, ultimately leading to diminished nutrient uptake. This intricate interplay highlights a mutual relationship.

Based on the analysis of variance (ANOVA) presented in the table, it is observed that the observed F value for the three samples exceeds the critical F value. This indicates that the difference between the groups is significant.

3.1.3. Amino Acids. Table 2 illustrates the proportions of amino acids influenced by three distinct treatments. It is noteworthy that the following amino acids are conspicuously absent: alanine, arginine, asparagine, glutamic acid, glycine, glutamine, methionine, pyrrolysine, cysteine, threonine, tyrosine, and tryptophan. Zandalinas et al. and Ostadi et al.[33,34] emphasize that climate change is detrimentally impacting plant life, posing a severe threat to agricultural production and food supplies.

Based on the analysis of variance (ANOVA) presented in the table, it is observed that the observed F value for the three

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samples exceeds the critical F value. This indicates that the difference between the groups is significant.

3.1.4. Secondary Metabolite. The graphical representation in Figure 2 reveals an increase in the concentration of secondary metabolites as water stress intensifies during the initial two years. However, as the temperature rises, the content of secondary metabolites diminishes. Furthermore, changes in solvent composition induce variations in secondary metabolite content, with alkaloids being the most prevalent in all three samples. Findings by Marone et al.[35] support these results, demonstrating increased counts of secondary metabolites under abiotic stress. Similarly, Shabankareh et al.[36] indicate that stress conditions trigger enhanced production of secondary metabolites, while Applequist et al.[37] emphasize that plants exposed to unfavorable conditions exhibit higher proportions of secondary metabolites. In a separate study, Li et al.[38] confirm an increase in secondary metabolite levels during abiotic stress. Notably, research by Takshak et al. and Pang et al.[39,40] illustrates that plants subjected to water stress elevate their bioactive compound levels. Consistent with this, Jactel et al.[41] demonstrate that plants under water stress increase their phenolic compound levels as a defense mechanism or adaptive response to harsh climatic conditions.

3.2. EO Yield. *3.2.1. Yield.* Evident from Figure 3 is the increased production of EOs in sample two in comparison to the other samples. This observation aligns with the research findings of Molotoks et al.,[42] who determined that plants subjected to mild stress display elevated concentrations of EOs compared to those experiencing severe stress. Furthermore, Ni et al.[43] demonstrated that rosemary under nonirrigated conditions exhibits the highest EO content.

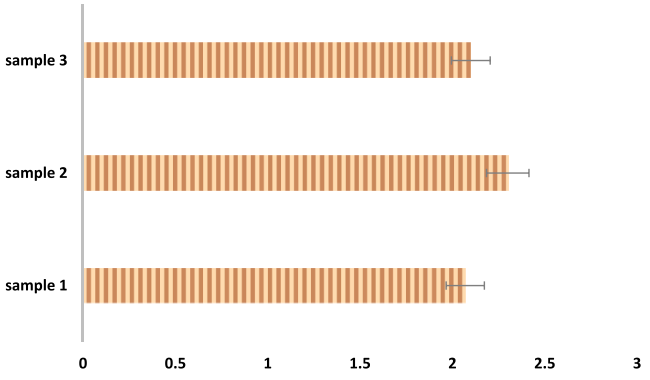

Figure 3. EO yield (%).

3.2.2. Physical Properties. EOs collectively exhibit organoleptic qualities, engaging our senses through taste, scent, appearance, and texture. Significantly, they maintain a liquid state at room temperature and possess high volatility, facilitating rapid evaporation. This volatility enables them to be easily carried by water vapor, enhancing their versatility for various applications. Beyond their utilitarian functions, EOs create an aromatic symphony, emitting alluring and distinct fragrances that captivate our senses. Their visual diversity is equally captivating and influenced by the specific extraction method employed. Microwave distillation with the Clevenger apparatus produces pale-yellow oils, while Clevenger-assisted distillation yields oils with a faint, delicate yellow color. In contrast, EOs obtained through simple hydrodistillation exhibit a striking and vivid red chromaticity.

Turning attention to the physicochemical attributes of three distinct rosemary EOs (outlined in Table 3), a panorama of variation emerges. Density ranges between 0.80 and 0.81, reflecting subtle differences. Similarly, the refractive index spans from 1.368 to 1.371, indicating disparities in optical behavior. The angle of rotation, indicating optical activity, varies within the range of +2 to +3°. These nuanced variations, akin to individual brushstrokes on a canvas, reveal intricate compositions that are unique to each EO. This array of distinctions carries significant implications, allowing researchers and industries to unravel the multifaceted identities characterizing each variant. Insights derived from these attributes deepen our understanding of potential applications across diverse realms, from perfumery to aromatherapy and pharmaceuticals.

Table 3. Physical Properties of EO under Climatic Conditions Different

physical properties	sample 1	sample 2	sample 3	AFNOR standard
relative density at 20 °C d	0.80	0.80	0.81	0.806−0.810
refractive index at 20 °C n	+1.371	+1.370	+1.368	$+1.3650 \leq n \leq +1.3701$
rotary power at 20 °C α	+2	+1	+1°	$-2° \leq \alpha \leq +5°$

Transitioning to the referenced studies, significant insights into plant responses to drought conditions are presented. Bettaib et al.[44] elucidate that drought manifestation, marked by elevated temperatures and limited water availability, induces notable biochemical changes in plant leaves. Specifically, a reduction in fatty acids and a decrease in the number of double bonds indicate a tangible shift in the plant's lipid composition under water-deficit conditions, likely an adaptive response to mitigate stress. Yang et al.'s[45] study expands our understanding of the far-reaching impacts of drought and water stress, revealing physiological, morphological, chemical, and physical alterations. These multifaceted adjustments underscore the plant's dynamic ability to respond to environmental challenges, optimizing resource allocation, enhancing water-use efficiency, and ensuring survival under adverse conditions. The collective findings highlight the complex and interconnected nature of plant responses to drought and water stress, providing insights crucial for understanding fundamental processes and informing strategies to enhance crop resilience and agricultural sustainability amid changing environmental dynamics.

3.2.3. Gas Chromatography−Mass Spectrometry. Table 4 unveils that the primary compound prevailing in the three examined EOs is 1,8-cineole, with varying percentages in each sample (S1: 48.83%, S3: 41.28%, and S2: 51.77%). Following 1,8-cineole, the subsequent significant compounds are camphor (S1: 17.35%; S3: 22.82%; S2: 22.31%) and α-pinene (S1: 10.66%; S3: 11.27%; S2: 9.84%).

It is noteworthy that the composition of constituents remains consistent among the three types of EOs, with monoterpenes encompassing all the identified compounds. Oxygenated monoterpenes predominate, constituting 72 to 83% of the compounds, while monoterpene hydrocarbons make up 16 to 27%.

However, the content of these compounds exhibits variability based on the extraction method employed. EO from sample 2

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Table 4. Chemical Compounds of Three Samples under Climatic Conditions Different (%)

	S1	S2	S3
α-pinene	9.49	8.15	10.11
camphene	4.53	4.17	4.47
β-pinene	3.72	0.19	8.03
aTerpinene	0.18	Tr	Tr
p-cymene	2.35	0.74	2.19
limonene	Tr	Tr	Tr
cineole	49.09	53.21	42.12
β-myrcene	2.54	1.94	1.32
linalool	0.13	0.1	0.21
camphor	17.93	22.53	22.68
borneol	1.17	2.8	0.96
aTerpineole	3.24	5.04	1.76
verbone	0.61	0.11	0.39
bornyl acetate	4.89	1.02	5.46
B-caryophyllene	Tr	Tr	0.11
a-caryophyllene	0.05	Tr	0.08

stands out as the richest in α-pinene (10.66%), camphene (4.71%), p-cymene (2.44%), and β-myrcene (2.40%).

These findings illuminate the chemical composition of the EOs, emphasizing the significant role of extraction methods in determining their specific compound content. The presence of common compounds across the samples underscores the consistent nature of the EOs, rendering them suitable for diverse applications in industries, such as cosmetics, aromatherapy, and pharmaceuticals. This aligns with similar results obtained by Sarmoum et al.,[46] who observed substantial variations in both qualitative and quantitative composition when applying diverse stress conditions to different rosemary oil plants. Minor constituents included camphor (1.159%) and caryophyllene oxide (1.739%), highlighting the considerable qualitative and quantitative divergence in chemical constituents among distinct rosemary oil plants, reflective of specific stress conditions imposed upon them.

Furthermore, in categorizing EO compounds into chemical groups, the outcomes indicate the prevalent dominance of three major groups: monoterpenes (31.41−35.57%), oxygenated monoterpenes (31.04−34.45%), and ketones (8.08−29.71%) in the oil composition across all experimental conditions. This aligns with the findings of García-Caparrós et al. and Haydari et al.,[47,48] who reported that water stress and salinity induce changes in the chemical compositions of plant EOs.

3.3. Antioxidant Activity. In our investigation, three samples of *R. officinalis* EO, namely S1, S3, and S2, were examined, all displaying significant antioxidant capacity (Table 5). The calculated IC_{50} values, representing the concentration needed to inhibit 50% of β-carotene, exhibited variations among the samples, with S2 having the highest value (23.34 ± 0.15), followed by S1 (23.02 ± 0.08) and S3 (18.56 ± 0.14). Notably, the IC_{50} values of 1.8-cineole (17.11 ± 0.07) and BHT (12.52 ± 0.06) were lower than those of the rosemary EO.

Attributing the antioxidant effect of a complete EO to specific active principles poses challenges, given that EOs consist of complex mixtures of various chemical compounds. In addition to major components, minor molecules may significantly contribute to the oil's antioxidant activity. Therefore, the observed antioxidant properties of the oils from this plant likely result from the combined activities of different major and minor components within the oil.

These findings align with Mumivand et al.,[49] confirming the significant impact of both drought stress and the synergistic interplay between drought stress and accessions on various physiological parameters, including superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, proline, drug yield, and EO yield (observed only in the second year). Additionally, Ghanbarzadeh et al., Kulak et al., and Ahmadi et al.[50−52] reported heightened activity in antioxidant enzymes, such as ascorbate peroxidase, guaiacol peroxidase, and superoxide dismutase, in response to water deficit stress and inoculations. These studies collectively underscore the intricate relationship among environmental stressors, antioxidant enzyme activity, and the biochemical composition of EOs in plants such as *R. officinalis*.

3.4. Antidiabetic Activity. The insights derived from the data depicted in Figure 4 highlight the presence of significant antidiabetic attributes within the three distinct samples, as evidenced by their impact on the inhibition of crucial enzymes, namely, α-amylase and α-galactosidase.

In terms of α-amylase inhibition, S2 demonstrates notable efficacy, displaying robust antidiabetic activity. This suggests its pronounced ability to impede the function of α-amylase, which is a key enzyme involved in starch hydrolysis. S3 also exhibits a commendable antidiabetic influence on α-amylase, albeit comparatively less potent than S2. On the other hand, sample one shows a relatively milder antidiabetic potential.

The shifting focus is shifted to α-galactosidase inhibition and the dynamics change. S3 demonstrates a subdued antidiabetic effect, indicating its limited ability to hinder α-galactosidase activity. In contrast, sample two takes the lead with conspicuously elevated antidiabetic efficacy against α-galactosidase compared to the other two samples. This suggests that S2 has significant potential for attenuating α-galactosidase function, making it a promising candidate for diabetes management.

In summary, the graphical representation in Figure 4 reveals the multifaceted antidiabetic attributes of the three samples. Sample two stands out for its formidable inhibitory impact on α-amylase and its comparatively elevated hindrance of α-galactosidase. Meanwhile, S3, while displaying modest inhibition against α-amylase, presents limited effectiveness in curtailing α-galactosidase. These findings provide valuable insights into the potential utility of these samples for diabetes modulation based on their intricate interactions with these enzymes.

In Figure 5, the substance acarbose demonstrates significant inhibitory effects on enzymatic activity. Specifically, its inhibition of α-amylase, a key enzyme in starch breakdown, resulted in an IC_{50} value of 0.285 mg/mL, indicating an effective reduction of the enzyme's activity. Acarbose also displayed the inhibition of α-glucosidase, another crucial enzyme in carbohydrate metabolism, with an IC_{50} value of 0.131 mg/mL, emphasizing its potency in impeding α-glucosidase activity.

Table 5. Antioxidant Activity of Three Samples

	sample 1	sample 2	sample 3	cineole	BHT
values of IC_{50} (mg/mL)	23.02 ± 0.08	23.34 ± 0.15	18.56 ± 0.14	17.11 ± 0,07	12.52 ± 0.06

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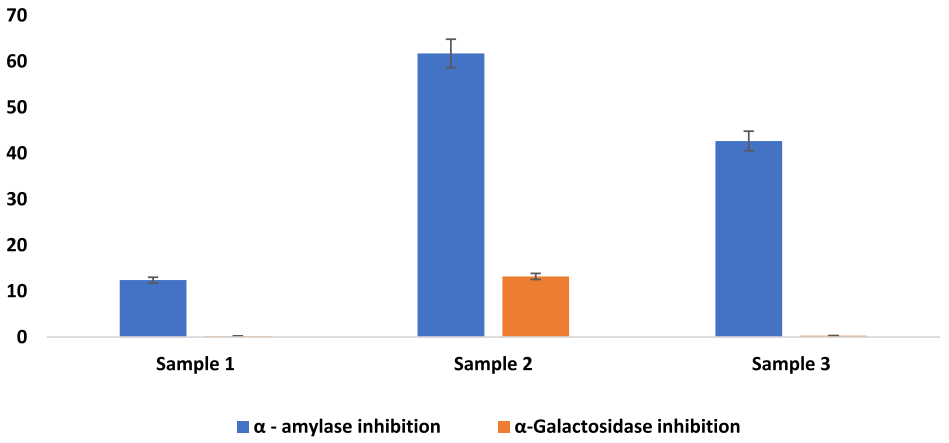

Figure 4. α-Amylase and α-glucosidase enzyme inhibition tests (IC_{50} (mg/mL)).

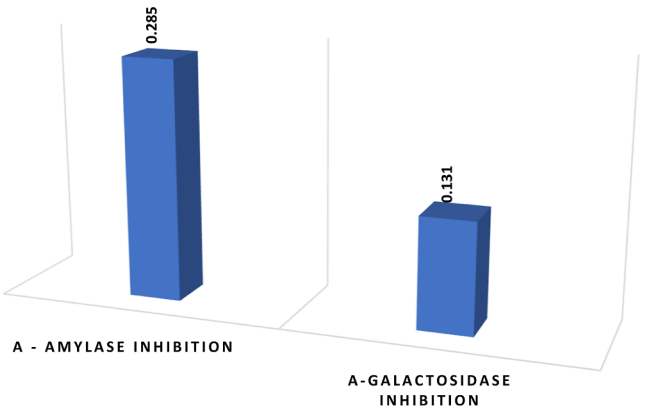

Figure 5. Acarbose inhibition for α-amylase and α-glucosidase (IC_{50} (mg/mL)).

These findings underscore acarbose's capability to modulate carbohydrate metabolism by interfering with the actions of α-amylase and α-glucosidase enzymes.

4. CONCLUSIONS

Water is a fundamental element for the survival of all living organisms and the maintenance of cellular homeostasis. However, the impacts of climate change and associated exacerbations restrict the availability of water, particularly the capillary water essential for plant survival. This results in various challenges at the plant level, leading to alterations in the physical, chemical, and morphological properties. This study was conducted to investigate the effects of climate change, manifested through drought and water scarcity, represented in three water stress regimes: sample one under 40% stress, sample two under 60% stress, and sample three under 80% water stress.

The results demonstrate that severe water stress causes a decrease in the contents of primary and secondary metabolites, EO yields, and major compounds. Additionally, water stress induces changes in the physical properties of *R. officinalis* EOs as well as their antioxidant and antidiabetic activities.

In conclusion, the remarkable findings of this study highlight the significant impact of climate change on the intricate chemical composition of plants, particularly in secondary metabolite production. These discoveries offer valuable insights for designing strategic interventions to mitigate the potential disruptions caused by climate change to the nutritional and medicinal values of these botanical constituents. However, as we conclude, a clear call for further investigation emerges, aimed at unraveling the complex mechanisms governing secondary metabolite production. These efforts unveil the mysteries of nature's symphony, revealing harmonies that adapt and evolve in response to the dynamic rhythms of the environment.

Finally, based on these results, our future line of research is to evaluate the impact of climate change on the morphology and IN VIVO activity of plants.

■ ASSOCIATED CONTENT

Supporting Information

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(PDF)

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