

# Impact of Climate Change on the Chemical Compositions and Antioxidant Activity of *Mentha pulegium* L.

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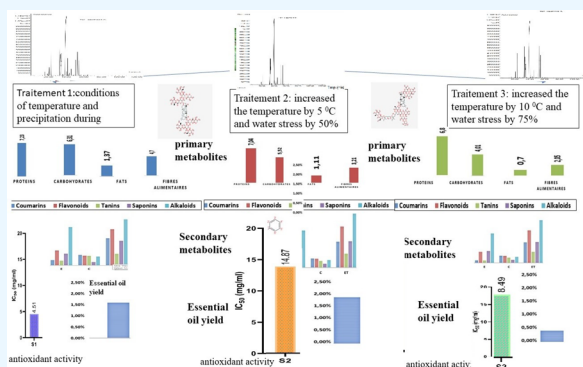
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**ABSTRACT:** A central position in Moroccan ethnobotany is held by the *Mentha* genus, serving as a vital reference for aromatic and medicinal plants within the Lamiaceae family. The profound importance of *Mentha* species in the daily lives of Moroccans is recognized, and the primary objective of this study is to assess the impact of rising temperatures and decreasing precipitation on the primary and secondary metabolites of *Mentha pulegium* under the following climatic conditions: sample 1, cultivated under standard temperature and precipitation conditions during the first year; sample 2, subjected to an 8 °C temperature increase and a 25% reduction in water supply; and sample 3, exposed to a 12 °C temperature rise and a 50% decrease in water availability. Phytochemical screening results reveal a progressive decline in primary metabolites from sample 1 to sample 3 due to the increase in temperature and decrease in precipitation. Conversely, a distinct trend is observed in secondary metabolites and the yield of essential oil, increasing from sample 1 to sample 2 as the temperature rises and precipitation decreases. Remarkably, in sample 3, the yield of essential oil decreases as climatic conditions further deteriorate. Additionally, GC analysis demonstrates that modifications in the chemical compositions of essential oils occur because of the disruption of climatic parameters, particularly in the major compounds. Similarly, changes in climatic parameters significantly influence antioxidant activity, with sample 2 exhibiting the highest activity, as reflected by an IC<sub>50</sub> value (half-maximal inhibitory concentration) of 14,874.04 µg/mL, followed by the third sample at 8488.43 µg/mL, whereas the first sample exhibits the lowest activity at 4505.02 µg/mL. In summary, the complex relationship between climatic factors and the chemical composition of *Mentha pulegium* is highlighted by our experiment, emphasizing its implications for medicinal properties within an ecological context.



## 1. INTRODUCTION

The first alert to humanity by the scientific community regarding the dangers of unsustainable activities in various ecosystems was issued in 1992.<sup>1</sup> The global modifications caused by anthropogenic activities, referred to as anthropization, have resulted in imbalances in the exploitation of natural resources.<sup>2</sup> These changes in the Earth's meteorological parameters are alarming consequences of climate change.<sup>3</sup> These changes are the results of the intensification of greenhouse gas emissions.<sup>4</sup> Furthermore, global warming has already reached 1.1 °C in recent years compared to the preindustrial era, and its impact on ecosystems is more severe than estimated in previous reports. In most cases, this will lead to the degradation of animal and plant biodiversity, potentially resulting in global-scale extinctions.<sup>5</sup> Recent studies have demonstrated that these climate changes have significant

impacts on the processes and functioning of ecosystems<sup>6</sup> as well as on the various interactions within ecosystems.<sup>7</sup> Additionally, they affect the chemical compositions of plants and the yield of essential oil.<sup>8</sup>

Traditional medicine that utilizes aromatic and medicinal plants is prevalent worldwide, particularly in rural areas. In Africa, discussing healing without mentioning aromatic and medicinal plants (MAPs) is nearly impossible, as up to 80% of

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the population relies on these plants for prevention or therapy.<sup>9</sup>

Morocco, as a northern African country, is situated between 21°–36° N latitude and 1°–17° W longitude. The country's climate is influenced by both the Mediterranean and Atlantic climates. Morocco's climate exhibits significant variability due to its diverse geographical features, including access to the sea, mountainous regions, and desert terrain. The variation in altitude across Morocco creates isolated zones with unique ecologies and climates, ranging from humid conditions to Saharan desert climates.<sup>10,11</sup> Morocco boasts of a very rich plant diversity, with approximately 42,000 registered plant species belonging to 150 different families and spread across 940 genera. Notably, Morocco is home to a significant number of endemic plant species, with around 900 species found exclusively within its borders. This diverse plant life contributes to the country's ecological and botanical significance.<sup>12</sup> The genus *Mentha* is highly prevalent in the Taounate region and finds extensive use in traditional medicine and various other fields. Consequently, the active compounds derived from this genus are employed in a wide range of biological activities and in the treatment of various disorders, diseases, and infections.<sup>14</sup>

Studies have indicated that the mountainous regions of the Atlas and Rif Mountains are the most significant areas for plant endemism in Morocco. These regions harbor a high number of plant species that are unique to these areas, highlighting their importance for biodiversity and conservation efforts in Morocco.<sup>13–15</sup> Moroccan plant resources hold significant potential for use in various fields, including medicine, pharmaceuticals, cosmetics, and agro-alimentary applications.<sup>16</sup> These resources offer a wide range of bioactive compounds and natural ingredients that can be harnessed for their therapeutic, cosmetic, and nutritional benefits, making them valuable assets for research and development in these industries.<sup>18,19</sup>

Given the significance of Morocco's natural resources and the adverse effects of climate change on the chemical compositions of plants, it is imperative to anticipate the impact of these changes on plant behavior. To address this concern, we conducted an ethnobotanical survey to identify the most commonly used aromatic and medicinal plants including *Mentha pulegium*. Subsequently, we cultivated these plant samples under varying climatic conditions. The results we obtained reveal that intensified meteorological parameters, rising temperatures, and reduced precipitation have indeed led to modifications in the chemical compositions of these plants.

Currently, the challenge lies in understanding that meteorological parameters can directly affect biomass production and indirectly affect the chemical compositions of plants. In this study, we conducted a 4 year analysis of changes in the chemical compositions of *Mentha pulegium* cultivated under different climatic factors.

The primary objective of this article is to enhance our understanding of how aromatic and medicinal plants, widely utilized by local residents, respond to climate change in terms of their chemical composition. Furthermore, we aim to provide recommendations for adapting to the effects of climate change in this context.

## 2. MATERIALS AND METHODS

**2.1. Study Area.** The province of Taounate, with a total area of 5616 km<sup>2</sup>, is situated in the northern part of the

Kingdom within the pre-Rifian and Rifian zone. Geographically, it is divided into 4 circles; 15 Caidats (administrative districts); 44 rural communes, out of which 22 are forestry areas; and 5 urban communes. The population of this province amounts to 662,246 inhabitants, with 87% residing in rural areas, giving it a predominantly rural character.

The climate in the province is of the Mediterranean type, characterized by the alternation of two seasons: one wet and cold and the other hot and dry. During the summer season, temperatures can rise above 45 °C, and the province receives an average annual rainfall of 790 mm, making it one of the wettest regions in the country. In some areas, such as Jbel Outka, the maximum annual rainfall can occasionally reach as high as 1800 mm.

**2.2. Methodology.** Data regarding the therapeutic use of the three plants were collected through a questionnaire administered to residents of the Taounate region. Furthermore, these plants were cultivated in the field to anticipate the potential impacts of climate change on their chemical compositions. This research was conducted because of the significant role these plants play in our daily lives and their various applications, emphasizing the need to understand how climate change might affect their chemical properties.

To assess the impact of climate change on the chemical composition of *Mentha pulegium* plants, we conducted a study involving three groups of wild specimens collected from the Taounate region. These specimens were subsequently cultivated in the same geographical area. Official identification of these samples was carried out by botanists from the National Scientific Institute, and a voucher specimen with the designation H37 was created for reference.<sup>20</sup>

The transplantation of the samples was conducted under the following conditions:

Sample 1: These specimens were exposed to normal seasonal average temperatures and received the typical precipitation levels observed in Taounate.

Sample 2: In a controlled closed environment, the temperature was artificially increased by 8 °C, and the plants experienced water stress conditions with a 25% reduction in irrigation.

Sample 3: Similarly, in another controlled closed environment, the temperature was raised by 12 °C, and the plants underwent a more severe water stress condition with a 50% reduction in irrigation.

These controlled conditions allowed us to observe and analyze the effects of the altered temperature and water availability on the chemical composition of the *Mentha pulegium* plants.

**2.3. Phytochemical Screening.** The phytochemical screening process consisted of a qualitative analysis employing standard methods and techniques. This analysis aimed to identify and characterize the presence of specific phytochemical compounds within the *Mentha pulegium* plants under the varying experimental conditions.<sup>14,18–20</sup> To identify the primary and secondary metabolite families in the plant samples, the screening process involved determining the presence or absence of specific compounds or compound groups.

Following the qualitative identification of major metabolite families, quantitative assays were conducted on secondary metabolites. These assays were carried out using established methodologies referenced in the research;<sup>21–24</sup> the quantification of these metabolites was achieved by employing

established methodologies, which offer a reliable framework for accurately measuring and determining the levels or concentrations of the identified metabolites in the plant samples. These methods ensured the precision and reliability of the quantitative data collected during the study.

#### 2.4. Essential Oil and Gas Chromatography (GC).

Essential oil is a natural chemical compound obtained through processes such as distillation or alternative techniques. These oils are derived from various sources, including aromatic elements found in roots, bulbs, rhizomes, stems, wood, barks, leaves, buds, saps, flowers, fruits, and seeds of plants.<sup>14</sup>

Extraction plays a crucial role in various industrial sectors including pharmaceuticals, cosmetics, perfumery, and the food industry. Over the past decade, there has been increased emphasis on ensuring the quality and safety of food and drugs, leading to regulatory measures that primarily address toxicity levels and other safety concerns.<sup>21</sup> As a result of these regulatory measures and a heightened focus on safety, there has been an increasing preference for "natural" products over synthetic substances.

Essential oils, with their intricate and diverse chemical compositions, require careful consideration when selecting extraction methods.

The chosen extraction technique should be capable of effectively capturing both polar and nonpolar compounds present in essential oils. It should also avoid triggering biochemical reactions; inducing thermal degradation, oxidation, reduction, hydrolysis, and pH alterations; or causing the loss of volatile compounds. Achieving these goals necessitates a thorough consideration of various parameters and attributes during the extraction process.<sup>22</sup>

As a result, various extraction methods have emerged to meet these criteria. These methods include cold expression, distillation, steam distillation, hydrodistillation, enfleurage, organic solvent extraction, CO<sub>2</sub> extraction, microwave-assisted hydrodistillation, and ultrasound-assisted hydrodistillation. Each of these techniques has its advantages and suitability for specific applications within the extraction of essential oils.<sup>23</sup>

In our study, the plant material comprised leaves that had been dried in the shade. Approximately 100 g of dried leaves was subjected to hydrodistillation for a duration of 3 h using a Clevenger-type apparatus.

For the analysis of the essential oil samples, a GC–MS (gas chromatography–mass spectrometry) system was employed. This system featured a multimode injector and a 123-BD11 (biodiesel column) with dimensions of 15 m in length, 320  $\mu$ m in diameter, and 0.1  $\mu$ m in film thickness. The essential oil samples were introduced into the column using the split 1/4 mode, with helium serving as the carrier gas at a flow rate of 2 mL/min.

The analysis involved evaluating the peak areas of the samples, which were then expressed as a percentage of the total compounds detected. This was accomplished using the full scan mode within the mass range of 30–1000  $m/z$ , employing a gain factor of 5 and electron impact ionization. The ion source and quadrupole temperatures were set at 230 and 150  $^{\circ}$ C, respectively.

To separate and identify the individual compounds present in the essential oil samples, a temperature program was employed. The initial oven temperature was set at 30  $^{\circ}$ C and was gradually increased until reaching a final temperature of 360  $^{\circ}$ C. The composition of the essential oil samples was

determined based on the data obtained from these experimental parameters.

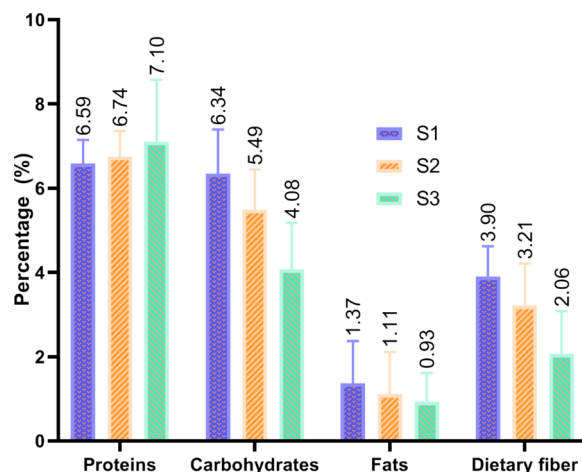
**2.5. Antioxidant Activity.** To perform the FRAP test (ferric reducing antioxidant power assay), various concentrations (0.5 mL) of each extract were combined with a 0.2 M phosphate buffer solution (pH 6.6) and a 1% potassium ferricyanide K<sub>3</sub>Fe(CN)<sub>6</sub> solution (2.5 mL each). The resulting mixture was incubated at 50  $^{\circ}$ C for 20 min to allow the reduction reaction to take place. Subsequently, the reduction reaction was terminated by adding 10% trichloroacetic acid (2.5 mL). Following the incubation and reaction termination steps, the mixture was centrifuged at 3000 rpm (revolution per minute) for 10 min. The supernatant from each concentration (2.5 mL) was combined with 2.5 mL of distilled water and a 0.1% aqueous solution of FeCl<sub>3</sub> (0.5 mL).

The resulting reaction medium was measured spectrophotometrically at a wavelength of 700 nm. An increase in absorbance signified a higher reduction capacity of the samples. Catechin served as the reference compound in the assay, and the test was conducted in triplicate. The IC<sub>50</sub> values, which represent the concentration needed for 50% reduction capacity, are reported as the mean  $\pm$  standard deviation (SD) based on the triplicate measurements.

**2.6. Statistical Analysis.** The data are expressed as means  $\pm$  standard errors and were statistically analyzed using GraphPad Prism 5 Software (San Diego, CA, USA). To compare multiple groups, we employed one-way analysis of variance (ANOVA).

### 3. RESULTS AND DISCUSSION

**3.1. Nutritional values.** Figure 1 illustrates that in all three samples, protein, carbohydrates, fats, and dietary fiber show



**Figure 1.** Protein, carbohydrate, fat, and dietary fiber contents in *Mentha pulegium* samples under three different treatments.

higher levels in sample 1 followed by sample 2 and lastly sample 3. Specifically, protein, carbohydrates, fats, and dietary fiber contents in sample 1 were 7.23, 6.31, 1.37, and 4.70%, respectively. These values decreased to 7.04, 5.52, 1.11, and 3.21% in sample 2 and further decreased to 6.80, 4.01, 0.70, and 2.05% in sample 3.

**3.2. Amino Acids.** Figure 2 reveals that certain amino acids, including alanine, arginine, asparagine, glutamate or glutamic acid, glutamine, methionine, pyrrolysine, selenocysteine, threonine, tyrosine, and tryptophan, are absent or found

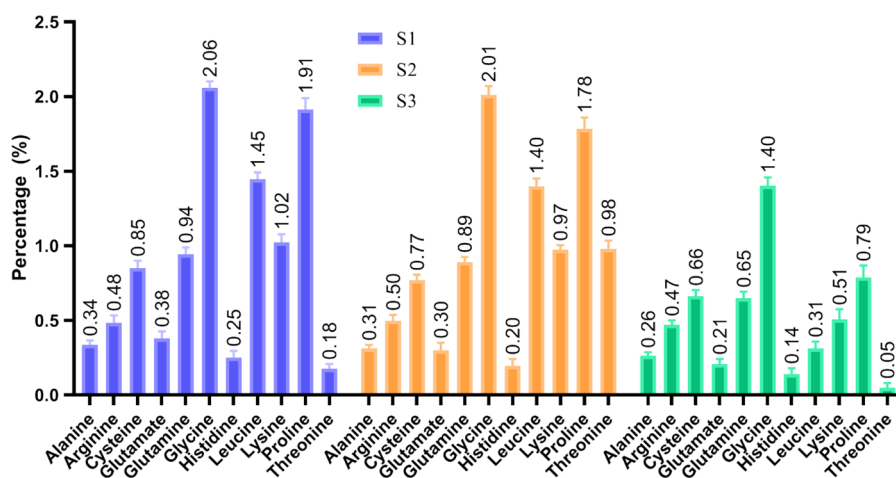


Figure 2. Amino acid content in *Mentha pulegium* samples under three different treatments.

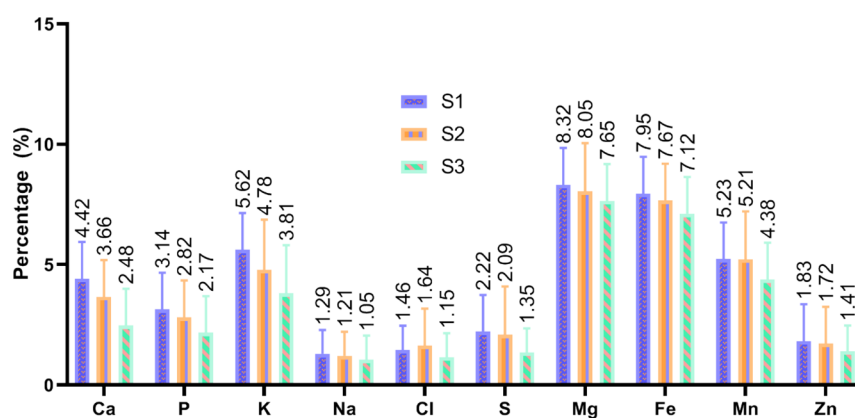


Figure 3. Mineral composition in *Mentha pulegium* samples under three different treatments.

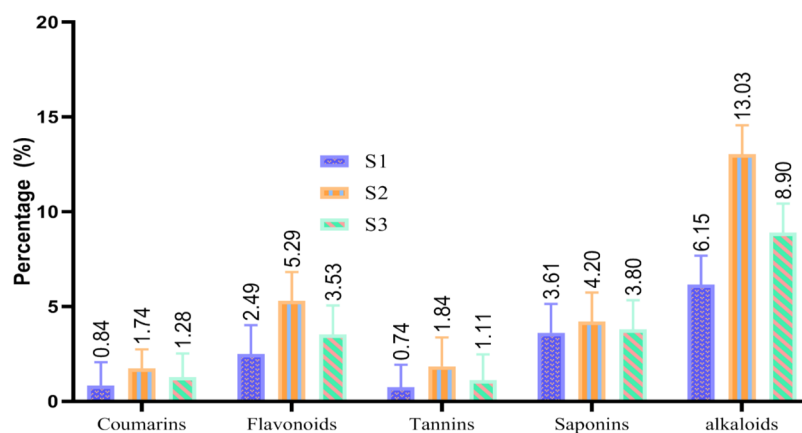


Figure 4. Secondary metabolites of extracts of *Mentha pulegium* under three different treatments.

in minimal amounts in all three treatments. The highest content of these amino acids is observed in treatment 1 followed by treatment 2 and lastly treatment 3.

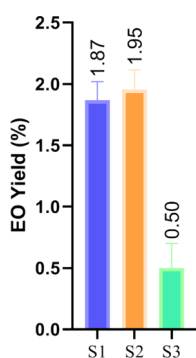
Conversely, glycine, histidine, leucine, lysine, phenylalanine, and serine are present in relatively higher quantities in treatment 1, with concentrations of 0.9, 0.4, 2.1, 0.3, 1.5, and 1.9%, respectively. These concentrations decrease to 0.8, 0.3, 2, 0.2, 1.4, and 1.8% in treatment 2 and further decrease to 0.7, 0.2, 1.4, 0.1, 0.3, and 0.8% in treatment 3.

**3.3. Mineral Compositions.** The results depicted in Figure 3 indicate that, in general, mineral compositions are

most abundant in sample 1 followed by sample 2 and lastly sample 3. Specifically, the concentrations of Ca, P, K, Na, S, Mg, Fe, Mn, and Zn were highest in treatment 1, measuring at 4.75, 3.74, 5.95, 1.29, 2.55, 8.65, 8.28, 5.56, and 2.16%, respectively. These concentrations decreased to 3.99, 3.15, 5.45, 1.21, 2.09, 8.05, 8, 5.21, and 2.05% in treatment 2 and further decreased to 2.81, 0, 3.81, 1.05, 1.35, 7.98, 7.45, 4.71, and 1.45% in treatment 3.

**3.4. Secondary Metabolites.** Figure 4 illustrates that secondary metabolites are more prevalent in treatment 2 followed by treatment 3 and finally treatment 1.

**3.5. Essential Oil Yield.** Figure 5 depicts the essential oil yield of the three samples of *Mentha pulegium* under varying



**Figure 5.** Comparison of essential oil yields of *Mentha pulegium* samples under three different treatments.

climatic conditions. It is evident that treatment 2 exhibits the highest yield at 2.0% followed by treatment 1 at 1.50% and finally treatment 3 at 0.40%.

**3.5.1. Gas Chromatography (GC).** The chemical composition of the essential oils of *Mentha pulegium* from the three samples is detailed in Table 1.

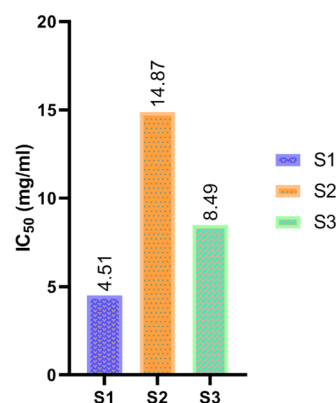
**Table 1. Chemical Compositions in Percentage of Essential Oils of *Mentha pulegium* of Three Samples in Different Climatic Conditions**

	sample 1	sample 2	sample 3
$\alpha$ -pinene	1.4	0.1	0.7
cyclohexanone-3-methyl	0.7	0.2	
$\beta$ -pinene	0.1	0.1	1.2
myrcene	0.5	0.1	0.1
octanol-3	2.4	2.1	0.7
$\delta$ -2-carene		0.3	0.9
limonene	2.4	3.5	2.5
<i>p</i> -mentha-3,8-diene	0.1	0.1	1.5
menthone	3.8	4.2	3.4
pinocarvone	4	0.1	2.9
isomenthol	0.4		0.1
menthol	5.2	0.1	4.2
dihydrocarvone	8.6		8.1
<i>R</i> (+)-pulegone	62.02	63.6	51.7
carvone	5.9		9.8
$\alpha$ -peperitone	0.6		0.1
piperitenone	2.1	25.1	10.3
caryophyllene	0.1	0.1	0.3
germacrene D	0.3	0.1	0.2
<i>g</i> -eudesmol	0.2	0.1	0.1
$\alpha$ -eudesmol	0.4	0.1	0.1

From the table, it is evident that the predominant compound in all three essential oils studied is pulegone, with the highest percentage observed in sample 2 at 63.6% followed by sample 1 at 62.02% and finally sample 3 at 51.7%.

**3.6. Antioxidant Activity.** Figure 6 reveals that sample 3 exhibits a relatively high antioxidant capacity. Conversely, sample 1 shows a moderately lower antioxidant capacity in comparison to that of sample 3. Interestingly, sample 2 demonstrates the highest IC<sub>50</sub> value among the samples.

Understanding the ethnobotanical uses and biological activities of essential oils is of great importance, particularly



**Figure 6.** Antioxidant activity of three samples in different treatments (FRAP: ferric reducing antioxidant power assay) (IC = half-maximal inhibitory concentration).

given the potential influence of climate change on these properties. Essential oils are concentrated extracts derived from plants and are rich in various bioactive compounds.<sup>24</sup> The diverse range of bioactive compounds found in essential oils is accountable for their manifold biological activities, encompassing antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant properties.<sup>25</sup> Essential oils have a long history of utilization for medicinal and therapeutic purposes spanning thousands of years, and they continue to be a prominent component of traditional medicine systems in various regions across the globe.<sup>26</sup> It is essential to acknowledge that the ethnobotanical uses and biological activities of essential oils can vary significantly based on factors such as the plant species, the plant part utilized, and the extraction method employed.<sup>27</sup> Moreover, these properties can also be influenced by environmental factors, including climate change.<sup>25</sup>

Climate change, driven by the rising concentration of greenhouse gases, is expected to have significant impacts on ecosystem functioning, including vegetation dynamics. These changes in ecosystems can, in turn, influence the chemical compositions of plants.<sup>28</sup> Although temperature is a crucial climatic factor, it is important to recognize that other factors such as water availability, air quality, and soil pH can also exert a profound influence on plant growth and chemical composition. These combined factors create a complex web of interactions in the response of plants to changing environmental conditions.<sup>29</sup> Therefore, aromatic and medicinal plants hold a significant role in our lives, particularly in developing countries and especially in countries with riverine regions. This prominence is attributed to the challenges associated with accessing modern medicine and the economic hardships faced by communities, leading them to rely on traditional medicine.<sup>30</sup> Nonetheless, the challenge lies in the fact that alterations in meteorological parameters have indeed affected the chemical compositions of these plants. This situation has compelled us to investigate the anticipated effects of rising temperatures and declining precipitation levels on the concentrations of primary and secondary metabolites in three plants that are widely utilized by countries with riverine regions.<sup>31</sup> Analysis of the obtained results reveals that the percentages of primary and secondary metabolites are typically disrupted as a consequence of the three experimental conditions. Sample 1, cultivated under normal temperature and precipitation conditions, demonstrates a decrease in the number of primary metabolites. In contrast, sample 2,

subjected to an 8 °C temperature increase and 25% water stress in the second year, and sample 3, under the fourth-year conditions of worsening climate change, show a consistent trend of decreasing primary metabolite concentrations with rising temperatures and reduced precipitation. This trend is substantiated by the data.<sup>32,33</sup> These findings align with previous research that has demonstrated a decrease in the rate of photosynthesis in response to water deficits. Similarly, the reduction in the percentages of proteins, carbohydrates, lipids, dietary fibers, amino acids, and mineral compositions with increasing temperatures and decreasing precipitation corresponds to the outcomes observed in this study.<sup>34,35</sup> Additionally, concerning secondary metabolites, the analysis of the results demonstrates that their percentages fluctuated across the three samples in response to the temperature and precipitation variations. Notably, the percentage of secondary metabolites increased in sample 1, which was cultivated under normal temperature and precipitation conditions during the first year, compared to sample 2, which experienced water stress in the second year<sup>36</sup> and<sup>37</sup> have reported that climate-related stresses, including drought and heat, can lead to an increase in the production of flavanols. However, in the fourth year, as the challenging climatic conditions continued to intensify, there was a noticeable onset of a decrease in the percentage of secondary metabolites.<sup>38</sup> Essential oils are highly concentrated bioactive substances found in plants, and they can be obtained through various methods, including cold expression, distillation (steam distillation, hydrodistillation, or water distillation), enfleurage, organic solvent extraction, CO<sub>2</sub> extraction, microwave-assisted hydrodistillation, and ultrasound-assisted hydrodistillation.<sup>39</sup> Hence, plants have remained a vital source in the pharmaceutical industry since ancient times. Over the centuries, human traditions have evolved, expanding our knowledge and utilization of medicinal plants for therapeutic purposes.<sup>40</sup> Indeed, the therapeutic properties of plants are attributed to the presence of numerous natural bioactive compounds, often comprising active ingredients in these plants, including essential oils. Given the significance of essential oils and their diverse applications, it is imperative to evaluate the influence of climate change on these compounds.<sup>41</sup>

To anticipate the potential impact of climate change on essential oil yield, a greenhouse study was conducted involving three plants subjected to varying climatic conditions.<sup>42</sup> Analysis of the obtained results reveals that the yield of essential oils increased in the first 2 years in all three plants, particularly in samples 1 and 2, in response to rising temperatures and decreasing precipitation, as observed in the study,<sup>37,43</sup> which indicate that water-stressed plants tend to exhibit higher essential oil yields. However, in sample 3 of the three plants during the fourth year, a gradual decrease in the essential oil yield is noticeable, corresponding to the worsening climatic conditions. This observation is corroborated by refs 17 and 44, which support the notion that drought conditions can lead to a reduction in oil content.

Climate change indeed serves as a significant driver of alterations in natural systems, and one of its effects is the modification of the key compounds found in plants. This aligns with the findings of ref 45, which indicate that water stress can result in an increase in the content of essential oils and a concurrent increase in the major compounds within those oils. The primary compounds in essential oils, including 1,8-cineole, menthone, pulegone, and piperitone, play significant roles in

the composition and function of these oils. As supported by ref 46, environmental stresses can trigger an increase in the concentration of major compounds like pulegone and isopulegone, which serves as an adaptive response to stress conditions. Reference 47 reinforces this idea, highlighting that pulegone serves as a primary component in the defense mechanisms of plants against various stresses. Moreover, pulegone exhibits positive correlations with secondary metabolites and plant morphology, as emphasized in ref 48. Findings reveal an interesting shift in composition, with buds accumulating menthone as the principal ingredient instead of menthone and menthol. This suggests that the chemical makeup of essential oils can adapt to changing environmental conditions.

Indeed, reversible reactions such as isomenthone conversion or menthone transformation are possibilities in response to changing environmental conditions. These transformations in the chemical composition of essential oils can be influenced by various factors, including plant development and environmental conditions. As mentioned in ref 49, changing environmental conditions can lead to modifications in the major compounds of essential oils in aromatic and medicinal plants as an adaptive response to stress conditions. Reference 50 provides evidence that GC/MS analysis of oil samples can show an increase in thymol content in response to water stress, whereas cymene content decreases. Reference 51 shows that altering irrigation levels can have significant effects on the quality and quantity of essential oils, impacting various components.

Moreover, ref 52 indicates that different water regimes can significantly influence the composition of essential oils, affecting the levels of oxygenated monoterpenes, monoterpene hydrocarbons, aldehydes, and alcohols. Lastly, ref 53 highlights that when plants experience stress, the total content of essential oil can increase significantly, up to three times higher compared to control plants.

In regard to the main compounds of essential oils, it is observed that the content of menthone, menthol, and pulegone increased with the various treatments applied. However, the concentrations of limonene and menthofuran remained unchanged. This suggests that the treatments had a notable impact on specific compounds within the essential oils, whereas others remained relatively stable.<sup>54</sup>

The gas chromatography analysis of essential oils from the aerial parts of the studied samples in the Taounate region (Morocco) under different climatic conditions reveals a richness in specific chemical compounds, notably, pulegone, dihydrocarvone, carvone, and menthone. Importantly, the concentrations of these chemical compounds exhibit variations depending on climatic parameters. Initially, in the first 2 years, the concentrations increased in response to rising temperatures and increasing water stress levels. However, in the fourth year, as the temperature and water stress intensify further, there is a decrease in the concentrations of these compounds. This pattern aligns with the findings reported by Mollaei et al.<sup>55</sup> Indeed, several environmental factors play a crucial role in influencing phytochemical compounds and antioxidant activity in plants. Temperature and altitude are among these factors, and they have been found to have a significant impact on the concentration of specific compounds including 1,8-cineole, limonene, and menthone.

Conversely, average precipitation levels are another influential factor, primarily determining the content of

compounds such as *trans*-piperitone epoxide, piperitone oxide, and pulegone in plants. These findings highlight the complex interplay of environmental conditions in shaping the chemical composition of plants and their bioactive compounds.

Cherrat et al.<sup>56</sup> also observed that the essential oil of *Mentha pulegium* was primarily composed of monoterpenes, which made up 86.40% of its total composition. Sesquiterpenes were present in lower quantities, accounting for 10.81% of the composition. Among the major components of this essential oil, pulegone was the predominant compound, with a substantial concentration of 33.65%. Additionally,  $\alpha$ -terpinenyl acetate was notable, representing 24.29% of the total composition. Other compounds, such as bicyclo[3.1.0]hexane and 6-isopropylidene, were also detected.

The characterization method used in analyzing essential oils is indeed a critical factor that can influence the determination of the chemical compound content in these oils. Different methods may yield varying results, emphasizing the importance of standardized and consistent analytical approaches. Mohammadhosseini<sup>57</sup> employed two different analysis techniques, MAHD-GC-MS and HS-SPME-GC-MS, to identify constituents in the essential oils and volatile compounds of the aerial parts of *Mentha pulegium* L. Using these techniques, the author identified a total of 30 and 28 constituents in the essential oils and volatile compounds, respectively.

In his analyses, a high prevalence of oxygenated monoterpenes was observed in the essential oils, whereas nonterpene hydrocarbons were dominant in the volatile compounds. More specifically, carvone constituted approximately 56.0% of the essential oil when the MAHD technique was used. In contrast, when the HS-SPME technique was employed, the main constituents of the volatile profile were oleic acid (20.1%), carvone (17.7%), and limonene (16.1%). These differences in chemical profiles highlight the influence of the analysis technique on the identification of compounds in essential oils.

Brahmi et al.<sup>58</sup> conducted a study on *Mentha pulegium* extract (MPE) and identified its dominant constituents. Pulegone was found to be the major compound, representing 70.4% of the composition. This was followed by neo-menthol at 13.4%, neo-menthol acetate at 3.5%, and menthone at 2.7%. These findings are consistent with research by Mohammadhosseini et al., Xu et al., and Roasa et al., who also noted variations in volatile fractions obtained from different plant organs. This highlights the diverse chemical profiles of *Mentha pulegium* and the significance of pulegone in its composition.<sup>59–61</sup>

The toxicological effect of changes in essential oil composition depends on the specific compounds that are present in the oil and the concentrations of these compounds.<sup>62</sup> Some essential oil components may have toxic effects at high concentrations or with prolonged exposure, whereas others may be safe for use in certain concentrations.<sup>63</sup> It is important to characterize the essential oil composition and conduct toxicity studies to assess the safety of its use.<sup>64</sup> Additionally, the toxicity of essential oils can vary depending on the method of administration. Therefore, further studies are needed to evaluate the toxicological effects of changes in essential oil composition.<sup>65</sup>

The antioxidant activity of this plant, resulting from various bioactive compounds, such as secondary metabolites and essential oils, is affected by the disruption of climatic parameters. These findings have been corroborated by refs 28 and 66–68. In sweet basil, an accumulation of antioxidant

compounds induced by stress was observed. This indicates that, under conditions of severe stress, antioxidant compounds can be synthesized by plants exposed to sweet basil.

These findings have been supported by previous studies.<sup>69–71</sup> These studies demonstrated that water stress can influence bioactive compounds, particularly the content of glucosinolates and the activities of antioxidant enzymes

#### 4. CONCLUSIONS

In summary, this study investigated the impact of climate change on the chemical composition and essential oil yield of *Mentha pulegium* over a 4-year period. Three samples were cultivated: one under normal conditions, one with increased temperature and water stress, and one with an even greater temperature increase and water stress.

The results indicated that as temperatures rose and precipitation decreased, there was a general decrease in the plant's protein, carbohydrate, lipid, fiber, amino acid, and mineral content. Secondary metabolites were highest in the sample grown under normal conditions in the first year but declined in subsequent years as the climate conditions worsened. Essential oil yield initially increased with higher temperatures and reduced precipitation but declined in the fourth year as extreme climatic conditions persisted.

These findings emphasize the significant impact of climate change on the chemical composition and essential oil production of *Mentha pulegium*.

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