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Effect of Climate Change on the Phytochemical Constituents, Essential Oil Yield and Chemical Composition of *Inula viscosa* Leaves

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ABSTRACT

Climate change poses a threat to plant and animal biodiversity. It induces physiological and morphological changes in plants. This study aimed to assess the effect of climate change on the phytochemical constituents, essential oil yield and chemical composition of Inula viscosa leaves. Three samples of I. viscosa from the Taounate region of Morocco were cultivated in a closed chamber for three years under different climatic conditions: sample 1 (I. viscosa cultivated in the first year under normal temperature and precipitation conditions of Taounate region), sample 2 (I. viscosa cultivated in the second year, at 5°C increase in temperature and 50% decrease in precipitation), and sample 3 (I. viscosa cultivated in the third year, at 10°C increase in temperature and 75% decrease in precipitation). Phytochemical and mineral analyses of the three samples were done according to standard procedures. Essential oils were extracted from the leaves of the three samples by hydro-distillation using the Clavenger apparatus. Chemical composition of the essential oils was identified by gas chromatography-mass spectrometric (GC-MS) analysis. Phytochemical screening showed that moderately intensified climatic conditions (sample 2) led to an increase in phytochemical content, mineral composition, and essential oil yield of Inula viscosa leaves. However, at adverse climatic conditions (sample 3), the phytochemical content, mineral composition, and essential oil yield of Inula viscosa leaves decreased significantly. GC-MS analysis revealed that changes in climatic condition affected the content of major compounds in the essential oil of I. viscosa leaves. Therefore, climate change can significantly impact the chemical composition of plants.

Keywords: Primary metabolites, Secondary metabolites, Climate change, Essential oil, Inula viscosa.

Introduction

Climate change denotes a persistent alteration in meteorological parameters. The global shifts in climate, attributed to human activities since the industrial revolution, has led to imbalance in the utilization of natural resource and substantial alterations in the earth's temperature and overall precipitation levels, presenting alarming consequences of climate change.¹ These modifications primarily result from the heightened emissions of greenhouse gases. Recent investigations indicate that the current rate of greenhouse gas emissions anticipates a temperature surge of 5.3°C by 2100 unless there is intervention.²

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Presently, climate change is recognized as the predominant factor posing a threat to global biodiversity. Furthermore, beyond the global warming already reaching 1.1°C over the last decade compared to the pre-industrial era, the impacts on ecosystems surpass both the extent and magnitude estimated in previous assessments.3 Global warming induced by humanization lead to imbalances in the exploitation of natural resources and to changes in meteorological parameters.4,5 Global warming are an alarming consequence of climate change.⁶ These changes are the result of the intensification of greenhouse gas emissions.⁷ In addition to the fact that global warming in recent years have already reached 1.1°C compared to pre-industrial times,8 the impacts on ecosystems have worsened over time.⁹ This will most likely lead to the degradation of plant and animal biodiversity, and even global extinction.¹⁰ Recent studies have therefore shown that these climatic changes affect the processes and functions of ecosystems and the multiple interactions between them,¹¹ as well as the chemical composition of plants and essential oils.¹²⁻¹⁴ Aromatic and medicinal plants are widely used in traditional medicine practices in different communities worldwide, especially in rural areas.¹⁵ In Africa, we cannot talk about healing without mentioning aromatic and medicinal plants. Up to 80% of the population in Africa use aromatic and medicinal plants to prevent and treat diseases.16

Inula viscosa have played a primordial role in the daily lives of the African population given its therapeutic, cosmetic and biological

effects.^{17,18} This plant was used for this study through an ethnobotanical study of selected medicinal and aromatic plants. The results of the study revealed a nuanced interplay between meteorological parameters, temperature rise, precipitation reduction, and the ensuing changes in the chemical composition of plants. This connection elegantly broaden the discourse on global climate change with its attendant effect on plant species.

A critical issue surfaces regarding the direct and indirect effects of meteorological parameters on biomass production and plant chemical composition. This unveils the multifaceted nature of the impact of climate change on ecosystems, emphasizing the intricate relationships between climatic factors and vegetation.

The main objective of this study is to deepen our understanding of how commonly used medicinal and aromatic plants respond to climate change. This knowledge isn't just academic; it holds tangible significance for local populations. The call to provide recommendations for adapting to climate change impacts, based on the chemical composition of these plants, will creates a meaningful bridge between global climate concerns and actionable insights for communities.

Materials and Methods

Plantation

The transplant was carried out in the Taounate region situated in the northern region of Morocco ($34^{\circ}39'38''N 4^{\circ}26'00''W$, 815 m), encompassing a landmass of 5,616 square kilometers within the Fez-Meknes region. The fields were cultivated a few weeks before transplanting. Weeds were removed every week after transplanting to ensure proper nutrition.

Three samples of *I. viscosa* from the Taounate region were planted in a closed chamber for three years under different environmental conditions (Table 1): sample 1 (*I. viscosa* planted in the first year under normal temperature and precipitation conditions of Taounate region),

sample 2 (*I. viscosa* planted in the second year, at 5°C increase in temperature with 50% decrease in precipitation), and sample 3 (*I. viscosa* planted in the third year, at 10°C increase in temperature with 75% decrease in precipitation).

Analysis of phytochemical constituents and mineral composition

The leaves of *I. viscosa* from the three samples were subjected to qualitative phytochemical screening and analysis of mineral composition according to standard procedures.^{19,20} Following the qualitative analysis, quantitative phytochemical analysis for the major secondary metabolites were performed following standard procedures.²¹⁻²³

Extraction of essential oil

The leaves of *I. viscosa* were dried in the shade. The dried leaves (100 g) were subjected to hydro distillation using a Clevenger-type apparatus for three hours.

Gas Chromatographic-Mass Spectrometric analysis (GC-MS) analysis of essential oil

The chemical composition of the three essential oil samples obtained from *I. viscosa* cultivated under various climatic conditions was analyzed using GC-MS instrument equipped with a flame ionization detector (FID) and two DB-1 fused silica capillary columns. Helium served as the carrier gas at a flow rate of 0.8 mL/min with a temperature gradient 50 to 200°C at 5°C/min increment.

Statistical analysis

The data were presented as mean \pm standard error of mean (SEM), and were subjected to one-way analysis of variance (ANOVA) and multiple comparison test using GraphPad Prism-5 software. P ≤ 0.05 was regarded as statistically significant.

Table 1: Climatic conditions of transplantation

	The seasonal average temperature in °C				Seasonal av	Seasonal average precipitation in mm			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	
Sample 1	16.25	34	21.25	6.75	42	21.75	70.25	55.25	
Sample 2	21.25	39	26.25	11.75	21	10.87	35.125	27.625	
Sample 3	26.25	44	31.25	16.75	14	7.25	23.41	18.41	

Results and Discussion

Effect of temperature and precipitation on the phytochemical constituents of Inula viscosa leaves

The primary metabolites (carbohydrates, proteins, amino acids, fats and fibre) contents of the three *I. viscosa* samples cultivated under different climatic conditions are presented in Table 2 and Figure 1. The percentage protein content in sample 1 was 10.13%, while samples 2 and 3 had significantly (p < 0.001) lower protein content of 9.06% and 7.01%, respectively. Similar trends were observed for fats, carbohydrates, and dietary fiber contents, all exhibiting a successive decline with increasing temperature and decreasing precipitation. Specifically, lipid content decreased successively from 1.56% in sample 1 to 1.2% in sample 2, and 0.7% in sample 3, carbohydrates content decreased from 8.21% in sample 1 to 7.55% in sample 2, and 6.3% in sample 3, while dietary fiber decreased from 4.43% in sample 1 to 4.01% in sample 2, and 3.2% in sample 3.

These findings agrees with the findings from previous studies; for instance, Yang et al. $(2022)^{25}$ reported similar results as obtained in this study, while others reported a decrease in the primary metabolites, antioxidant, and metabolic activities of plants due to a combined effect of water deficit and other abiotic stressors, and these changes were attributed to drought-induced decrease in primary metabolite content.²⁶⁻²⁸ The consistency across these studies underscores the relevance of our findings and strengthens the link between temperature, precipitation, and metabolic changes in the studied plant.

Table 3 and Figure 2 present the percentage amino acids content of the three samples of *I. viscosa*. Certain amino acids, including arginine, alanine, asparagine, glutamine, methionine, pyrrolysine, valine, threonine, Selenocysteine, tyrosine, and tryptophan were absent in one, two or the three samples, whereas, amino acids such as aspartate, cysteine, glycine, glutamate or glutamic acid, histidine, isoleucine, leucine, lysine, phenylalanine, proline, and serine were consistently present in all three samples (Table 3). Predominantly, phenylalanine, leucine, serine, and proline emerged as the key amino acids in *I. viscosa* across the three samples: Leucine (sample 1: 2.36%, sample 2: 2.03%, sample 3: 1.15%), Phenylalanine (sample 1: 2.45%, sample 2: 1.42%, sample 3: 0.3%), Proline (sample 1: 1.33%, sample 2: 1.03%, sample 3: 0.51%), and Serine (sample 1: 2.34%, sample 2: 1.63%, sample 3: 0.45%) (Figure 2).

Table 2: Primary metabolites of the samples of I. viscosa
cultivated under different climatic conditions

Primary metabolites	Inference						
	Sample 1	Sample 2	Sample 3				
Proteins	+	+	+				
Carbohydrates	+	+	+				
Fats	+	+	+				
Dietary fiber	+	+	+				

+; indicates presence of constituents

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Additionally, the content of certain amino acids, notably, glutamate or glutamic acid, isoleucine, leucine, lysine, phenylalanine, proline, serine, tyrosine, and valine exhibited significant variations in the three samples with a successive decrease in samples 2 and 3, that is with a rise in temperature and decrease in precipitation. This observation aligns with previous studies that emphasized the adverse impact of climate change on plants, posing a serious threat to crop yields and food supply. These findings further corroborate the broader effect of climate change on plant biochemical composition.²⁹⁻³¹

The secondary metabolites present in the three samples of *I. viscosa* are presented in Table 4 and Figure 3. The results revealed a significant (p < 0.001) disparity in the extraction capacity among the solvents used, for the secondary metabolites with ethanol emerging as the most effective solvent. Ethanol extracted the highest percentage of various secondary metabolites, including alkaloids, flavonoids, saponins, tannins, and coumarins. In Sample 1, the percentage contents of secondary metabolites were as follows: alkaloids (13.3%), coumarins (4.36%), tannins (4.34%), flavonoids (6.83%), and saponins (6.33%). Sample 2 exhibited percentage content of alkaloids as 15.1%, coumarins (4.55%), tannins (4.99%), flavonoids (13.07%), and saponins (8.15%). While sample 3 showcased alkaloids (14.00%), coumarins (4.44%), tannins (4.62%), flavonoids (9.58%), and saponins (7.00%).

Surprisingly, the results did not reveal a significant and consistent increase in secondary metabolites with changes in temperature and precipitation. This observation contradicts the expectation that alterations in abiotic factors, such as temperature and precipitation, would directly influence the production of secondary metabolites. Nevertheless, these findings align with prior research that reported similar outcomes, emphasizing the impact of modified abiotic factors on secondary metabolites.³² However, several other studies have established a connection between abiotic factors and secondary metabolite modulation.³³⁻³⁶ Moreover, existing literature consistently supports the notion that increased temperature can lead to a reduction in the levels of secondary metabolites.^{39,40} This inverse relationship, indicates that environmental stresses, including temperature variations, may impede the production of secondary metabolites. This aligns with the well-established understanding that environmental stressors play a pivotal role in influencing the biochemical composition of plants, offering valuable insights into the intricate relationship between plant physiology and external environmental conditions.

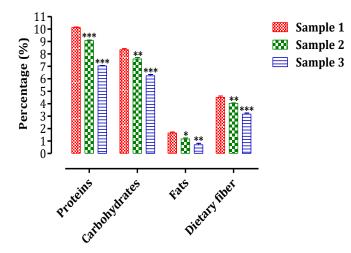


Figure 1: Quantitative primary metabolites (protein, carbohydrate, fat and dietary fiber) content of samples of *I. viscosa* cultivated under different climatic conditions

Table 3: Qualitative Amino acid composition of samples of *I.*

 viscosa cultivated under different climatic conditions

Amino acid	Inference					
-	Sample 1	Sample 2	Sample 3			
Asparagine	-	-	-			
Alanine	-	-	-			
Arginine	-	-	-			
Aspartate	+	+	+			
Cysteine	+	+	+			
Glutamate or Glutamic Acid	+	+	+			
Isoleucine	+	+	+			
Glutamine	-	-	-			
Glycine	+	+	+			
Histidine	+	+	+			
Proline	+	+	+			
Phenylalanine	+	+	+			
Serine	+	+	+			
Leucine	+	+	+			
Lysine	+	+	+			
Methionine	-	-	-			
Threonine	-	-	-			
Selenocysteine	-	-	-			
Pyrrolysine	-	-	-			
Valine	+	+	-			
Tyrosine	+	-	-			
Tryptophan	-	-	-			
+: indicates presence of constituer						

+; indicates presence of constituents

-; indicates absence of constituents

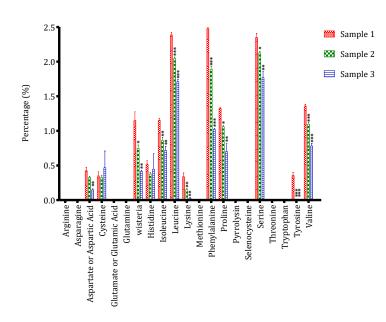


Figure 2: Quantitative amino acids content of three samples of *I. viscosa* cultivated under different climatic conditions *** significant difference at p < 0.001; ** significant difference at p < 0.05, compared to sample 1.

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Effect of temperature and precipitation on the mineral composition of Inula viscosa leaves

The mineral compositions of the three I. viscosa samples cultivated under different climatic conditions are presented in Table 5 and Figure 4. Analysis of the results revealed significant variations in the percentage mineral composition across the different samples. Calcium, potassium, magnesium, phosphorus, iron, and manganese were the most predominant elements in the three samples, while other elements including sodium, chlorine, sulphur, lead, selenium, copper, zinc, and cobalt were found in lower quantities. In sample 1, for instance, the percentages of calcium, potassium, magnesium, phosphorus, iron, and manganese were 4.25%, 9.55%, 11.6%, 3.35%, 11.2%, and 5.54%, respectively. Sample 2, on the other hand, exhibited calcium, potassium, magnesium, phosphorus, iron, and manganese contents of 4.25%, 7.93%, 10.1%, 3.15%, 9.38%, and 3.81%, respectively, while sample 3 showed the predominant elements as follows; calcium (4.18%), potassium (5.6%), magnesium (9.01%), phosphorus (3.08%), iron (7.03%), and manganese (3.6%) (Figure 4).

However, a noticeable decline in these elements were observed in samples 2 and 3. This decrease is unargueably influenced by the combined effect of elevated temperature and reduced precipitation, which significantly (p < 0.001) impacted all mineral compositions except for calcium and phosphorus. Previous research has reported that the content of certain minerals tends to decrease with rising temperatures and increasing water stress, as observed in this study.³⁷ These findings align with similar results of previous studies, providing further validation and support for the impact of climate-related factors on the mineral composition of plants.³⁸

Effect of temperature and precipitation on Inula viscosa leaves essential oil yield

As shown in Figure 5, the essential oil yields were calculated for the three samples of *I. viscosa*. Specifically, the essential oil yield in sample 1 was approximately 0.38%, an increased yield was observed in sample 2, and a decreased yield in sample 3 compared to that of sample 2, but higher than that of sample 1. Overall, the rise in temperature and the decline in precipitation significantly (p < 0.001) influenced the essential oil yield in samples 2 and 3, with an increase in sample 2, and a subsequent drop in sample 3. This fluctuation may be attributed to the detrimental impact of water stress and drought on essential oil yield, a phenomenon well-supported by existing literature, where water stress have been reported to negatively affects essential oil yield.^{43,44} Furthermore, numerous studies have demonstrated that severe water stress can enhance the concentration of essential oils, with the highest yield occurring under conditions of moderate water stress,⁴⁵⁻⁵⁰ further reinforcing the findings from the present study.

Effect of temperature and precipitation on Inula viscosa leaves essential oil composition

GC-MS analysis of the essential oils from the leaves of three samples of *I. viscosa* are presented in Figures 6, 7, and 8, along with the corresponding details in Table 6. The analysis identified 47 compounds in each sample. Notably, sample 2 was distinguished by the presence of compound **17**(β -selienene) which was absent in samples 1 and 3, and the absence of compound **18** (10,11-epoxy-calamenene), which was present in samples 1 and 3. The GC-MS results underscore the impact of climatic conditions on the chemical compositions of plants, particularly the major compounds.

The findings highlighted a notable variation in the chemical composition of *I. viscosa* cultivated under different climatic conditions, particularly the major compounds. For instance, the percentage of compound **28** [(E)-nerolidol] was 10.89% in sample 1, with a decrease to 6.90% in sample 2, and to 6.87% in sample 3. These changes in compound percentages are in line with established knowledge that stresses, including climatic conditions, can influence the content of various compounds within plants,⁵¹ which emphasizes the dynamic response of plant chemistry to environmental factors. Furthermore, the

results from the present study reinforce the significant impact of water stress on the major compounds of plants, highlighting the influential role of environmental factors in shaping the chemical profile of plants.⁵²

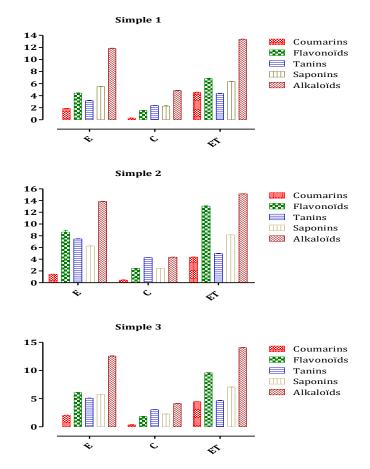


Figure 3: Quantitative phytochemical constituents of three samples of *I. viscosa* cultivated under different climatic conditions

E = Ether extract; C = Chloroform extract; ET = Ethanol extract

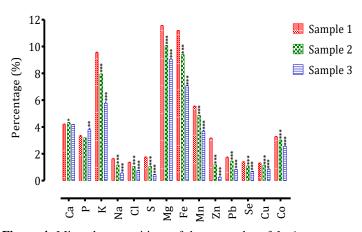


Figure 4: Mineral compositions of three samples of *I. viscosa* cultivated under different climatic conditions *** significant difference at p < 0.001; ** significant difference at p < 0.05, compared to sample 1.

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Table 4: Qualitative phytochemical constituents of three samples of I. viscosa cultivated under different climatic conditions

Phytochemical	Inference									
	Sample 1			Sample	Sample 2			Sample 3		
	Е	С	ЕТ	Е	С	ET	Е	С	ET	
Coumarins	+	+	+	+	+	+	+	+	+	
Flavonoids	+	+	+	+	+	+	+	+	+	
Tannins	+	+	+	+	+	+	+	+	+	
Saponins	+	+	+	+	+	+	+	+	+	
Alkaloids	+	+	+	+	+	+	+	+	+	

E = Ether extract; C = Chloroform extract; ET = Ethanol extract

Table 5: Mineral composition of three samples of *I. viscosa*

 cultivated under different climatic conditions

Mineral	Inference						
	Sample 1	Sample 2	Sample3				
Mn	+	+	+				
Na	+	+	+				
Cl	+	+	+				
Р	+	+	+				
Mg	+	+	+				
Fe	+	+	+				
Pb	+	+	+				
S	+	+	+				
Co	+	+	+				
Zn	+	+	+				
Cu	+	+	+				
Se	+	+	+				
Κ	+	+	+				
Ca	+	+	+				

Ca: calcuim; P: phosphorus; K: potassium; Na: sodium; Cl: chlorine; S: sulfur; Mg: magnesium; Fe: Iron; Mn: manganese; Zn: zinc; Pb: Lead; Se: selenium; Cu: copper; Co: cobalt;

Conclusion

The results of the present study have revealed that alteration in climatic conditions induces a variation in the chemical composition of *Inula viscosa*. Particularly, a rise in temperature and a decrease in precipitation have caused a significant modification to the primary and secondary metabolites, the mineral composition, as well as essential oil yield of the plant leaves. In addition, GC-MS analysis demonstrated that climatic conditions have a detrimental impact on the chemical compositions of essential oils. Therefore, it could be affirmed that climate change currently has an adverse effect on the ecosystem, emphasizing the need to consider climate change measures to mitigate its detrimental effects on humanity as a whole.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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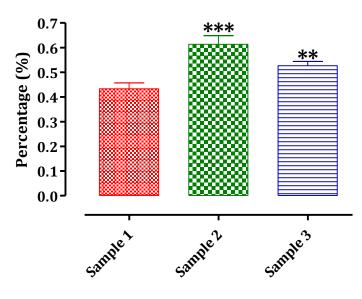


Figure 5: Essential oil yield of three samples of *I. viscosa* cultivated under different climatic conditions. *** significant difference at p < 0.001; ** significant difference at p < 0.01, compared to sample 1

Table 6: Chemical composition of the essential oils of three samples of I. viscosa cultivated under different climatic conditions

S/N	Compound name	Retention	Literature	Percentage composition (%)		
		index HP5	Retention Index	Sample 1	Sample 2	Sample 3
1	1,8-dehydro-cineole	992	991	0.09	0.21	0.17
2	n-nonanal	1104	1100	0.09	0.34	0.21
3	para-mentha-1,5-diene-8-ol	1168	1170	0.06	1.92	0.64
4	α-terpineol	1191	1188	1.09	2.45	1.20
5	α-copaene	1376	1376	3.28	4.23	2.45

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6	(E)- β -damascenone	1384	1384	2.37	3.35	1.97
7	(Z)- β -damascenone	1388	1387	0.93	0.03	0.04
8	1-tetradecene	1392	1389	1.88	0.56	1.79
9	α-cedrenes	1418	1419	4.01	1.95	2.49
10	(E)-caryophyllene	1443	1441	1.43	2.05	1.78
11	the aromatics	1454	1455	0.09	1.78	1.11
12	geranylacetone	1460	1460	1.61	2.09	1.43
13	allo-aromaticdendrines	1472	1467	1.19	2.05	1.94
14	cis-support adiene	1475	1477	0.04	0.73	1.05
15	β -chamigrenes	1476	1479	1.49	0.51	1.09
16	γ-muurolene	1485	1490	0.05	0.90	1.07
17	β-selienene	1490	1491	-	0.57	-
18	10,11-epoxy-calamenene	1490	1492	3.98	-	2.85
19	δ-selienene	1493	1493	2.43	1.89	1.56
20	cis-β-guaiene	1498	1500	1.67	0.98	1.78
21	α-muurolene	1501	1501	1.09	0.07	1.05
22	epizonarene	1505	1505	0.71	0.02	1.03
23	α-cuprenene	1523	1523	2.89	1.89	3.80
24	δ-cadinene	1535	1538	0.01	0.81	1.08
25	α-cadienene	1540	1540	1.96	2.82	2.07
26	α-copaen-11-ol	1542	1545	1.35	0.05	2.32
27	α-calacorene	1565	1563	7.08	26.07	11.90
28	(E)-nerolidol	1581	1580	10.89	6.90	6.87
29	caryophylleneoxide	1592	1589	2.28	1.95	2.92
30	1-hexadecene	1597	1596	3.05	1.81	1.06
31	phokienol	1600	1600	1.95	1.81	1.85
32	guaiol	1616	1619	10.3	1.68	1.39
33	isongifolan-7-α-ol	1627	1628	0.06	1.38	1.09
34	1-epi-cubenol	1627	1631	2.63	0.05	2.93
35	muurola-4,10(14)-diene-1- β .	1630	1632	1.92	3.06	3.92
36	gymnomitron	1641	1640	0.05	1.54	1.01
37	epi-α-cadinol	1648	1651	1.45	1.29	2.27
38	cedr-8(15)-en-9-a-ol	1653	1653	9.17	4.75	7.98
39	α-eudesmol	1656	1654	1.39	0.98	1.01
40	α-cadinol	1669	1667	0.02	0.43	0.04
41	14-hydroxy-(Z)	1673	1669	0.91	1.47	1.61
42	14-hydroxy-9-epi-(E)	1684	1681	1.03	1.19	1.17
43	ishwarone	1695	1699	0.03	0.48	0.03
44	epi-nootkatol	1745	1747	0.04	0.69	0.98
45	8-α-11-elemodiol	1769	1767	0.06	2.03	1.72
46	β -costol	1769	1768	1.27	1.28	1.09

1790

Total

2.79

94.91

2.69

96.84

2.89 **95.37**

1792

47

13-hydroxy-valencene

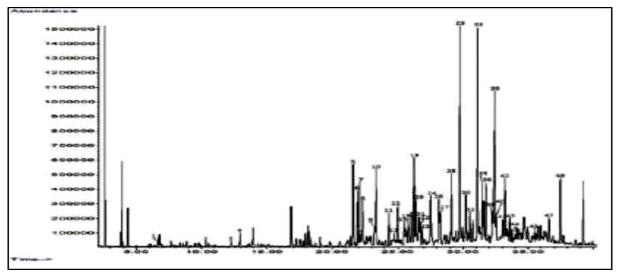


Figure 6: Gas chromatogram (GC) of essential oil extracted from sample 1

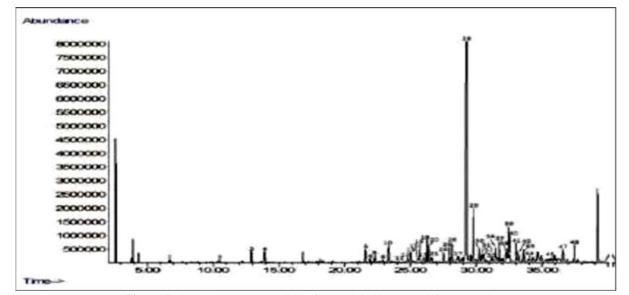


Figure 7: Gas chromatogram (GC) of essential oil extracted from sample 2

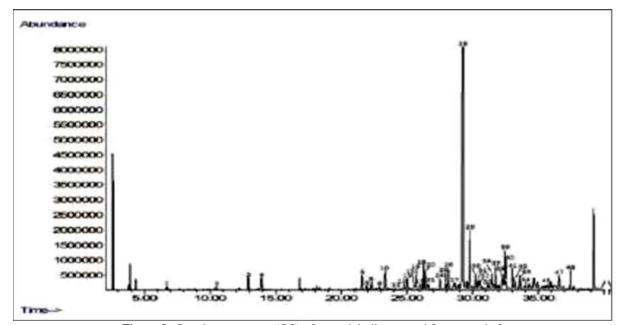


Figure 8: Gas chromatogram (GC) of essential oil extracted from sample 3

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