Section A-Research paper

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Forensic analytical characterization of Cannabis Sativa using GC-MS from the Dhauladhar range and lower Shivalik range.

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Abstract

Drug Abuser use Cannabis very commonly all over the world that has increased its usage annually very drastically. The analysis of cannabis by using high precision instrumental techniques such as Gas chromatography integrated with mass spectrophotometer (GCMS) permits for the analysis of cannabis that shows the variations of the constituents of this plant. It has been reported that data of its constituents varied with the prevalence of this plant. In the present study the plant of cannabis Sativa from two regions of near Himalayan range has been studied using GC-MS. This technique proved to be very useful and distinguished the constituents of cannabis sativa from lower Himalayan Region to the Upper Himalayan range. The outcomes obtained from the study will really be helpful in GCMS could be a helpful technique for the comparison of constituents of this drug of abuse in both ranges dhauladar range and lower shiwalik range which is able to assist the investigator regarding the origin of plant. Comparison additionally aids within the understanding and acquaintance of similarities of various samples of cannabinoid

Key words: Gas chromatography, mass spectrophotometer, cannabis sativa, forensic chemistry, drug of abuse.

Introduction

Cannabis plant is popularly known as marijuana or mind altering drug plant. Cannabis has numerous physical as well as mental effects that cause state of mind

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or elation feeling. The most hallucinogenic part of cannabis is THC tetrahydrocannabinol that is one in all the 483 compounds within the plant. Cannabis and cannabinoid: pharmacology, toxicology and therapeutic potential including at least 65 other cannabinoid

THC,Δ^9-THC

THC (tetrahydrocannabinol) is that chemical accountable for most of the marijuana's psychological effects. It acts very similar to the cannabinoid chemical created naturally by the body. Cannabinoid receptors are conc. In bound areas of the brain related to thinking, memory pleasure co-ordination and time perception. Consciousness alerting drug attach to those receptors and activates them and affects a person's memory pleasure movement thinking concentration co-ordination and sensory and time perception

THC is one in all several compounds founds in resins secreted by glands of the marijuana plant a lot of those glands of the marijuana are around the reproductive organs of the plant than on other areas of the plants alternative compounds distinctive to marijuana cannabinoid are present in resin . One cannabinoid CBD is non hallucinogenic and truly blocks the highness related to high associated with THC

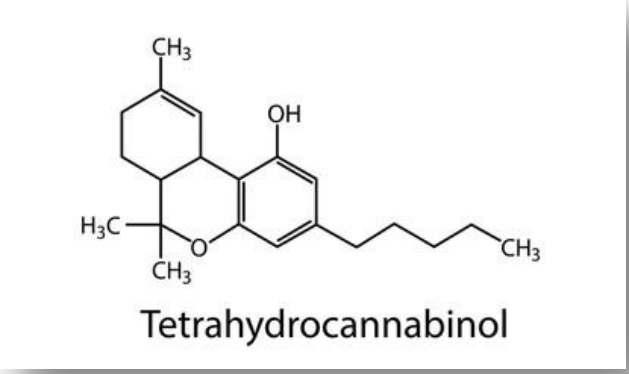

Figure 1 depicts the chemical structure of delta-9-tetrahydrocannabinol (THC)

CBD

CBD or cannabidiol is the second rifest active ingredient in cannabis. Cannabidiol (CBD). May be a phytocannabinoid discovered in 1940. It's one in all 113 known cannabinoid in cannabis plants at the side of psychoactive drug (THC) and

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accounts for up to 40% of the plant extraction as of 2019 , clinical analysis on CBD enclosed studies associated with anxiety , cognition, movement disorders and pain , however there's inadequate high quality proof that cannabidiol is effective for these conditions

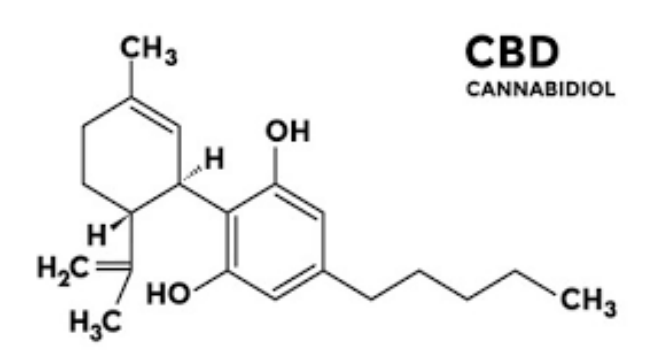

Figure 2 depicts the chemical structure of cannabidiol (CBD

CBC

CBC may be one in all the foremost long no psychotropic CBs found in strain or kind of cannabis as cannabichromene (CBC) conjointly known's as cannabichrome , cannabichromene pentylcannabichromene or cannabinochromene is associates in nursing medicinal drug which can contributes to the pain killing results of cannabis its one in all the many cannabinoid found within the cannabis plants and thus a phytocannabinoids .it bears structural similarity to the opposite natural cannabinoid as well THC , tetrahydrocannabivarian (THCV) , cannabidiol among others . CBC and its derivatives are as long as cannbinols. It's not regular by the convention in cannabis on mind alerting substances. It's a lot of common in tropical cannabis varieties

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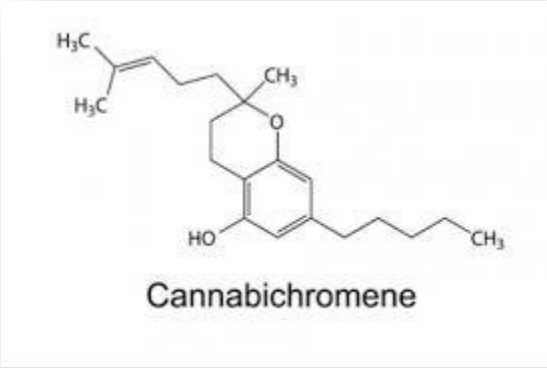

Figure 3 depicts the chemical structure of cannabichromene (CBC),

Experimental

General

The primary objective of this investigation is to conduct a comparative analysis of the chemical and biological constituents present in cannabis sativa sourced from the Dhauladar and lower Shiwalik ranges. Through this study, we aim to elucidate any variations in the components of cannabis obtained from different regions within these two ranges. To ensure the presence of cannabinoids in our samples, a preliminary color test was conducted prior to analysis. Subsequently, all 10 samples were subjected to gas chromatography-mass spectrometry (GC-MS) analysis using established scientific protocols.

Equipment

The experimental setup utilized in this study involved the use of a Shimadzu Corporation model QP2020 gas chromatography-mass spectrometry (GCMS) system. This equipment was employed for the purpose of comparing the various samples extracted from different regions within the Dhauladar and lower Shiwalik ranges. The GCMS system was chosen based on its ability to provide high resolution and sensitivity, making it a suitable choice for conducting a detailed and accurate analysis of the samples. This selection of equipment was made in accordance with established scientific standards

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Material

The resin extracted from cannabis plants is sourced from various regions of the Dhauladar and lower Shiwalik ranges, facilitated by local communities. A total of 10 samples are collected, with 1 to 5 samples obtained from the Dhauladar range and 6 to 10 samples collected from the lower Shiwalik range. The first sample is acquired from the Dharmkot region, followed by the second sample from Nagrotabagwan, the third from Yolcantt, the fourth from Chamunda, and the fifth from Dharmshala. The sixth sample is collected from Ambwala, the seventh from Banog, the eighth from Bogriya, the ninth from Banethi, and the tenth and final sample from Jamta. This collection process adheres to scientific standards.

Extraction

01 gram of cannabis resin was dissolved in 10 mL of ethanol and allowed to stand for 5 hours. The mixture was then sonicated and the solvent was removed using a rotary evaporator. The resulting sample was subjected to analysis using the appropriate analytical technique

GCMS

Afterward, we conducted GC-MS analysis to compare the cannabis samples collected from the Dhauladar and lower Shiwalik ranges. Prior to running the GC-MS analysis, sample preparation was required. The sample preparation process is outlined below.

Sample preparation

A 0.1 g resin sample was added to 10 mL of ethanol and mixed at room temperature. The filter paper and valve were washed with ethanol and the sample was filtered using filter paper. The resulting solution was transferred to a 2 mL GC vial and shaken well. The solution was then successfully analyzed by GC-MS in a scientific manner

Following are the conditions for instrument

- Column: 15m.0.25m, 0.25 micron
- Phase:5% diphenyl – 95% dimethylpolysiloxan
- Vector: a hydrogen atom, 1.1ml/min , constant flow
- Injector: split/inseparable, 280_c
- Division ratio- 20:01
- Oven: 2 mint at 200_c, 10c/min 200-240_c, 2mint at 240_c

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- Detector: MS300_C, H
- 35ml/min, air 350ml/min
- Internal standard : tribenzylamine (TBA) in ethanol (0.5mg/ml)
- Injection : 1.5 micro liters , split
- The order of elution: CBD, THC, CBN
- Instrumentation GC-MS

Result

The result of GCMS analysis of the sample obtained from the dhauladar range and lower shiwalik range are as follows

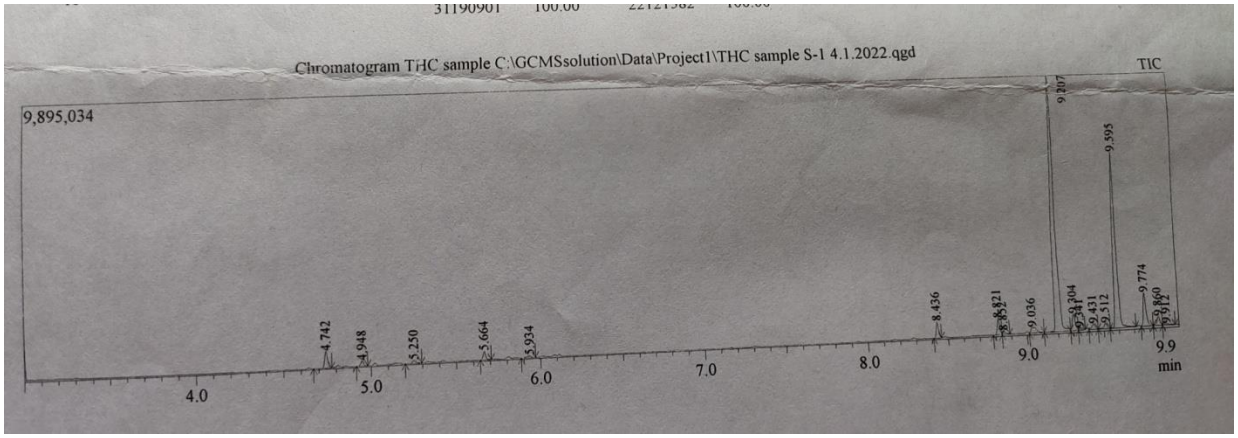

Figure 1 depicts the chromatogram of seven distinct constituents obtained from the cannabis plant originating from the Dharmkot region of the Dhauladar region. The varying heights of the peaks indicate that the quantity of each constituent present in the plant is unique. Additionally, the position of the peaks reflects the time of elution for each constituent, which differs based on their distinct chemical structures. Hence, each constituent exhibits a unique chemical profile that distinguishes it from other constituents present in the plant.

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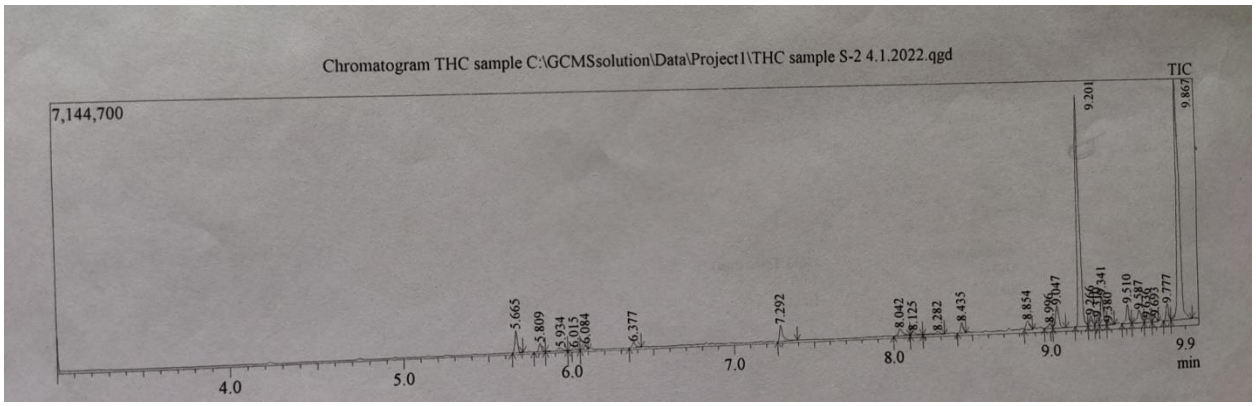

Figure 2 presents the chemical composition of a sample obtained from the Nagrota Bagwan region of the Dhauladar region, which comprises seven distinct constituents. The first constituent identified in the sample is caryophyllene, which is an essential oil present in Cannabis sativa. Its molecular formula is C15H24

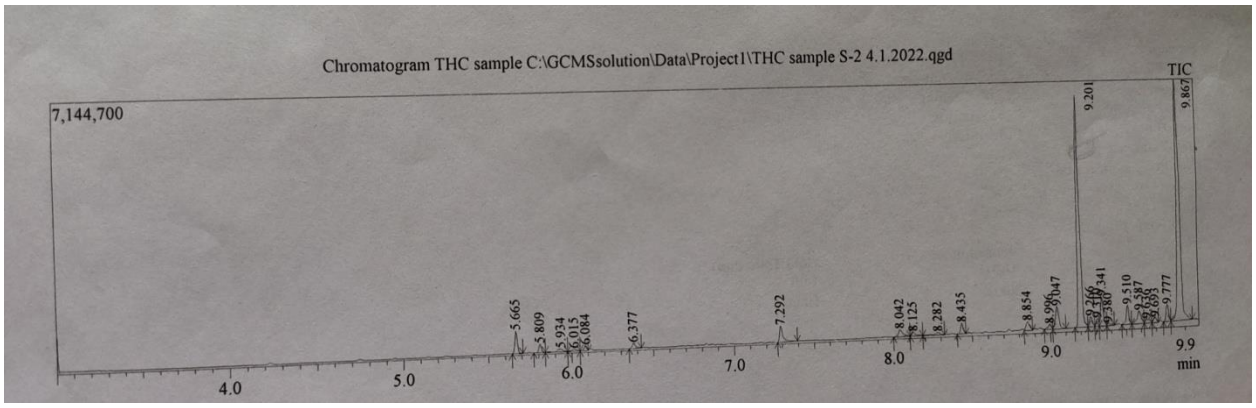

Figure 3 illustrates the analysis of a sample obtained from the Yol Cantt region of the Dhauladar region. The results indicate that there is an absence of THC content in the sample, as determined by the analytical method employed

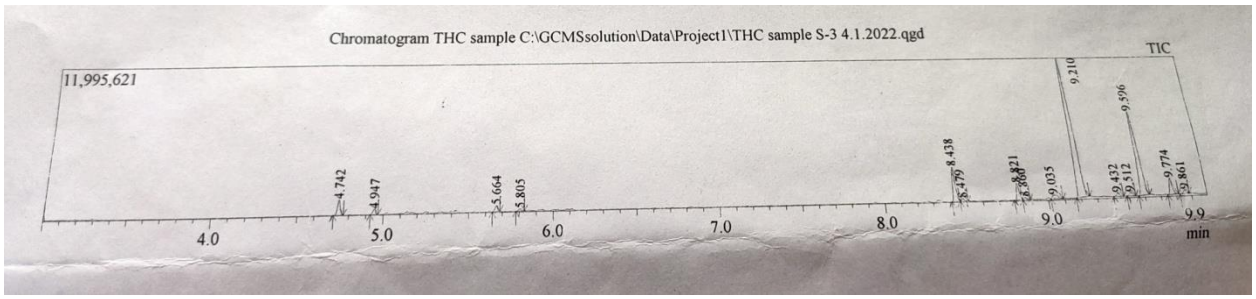

Figure 4 displays the comparison of two samples obtained from the Chamunda region and another region of the Dhauladar region. The results suggest that there is a minimal difference between the sample no 2 and this sample , indicating that Cannabis sativa plants in both areas possess similar constituents

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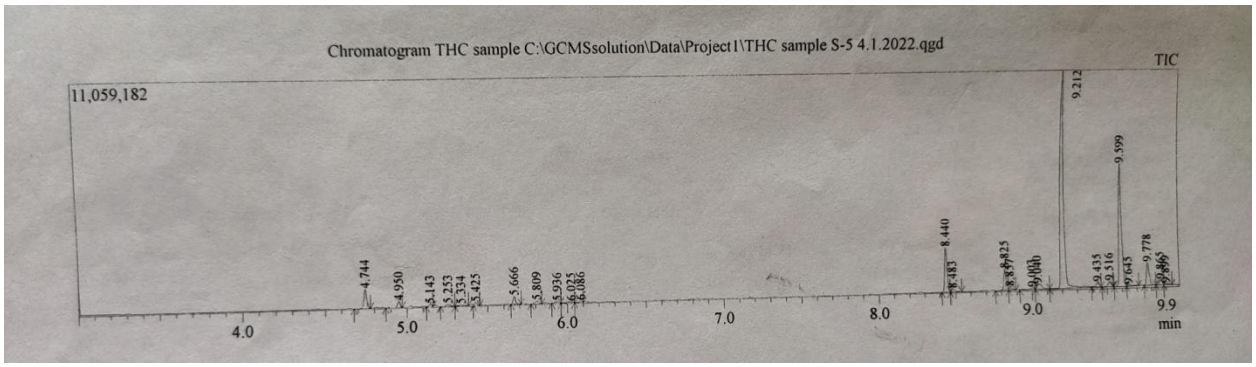

Figure 5 presents the analysis of a sample obtained from the Dharamshala region of the Dhauladar region, revealing the presence of seven distinct constituents in the sample. These seven constituents are also found in other samples obtained from the Dhauladar region, indicating that they are common constituents of Cannabis sativa plants in this region

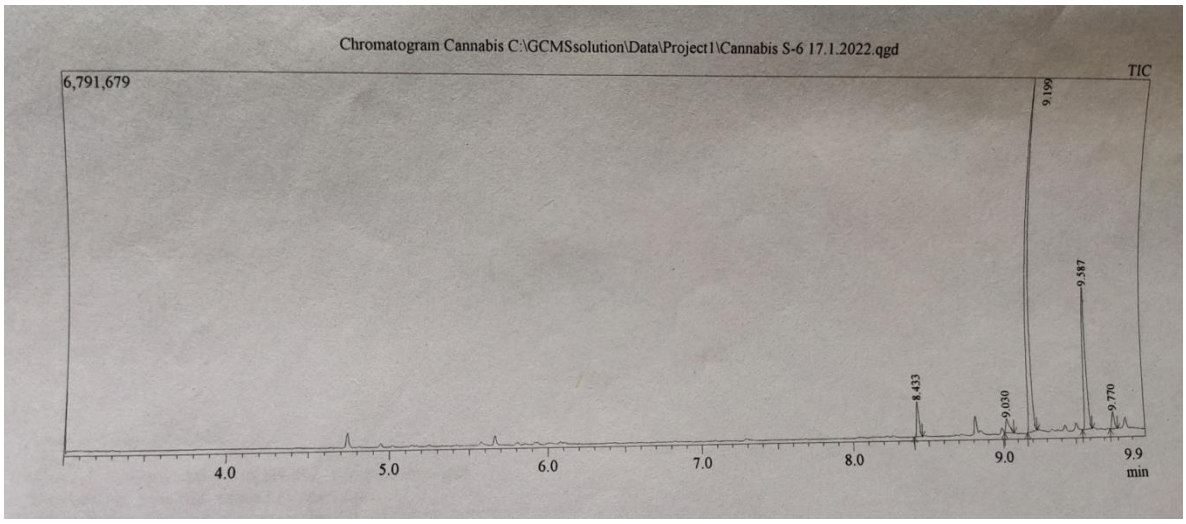

Figure 6 depicts the analysis of a sample obtained from the Ambwala region of the lower Shivalik range. The results indicate that the sample of Cannabis sativa obtained from this region contains only four components. Notably, the analysis revealed the absence of THC and cannabinol constituents in this sample

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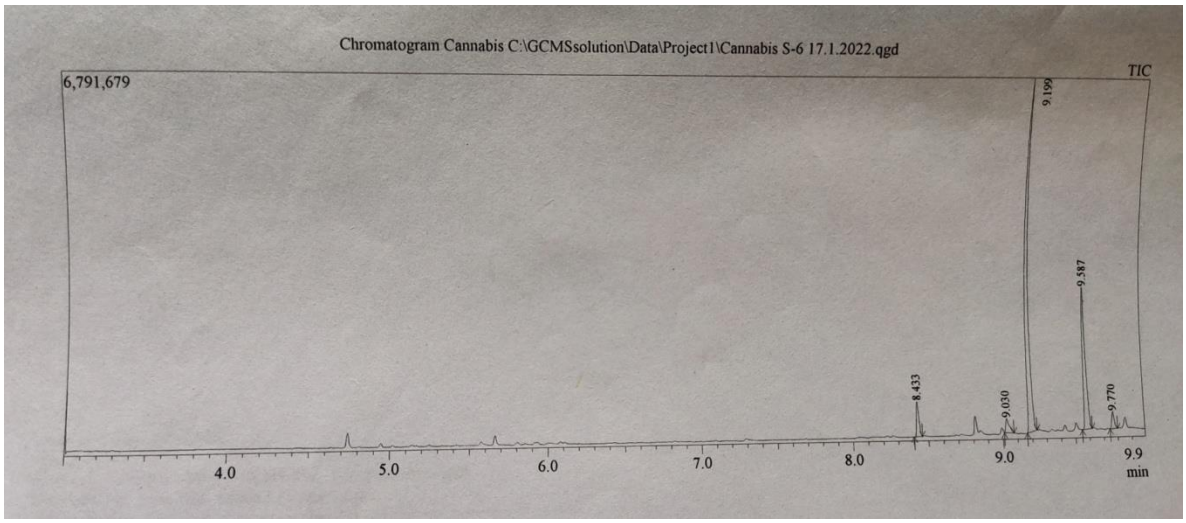

Figure 7 displays the analysis of a sample obtained from the Banog region of the lower shiwalik range, revealing the presence of seven distinct constituents of Cannabis sativa in the sample.

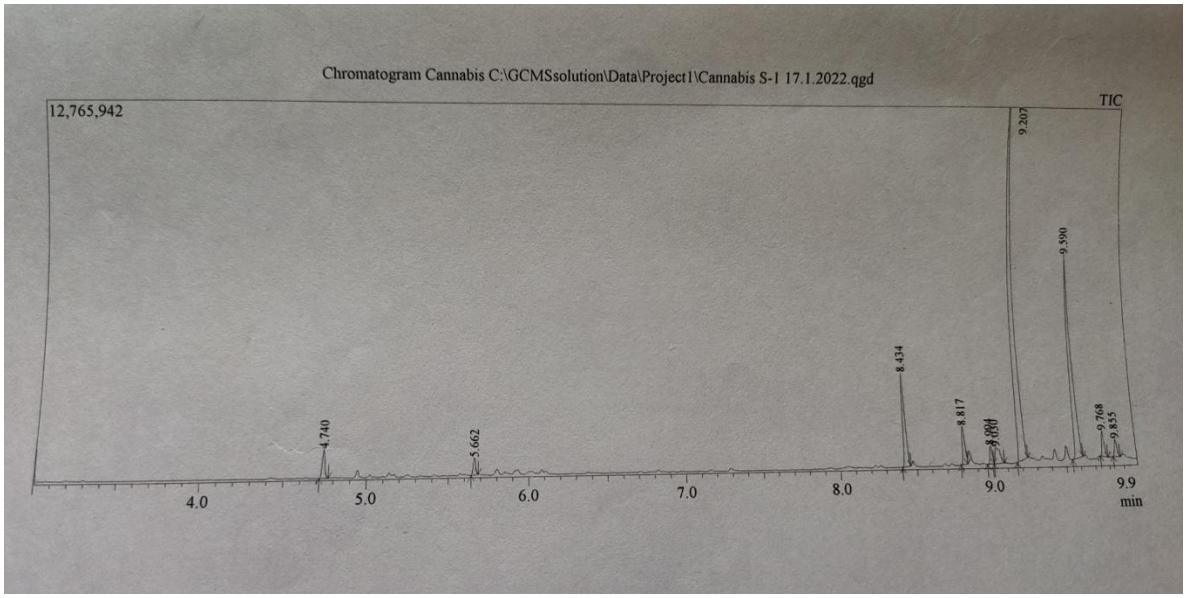

Figure 8 presents a comparison of two samples obtained from the Bogriya and Ambwala regions, respectively. The analysis indicates that both samples contain similar components of Cannabis sativa, suggesting that the plants in these two regions share similar chemical profiles

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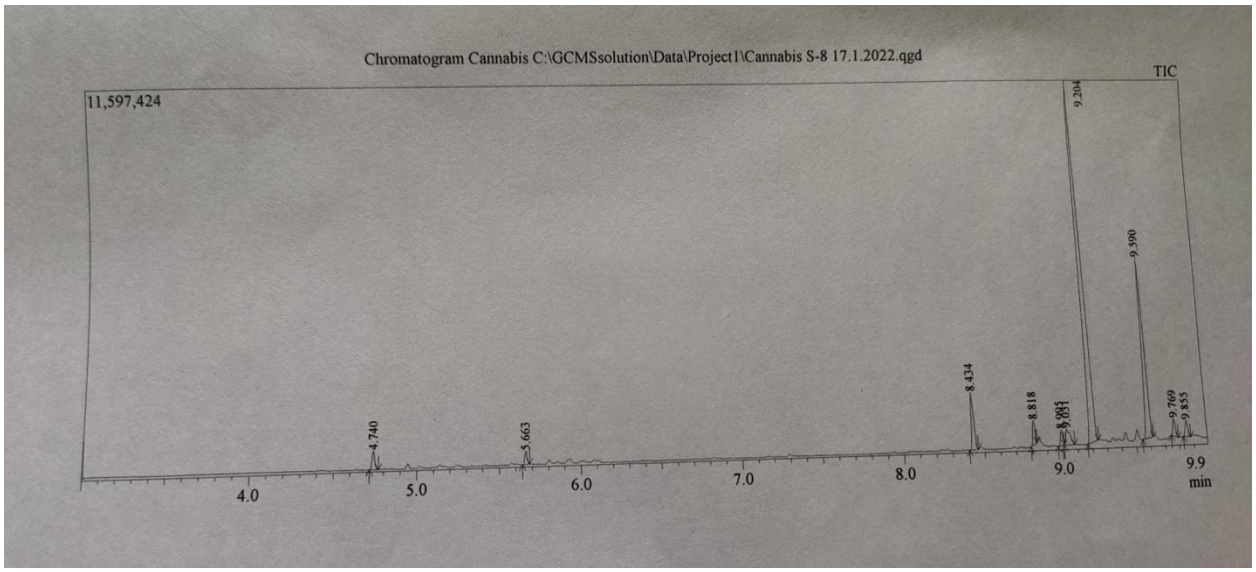

Figure 9 illustrates the analysis of a sample obtained from the Banethi region of the lower Shivalik range, revealing the presence of only five components of Cannabis sativa in the sample. Notably, the concentrations of THC and three other components were found to be the same in this sample.

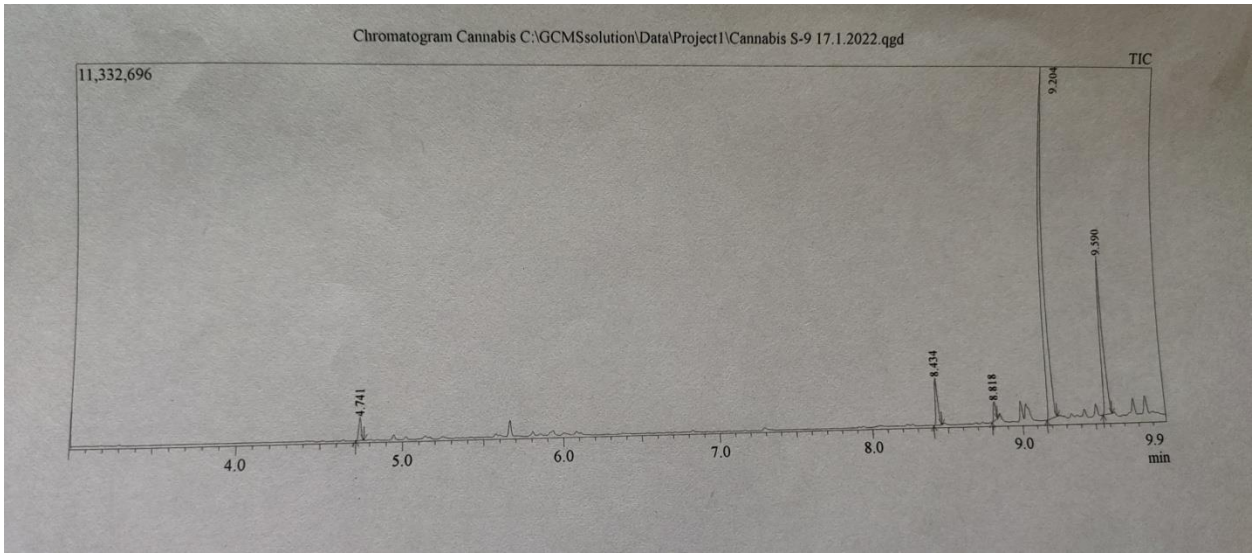

Figure 10 displays the analysis of a sample obtained from the Jamta region of the lower Shivalik range, revealing the presence of five distinct components of Cannabis sativa in the sample

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Interpretation of GC-MS for Dhauladar region

1. Dharmkot region

Peak number	Retention time	Name of constituent	Peak area
1	4.742	Caryophyllene	2.26
2	8.436	p-Heptylacetophenone	2.11
3	8.821	.delta9Tetrahydrocannabivarin	2.12
4	9.207	Cannabidiol	47.05
5	9.431	Dronabinol	1.02
6	9.774	Cannabigerol	5.50
7	9.860	Cannabinol	1.64

2. Nagrota Bagwan

Peak number	Retention time	Name of constituent	Peak area
1	5.665	Caryophyllene	2.30
2	8.435	p-Heptylacetophenone	1.19
3	-	.delta9Tetrahydrocannabivarin	-
4	9.201	Cannabidiol	0.80
5	9.587	Dronabinol	28.99
6	9.777	Cannabigerol	2.25
7	8.996	Cannabinol	2.25

3. Yol Cantt

Peak number	Retention time	Name of constituent	Peak area
1	4.742	Caryophyllene	3.30
2	8.438	p-Heptylacetophenone	8.03
3	8.821	.delta9Tetrahydrocannabivarin	3.95
4	9.210	Cannabidiol	47.79
5	9.432	Dronabinol	0.86
6	9.774	Cannabigerol	4.31
7	9.861	Cannabinol	1.04

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4. Chamunda

Peak number	Retention time	Name of constituent	Peak area
1	4.742	Caryophyllene	2.53
2	8.438	p-Heptylacetophenone	7.12
3	8.22	.delta9Tetrahydrocannabivarin	3.63
4	9.206	Cannabidiol	50.48
5	9.433	Dronabinol	0.86
6	9.774	Cannabigerol	4.01
7	9.861	Cannabinol	1.16

5. Dharmshala

Peak number	Retention time	Name of constituent	Peak area
1	4.744	Caryophyllene	2.85
2	8.483	p-Heptylacetophenone	0.57
3	8.825	.delta9Tetrahydrocannabivarin	3.43
4	9.212	Cannabidiol	47.30
5	9.435	Dronabinol	0.88
6	9.778	Cannabigerol	4.55
7	9.865	Cannabinol	1.15

Interpretation of GC-MS for lower shiwalik range

6. Ambwala

Peak number	Retention time	Name of constituent	Peak area
1	8.433	Caryophyllene	5.57
2	-	p-Heptylacetophenone	-
3	-	.delta9Tetrahydrocannabivarin	-
4	9.199	Cannabidiol	61.20
5	9.587	Dronabinol	2.76
6	9.770	Cannabigerol	3.15
7	-	Cannabinol	-

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7. Banog

Peak number	Retention time	Name of constituent	Peak area
1	4.740	Caryophyllene	2.44
2	8.434	p-Heptylacetophenone	8.65
3	8.817	.delta9Tetrahydrocannabivarin	3.79
4	9.207	Cannabidiol	50.78
5	9.590	Dronabinol	23.49
6	9.768	Cannabigerol	2.62
7	9.855	Cannabinol	1.86

8. Bogriya

Peak number	Retention time	Name of constituent	Peak area
1	4.740	Caryophyllene	2.03
2	8.434	p-Heptylacetophenone	6.37
3	8.818	.delta9Tetrahydrocannabivarin	2.63
4	9.204	Cannabidiol	54.21
5	9.590	Dronabinol	24.06
6	9.769	Cannabigerol	2.12
7	9.855	Cannabinol	2.03

9. Banethi

Peak number	Retention time	Name of constituent	Peak area
1	4.741	Caryophyllene	2.87
2	8.434	p-Heptylacetophenone	6.48
3	8.818	.delta9Tetrahydrocannabivarin	2.52
4	9.204	Cannabidiol	62.55
5	9.590	Dronabinol	25.58
6	-	Cannabigerol	-
7	-	Cannabinol	-

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10.Jamta

Peak number	Retention time	Name of constituent	Peak area
1	-	Caryophyllene	-
2	8.435	p-Heptylacetophenone	8.23
3	8.818	.delta9Tetrahydrocannabivarin	3.24
4	9.202	Cannabidiol	60.96
5	9.589	Dronabinol	24.73
6	9.770	Cannabigerol	2.85
7	-	Cannabinol	

Conclusion

The analysis of the Cannabis samples collected from the Dhauladar range indicates that all five samples contain the psychotropic constituent, THC. In contrast, samples taken from the lower Shivalik range show slight variations in their chemical profiles. Some samples contain only four components, while others contain seven. However, THC is the most abundant constituent present in all 10 samples analyzed. Additionally, cannabidiol and cannabinol are also present in most of the samples obtained from both the lower dhauladhar and lower Shivalik ranges.

Funding

None

Conflict of interest

None declared

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