

Phytochemical screening and Spectroscopic characterization of cannabis sativa L, cultivated in Mt Kenya region, Kenya

John Wamumwe Mwangi

d.mwangi@g.nsu.ru

Novosibirsk State University

Research Article

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Abstract

The aim of this research is to determine the phytochemicals and characterize *cannabis sativa* leaf extracts because they are the one consumed with HPLC, GC-MS, VV-VIS, FT-IR and AAS. phytochemical screening showed that phenols and tannins were the determinant phytochemicals, FT-IR analyzed functional group present, HPLC showed that Catechin acid di-hydrate was the major compound in the dichloromethane extract and ethanolic extract, while Naringin was the major component in the aqueous extract, GC-MS showed that palmitic acid, Linoleic acid, 7-octadecanoic acid and Linolenic acid are the major compound and it was confirmed by UV-VIS and AAS showed that *Cannabis sativa L.* was found to contain higher levels of heavy metals of Fe, Zn, Mn, and Cu.

1. Introduction

Cannabis sativa is a species of cannabis plant as shown in Fig. 1 distinguished by its tall size, slender leaves, and pronounced psychotropic characteristics [1]. It is one of the most common forms of cannabis used for both medical and recreational uses, with higher levels of THC (tetrahydrocannabinol) than its counterpart, *Cannabis indica* [2]. Sativa strains are known for their energizing and uplifting qualities, making them popular candidates for daytime use. *Cannabis sativa* is grown for industrial uses, including hemp production, which can be used in textiles, construction materials, and biofuels [3].

Cannabis sativa L. often known as hemp, is an African herb that belongs to the *Cannabaceae* family and is among the earliest plants to be cultivated[5]. With its intoxicating and psychedelic properties, cannabis is regarded by the Kenyans as the sacred grass. Members of the religious community utilize it for rituals and ceremonies, in Mount Kenyans, a traditional medical practice, uses it for therapeutic purposes [6]. It was used medicinally by the Africans in the 15th century BCE as an analgesic and to cure rheumatic pain.

According to Fordjour, E., et al., [4], the *Cannabis sativa L* plant is rich in a variety of compounds including polyphenols, cannabinoids, flavonoids, terpenoids, and sterols, with more than 1,000 phytochemicals identified in its leaves, flowers, bark, and roots. Bioactive substances such as CBD and 9-tetrahydrocannabinol (THC) are present in the flowers, leaves, and roots.

This study intends to analyze the extracts from the leaves of *Cannabis sativa L* (Cannabaceae) grown in the Mt. Kenya region. The research will focus on examining the chemical composition of the hexane extract through the GC-MS method, followed by a qualitative analysis of the organic extracts using the HPLC method. Additionally, the study will involve the identification of functional groups through FT-IR spectroscopy, assessment of heavy metals using AAS spectroscopy, and screening for phytochemicals.

2. Materials and methods

2.1 Plant material

The cannabis leaves were collected in the Mt Kenya region, specifically in the cultivation areas located in the mountain region, Kenya's main cannabis production region. The leaves samples were sampled in July 2024. The leaves are dried for two weeks at room.

2.2. Preparation of extracts

Before extraction, *cannabis sativa* leaves were washed many times with deionized water. The extract was obtained by boiling 10g of finely cut leaves in 100ml of de-ionized water for 5 minutes and filtering the liquid. The filtrate was refrigerated at -4°C ready for analysis.

2.4. Phytochemical study

In this study, the phytochemicals were analyzed according to Mwangi [7]. For the extraction process, 3 grams of coarsely powdered crude samples were placed in separate stoppered containers, each containing hexane, water, ethanol, chloroform and acetone.

2.5 Analysis by GC–MS method

The fatty acid methyl esters of the hexane extract of *Cannabis sativa L* leaves were analyzed according to the NF T60-233 standard methodology [8].

2.6 Analysis by HPLC method

Cannabis sativa leaf was analyzed using HPLC where extracts of ethanolic extract, dichloromethane extract, and aqueous extract were prepared. A variety of compounds, including gallic acid, vanillic acid, naringenin, rutin, catechin, and cannabis sativa leaf extracts (ethanolic extract, dichloromethane extract, and aqueous extract), were employed for the HPLC profile.

2. Heavy Metal Analysis

The samples underwent acid digestion to remove moisture, weighed portions of crushed and powdered plant samples and heated in an oven at 105°C. After charring, the dried sample was heated for 5 hours at 350°C. The sample was cooled in a desiccator before 2.5 mL of 6M HNO₃ was added to dissolve them. The solution was filtered, then transferred to a 20 ml flask and diluted to the mark 15ml. Heavy metal estimation was carried out using an atomic absorption spectrophotometer [AAS].

Calibration of Equipment For the elements under examination, the following sensitivity and detection limits for the AAS equipment were determined. lead 0.2 and 2.0 ppm; chromium 0.4 and 5.0 ppm; cadmium 0.3 and 1.5 ppm; iron 0.5 and 3.5 ppm; Copper 0.5 and 4.0 ppm; Manganese 0.5 and 2.50 ppm; Zinc 0.05 and 4.5 ppm; and Nickel 0.5 and 3.0 ppm.

3. Result and discussion

3.1 phytochemical analysis

Table 1
phytochemical analysis of *Cannabis sativa* L

test	Water extract	Hexane extract	Acetone extract	Ethanol extract	Chloroform extract
steroids	+	+	+		
triterpenoids	+				
saponins		+			
phenol	++	++	++	++	++
flavonoids					+
tannin	+		+	+	
resin		+			+
Alkaloids	++	++		++	++
Glycosides	+		+	+	
Carbohydrates	+	+	+		
Reducing sugar	++	++	++	++	++
Fixed oil and fats			+	+	+
Proteins and amino acids					

Positive (+) means Present, negative (-) means Absent

The extracts were found to contain large number of phenols, alkaloids and reducing sugar. Phenol plays a significant role in pharmaceutical science due to its foundational presence in the synthesis of various drugs and as an antiseptic agent. Its structural properties allow for the modification and derivation of numerous pharmaceutical compounds, enhancing the efficacy of active ingredients [9]. The presence of alkaloids plays crucial role in pharmaceuticals due to their diverse pharmacological properties and therapeutic applications [10]. These nitrogen-containing compounds, exhibit a wide range of biological activities, including analgesic, antimalarial, antitumor, and antidiabetic effects [11]. For instance, well-known alkaloids such as morphine and codeine are vital in pain management, while quinine is a key treatment for malaria [12]. Their unique chemical structures allow for the development of various medicinal formulations, making alkaloids a valuable source of inspiration for drug discovery and the advancement of pharmacotherapy. The presence of these compounds like reducing sugar, steroids, Glycosides, Carbohydrates and Proteins and amino acids in the *Cannabis sativa* leaf's is that they are key contributors to the medicinal value and their physiological effects [13].

3.2. Total phenolic content

The total polyphenols were analyzed, and the results are shown in Fig. 2 and Table 2. Figure 2 demonstrates the total polyphenol content of cannabis leaf extracts in ethanolic extract, dichloromethane extract, aqueous extract, and hexane extract is as follows: The gallic acid equivalent levels varied from 150.05 mg/g $\pm$ 0.06 to 47.09 mg/g $\pm$ 0.04, 37.75 mg/g $\pm$ 0.04, and 10.71 mg/g $\pm$ 0.02. The ethanolic and aqueous extracts contained the most polyphenols (150.05 $\pm$ 0.06 and 47.09 $\pm$ 0.04 mg GAE/g) in comparison to the other extracts.

According to Izzo, L., et al., [14] study, methanol and distilled water had the highest phenolic content in *cannabis Sativa L* leaves (36.58 $\pm$ 0.07 and 38.03 $\pm$ 0.04 mg of GAE/g). Ethyl acetate and ethanol had the lowest phenol levels (14.27 $\pm$ 0.06 and 3.0 $\pm$ 0.04 mg GAE/g), while hexane and acetone solvents showed negative phenol levels.

Table 2
results of the total
polyphenols μ g gallic acid
equivalent per mg of
extract (μ g EAG/mg of
extract)

Extract	Polyphenol
EHx	10.71 $\pm$ 0.02
EDm	37.75 $\pm$ 0.04
EEt	150.05 $\pm$ 0.06
EAq	47.09 $\pm$ 0.04

3.3 HPLC and GC-MS analysis of extracts

The qualitative investigation of EEt, EDm, and EAq extracts from Cannabis sativa leaves using HPLC allowed us to identify distict compounds, which were determined by comparing the retention time of each component to that of the standards used.

The chemical composition of the EDm, EEt, and aqueous extracts was analyzed using HPLC and GC-MS, with results given in Figs. 3, 4, and 5, as well as Tables 3, 4, and 5.

Table 3
phytochemical investigation of dichloromethane extract.

Polyphenolics compounds	Retention time	% Area
Hesperidin acid	4.53	3.77
8-methoxy flavon	20.83	6.24
Cinnamic acid	22.98	0.89
6-hydroxy flavon	23.40	7.23
Catechin acid dihydrate	24.50	53.0
Ferulic acid	25.01	3.18
Rutin	25.20	2.39

Table 4
phytochemical investigation of ethanolic extract.

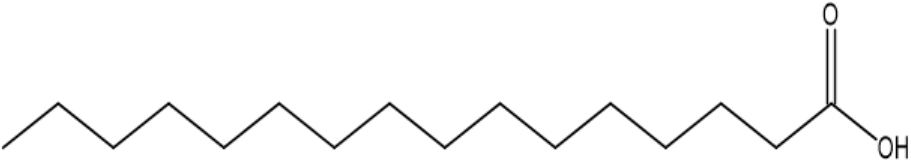
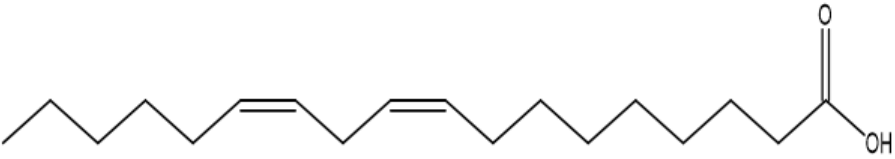
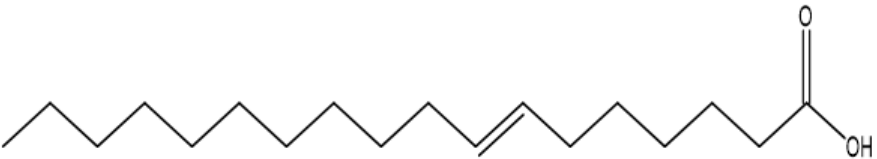
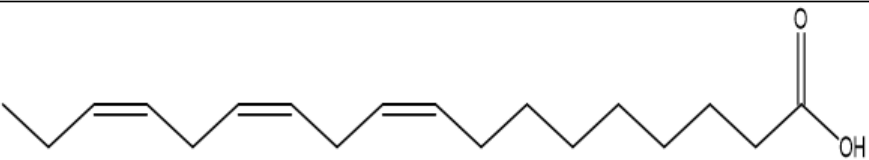
Polyphenolics compounds	Retention time	% Area
coumarin	9.11	1.40
Catechin acid dihydrate	11.30	1.09
Naringin	13.89	15.02
Rutin	14.26	10.20
Chlorogenic acid	16.50	1.50
Cinnamic acid	23.30	1.0
Ferulic acid	25.69	4.30
Hesperidin acid	30.11	8.99

Table 5
phytochemical investigation of aqueous extract.

Polyphenolics compounds	Retention time	% Area
Catechin acid dihydrate	4.2	25.11
Dianisidine	12.22	3.98
Caffeic acid	13.75	2.06
Benzoic acid	16.51	3.51
Cinnamic acid	15.20	3.35
Coumaric acid	17.19	3.45
Sinapinic acid	17.88	1.87

Using GC-MS analysis six compounds were detected where four compounds were in large amount and their structure and names are as shown in Table 6.

Table 6: Major fatty acids in Cannabis sativa leaf extract.

	Palmitic acid (peak 1)
	Linoleic acid (peak 2)
	7-oxtadecanoic acid (peak 3)
	Linolenic acid (peak 4)

This phytochemical study of Cannabis sativa L by matsunaga, T., et al. [15] was able to detect six non-cannabinoid components in cannabis leaves: gallic acid (44.8%), catechin acid dihydrate (5.8%), caffeic acid (5%), benzoic acid (4.7%), coumaric acid (2%), and ferulic acid (1.9%), with gallic acid accounting for the majority. As can be seen above, we were able to identify 14 non-cannabinoid components in the

majority of Cannabis sativ leaf extracts, with Catechin acid dihydrate being the most abundant in the dichloromethane extract and Naringin being the most abundant in the aqueous extract. These phenolic compounds are important in a variety of disorders, as well as cosmetics and antioxidants [16]. The primary components identified by GC-MS were palmitic acid, linoleic acid, 7-octadecanoic acid, and linolenic acid, as illustrated in Figs. 5 and Table 6.

3.4. Heavy Metal analysis by AAS

Cannabis sativa L. was found to contain higher levels of heavy metals as shown in and Fig. 5 of Fe, Zn, Mn, and Cu, as well as highly toxic metals like Pb, AS, and Cd, which exceed the WHO's permissible limits. These toxic metals will have a negative impact on human health, so it is necessary to monitor their levels in medicinal herbs and ensure that they fall within safe limits for consumption [17]. Prolonged exposure to heavy metals such as lead (Pb), arsenic (As), and cadmium (Cd) can lead to serious health issues, including neurological disorders, kidney damage, and an increased risk of cancers [18]. Additionally, essential trace elements like iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) are crucial for various biological functions, but their elevated levels, when coupled with toxic metals, can pose risks to human health [19].

To mitigate these risks, it is essential for regulatory agencies to establish and enforce guidelines for the permissible levels of heavy metals in medicinal herbs, including Cannabis sativa. Regular monitoring and testing of herbal products should be implemented to ensure that they meet safety standards. Furthermore, educating consumers and healthcare practitioners about potential contamination *cannabis sativa* will promote informed decision-making regarding their use.

3.5 functional group analysis by FT-IR spectroscopy

The following Table 7 summarizes the interpretation of the FT-IR spectrum

Table 7
interpretation of FT-IR spectrum in Fig. 6

Functional group	Stretching reason	Peak (cm ⁻¹)	reference
OH COOR	Due to vibration of acid and basic groups present in the extract	3437	[20]
carbonyl (C = O)	Due to vibration of acid groups present in the extract	1741	[7, 21]
	due to carbonyl stretching in proteins	1635	[22, 23]
N-H N = O	stretching typical of the nitro compound	1436 1242	[24]
C-N	stretching vibration of the aliphatic amines	1045	[25]
C-Cl C-Br	alkyl halides	769 687 526	[26–28]

3.6 UV–VIS analysis

The plant samples contain elemental elements in the form of compounds/molecules, which the LIBS approach cannot detect [29]. As a result, the UV-VIS spectroscopy technique is utilized to conduct molecular analyses on Cannabis sativa plant materials. The UV-VIS spectra of all samples were obtained and shown in Fig. 8

From the Table 6 the confirmation of palmitic acid at 280 nm, Linoleic acid at 410 nm, 7-octadecanoic acid at 616 nm and Linolenic acid at 670 nm were confirmed and affirmed the result from GC-MS in Fig. 6.

Conclusion

The purpose of this work was to perform phytochemical screening and spectroscopic characterization of *cannabis sativa* L grown in the Mt Kenya region of Kenya. Phytochemical analysis showed that *cannabis sativa* leaf extract is rich in alkaloids, phenols, carbohydrate and reducing sugar in large amount which facilitate medicinal properties. Cannabis sativa alkaloids, such as cannabinoids, have a variety of medicinal benefits, including analgesic, anti-inflammatory, and neuroprotective characteristics. Phenolic components enhance its antioxidant potential, aiding in the fight against oxidative stress and promoting general health. Carbohydrates and lowering sugars can also help provide energy and boost the extract's bioactivity. FT-IR analyzed functional group present which showed that's *cannabis sativa* leaf extract is rich in various functional groups, indicating the presence of diverse phytochemicals that contribute to its therapeutic properties. The FT-IR (Fourier Transform Infrared) spectroscopy analysis

typically reveals key functional groups such as hydroxyl (–OH), carbonyl (C=O), alkene (C=C), and ester (–COOR) groups, suggesting the presence of phenolic compounds. HPLC showed that Catechin acid dihydrate was the major compound in the dichloromethane extract and ethanolic extract, while Naringin was the major component in the aqueous extract, GC-MS showed that palmitic acid, Linoleic acid, 7-oxadecanoic acid and Linolenic acid are the major compound and it was confirmed by UV-VIS and AAS showed that *Cannabis sativa L.* was found to contain higher levels of heavy metals of Fe, Zn, Mn, and Cu.

Abbreviations

1. CBD-Cannabidiol
2. THC- 9-tetrahydrocannabinol
3. FT-IR- Fourier transform infrared spectroscopy
4. XRD- x-ray diffraction
5. HPLC- High-performance liquid chromatography
6. Gc-MS- Gas chromatography -mass spectroscopy
7. AAS-atomic absorption spectrophotometer
8. EHx- hexane extract
9. EDm- dichloromethane extract
10. EEt- ethanolic extract
11. EAq- aqueous extract

Declarations

Acknowledgments

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Declaration of interest

The authors declare that there are no conflicts of interest.

Funding Sources

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Data Availability Statement

All the data associated with this research has been provided herein.

Consent to Publish declarations

I all agree with Consent to Publish declaration

Ethics and Consent to Participate declarations

I all agree with Ethics and Consent to Participate declarations

Author Contribution statement

I JOHN WAMUMWE MWANGI solely contributed in researching and writing this research.

Clinical trial test

Not applicable

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Figures



Figure 1

cannabis sativa plant [4]

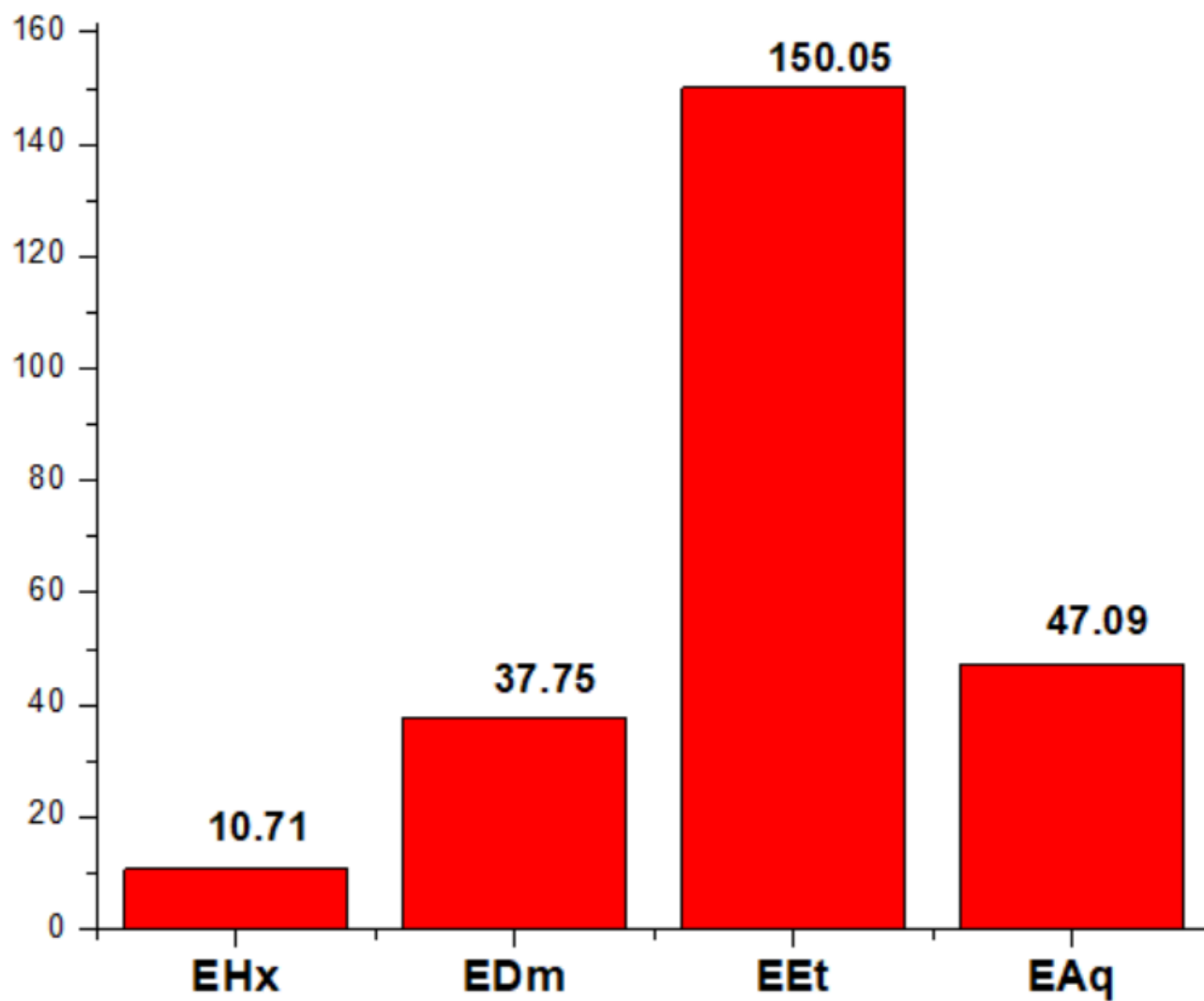


Figure 2

Histogram of total polyphenols present cannabis sativa extracts

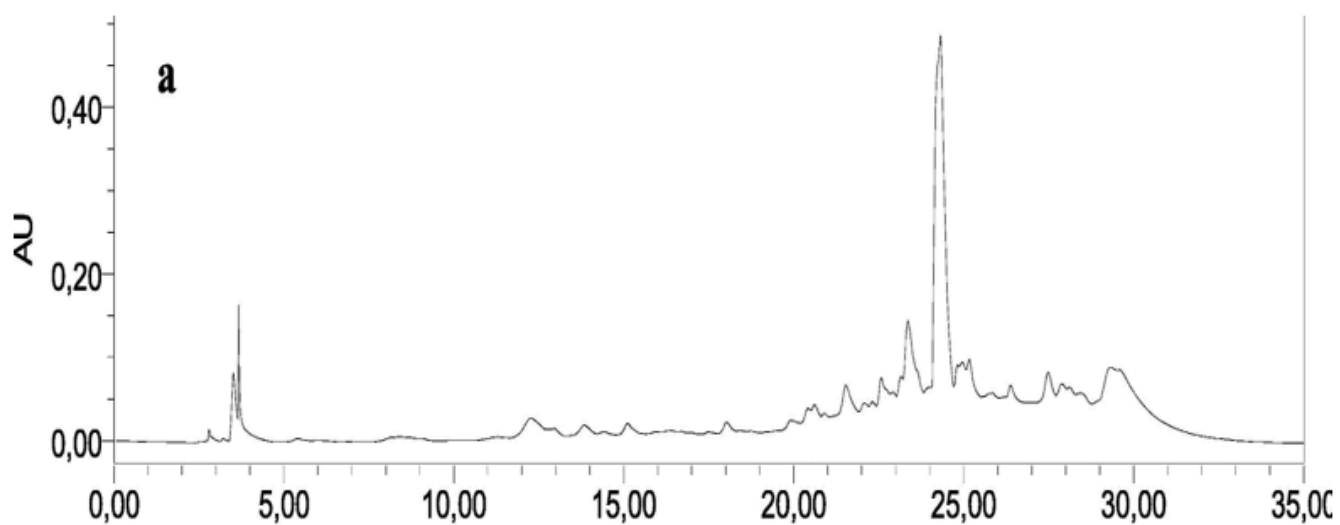


Figure 3

Dichloromethane chromatogram of the leaf of *Cannabis sativa*.

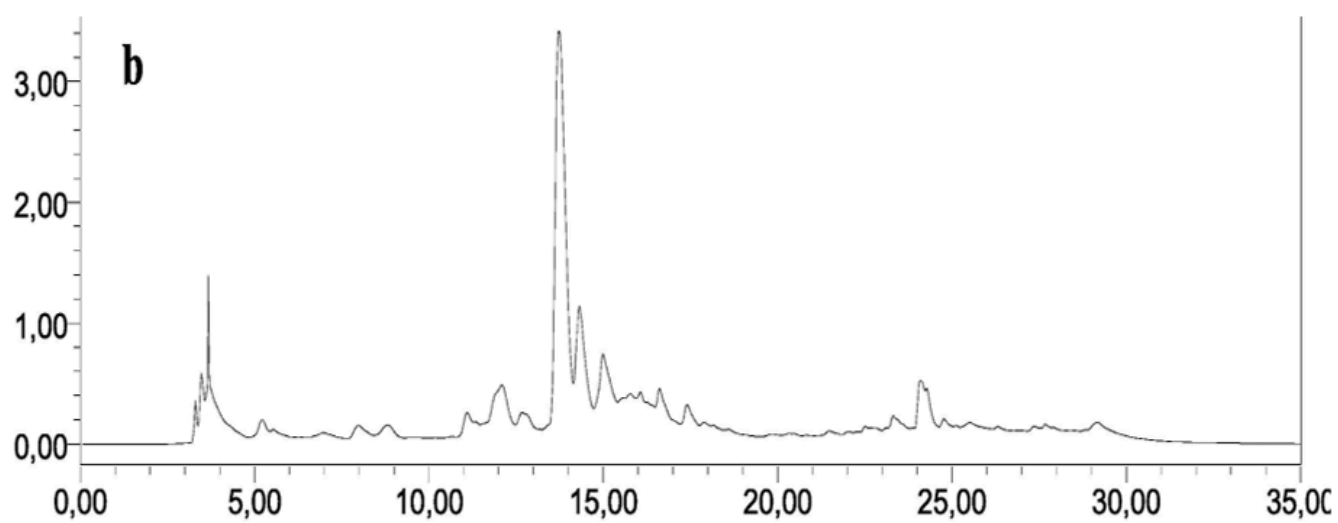


Figure 4

Ethanol chromatogram of the leaf of *Cannabis sativa*

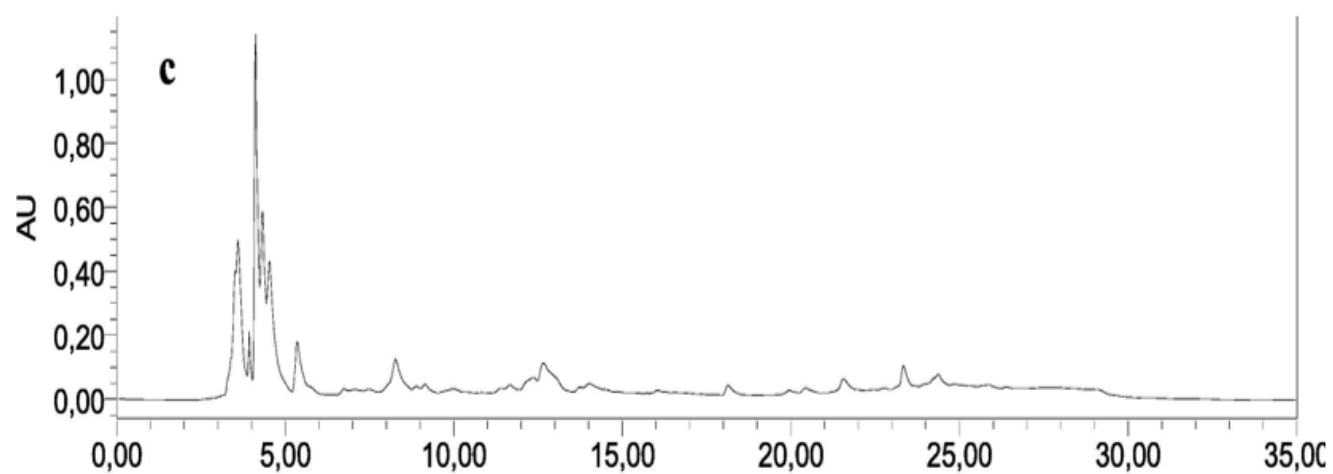


Figure 5

Ethanol chromatogram of the leaf of *Cannabis sativa*

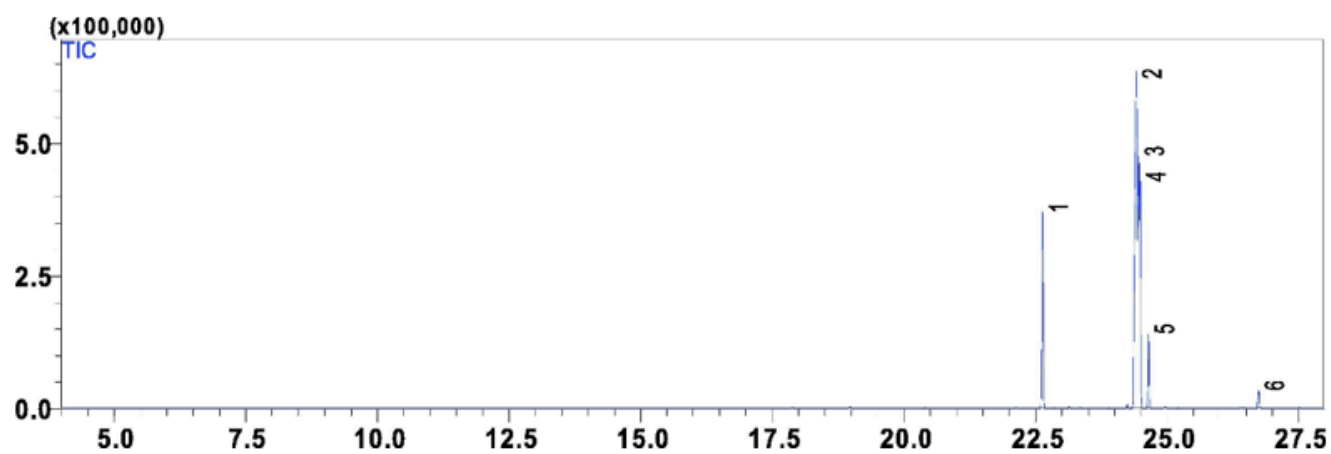


Figure 6

GC-MS analysis of cannabis sativa leaf hexane extract using

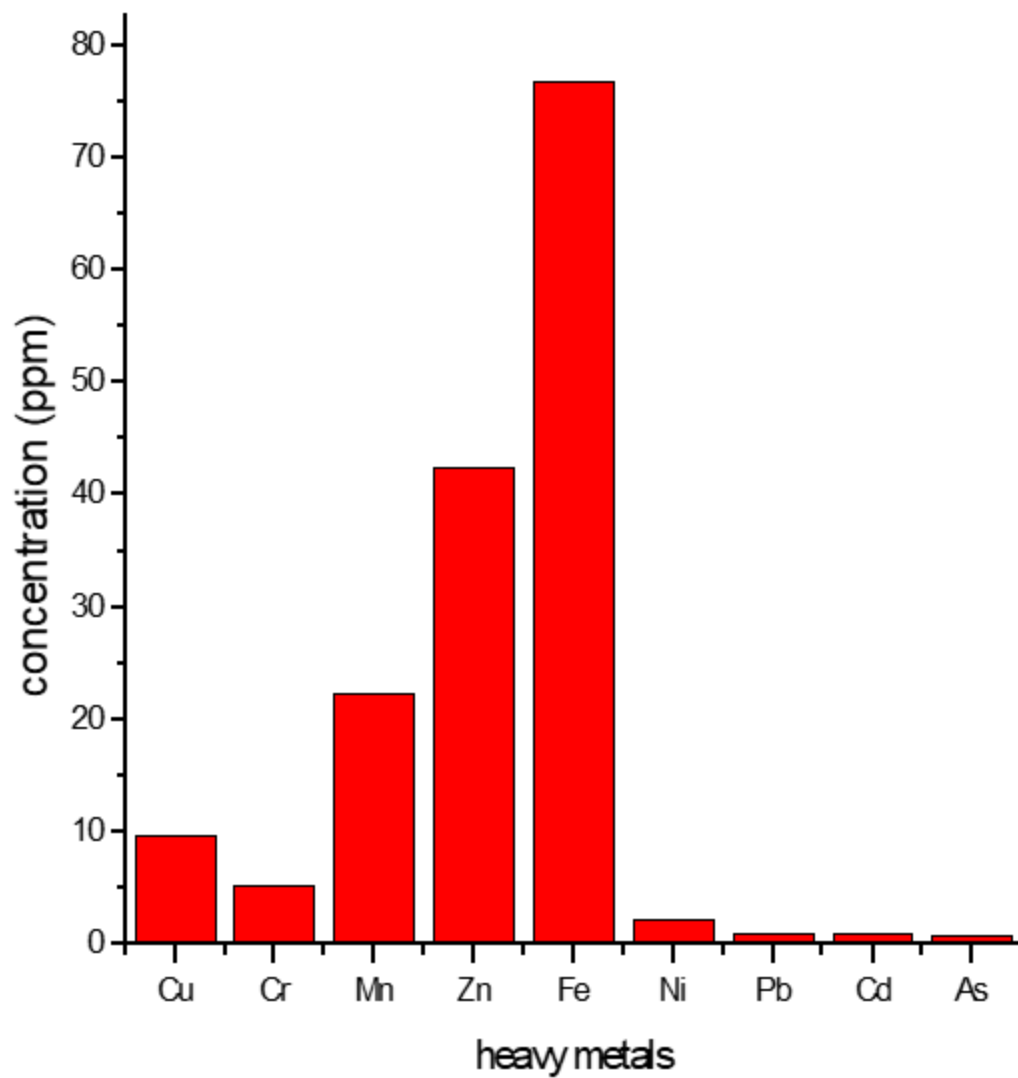


Figure 7

Figure 6: histogram of Heavy Metal and Concentration (ppm) in Cannabis sativa L

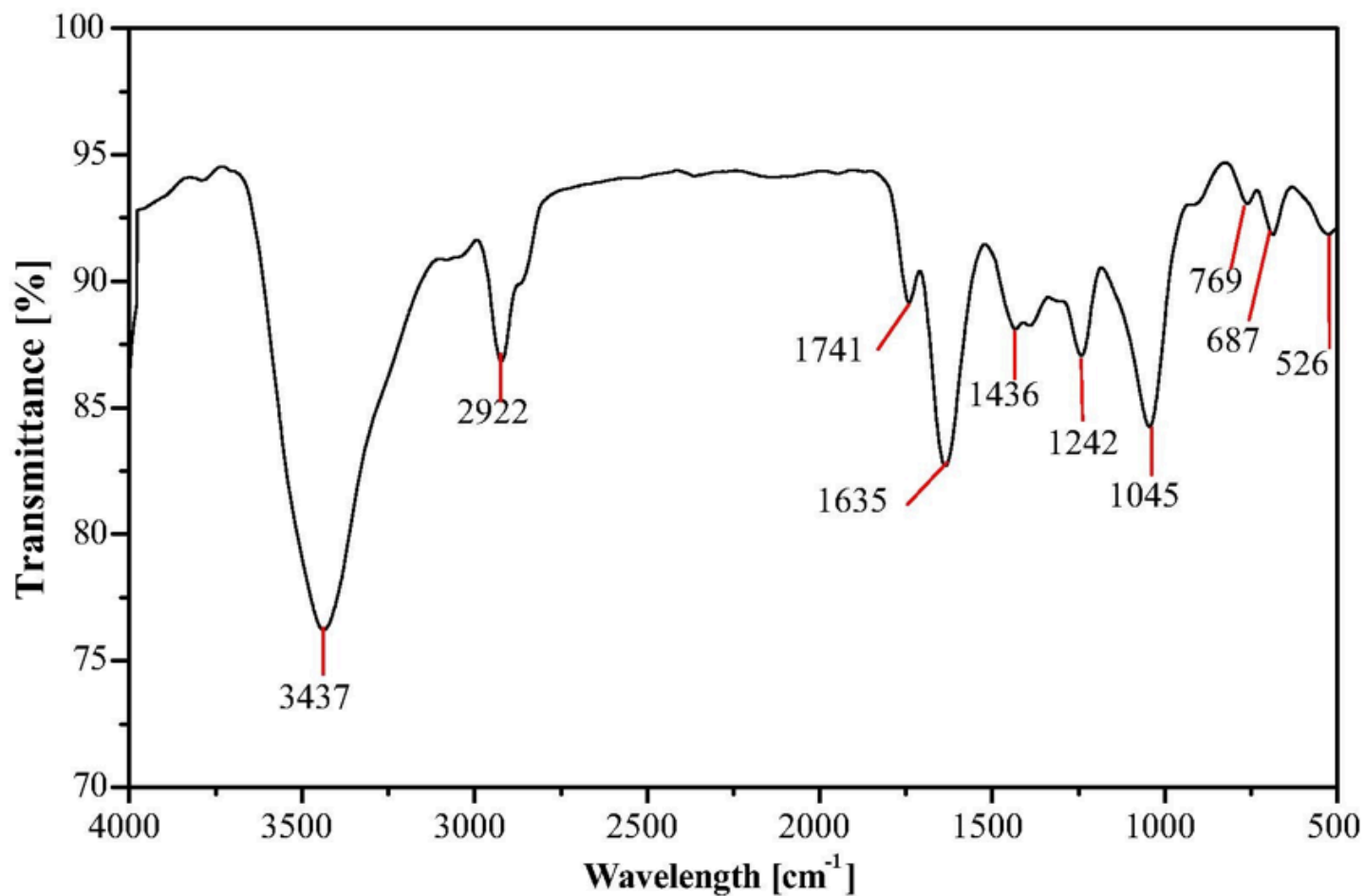


Figure 8

Figure 7: FT-IR spectrum of cannabis sativa extract

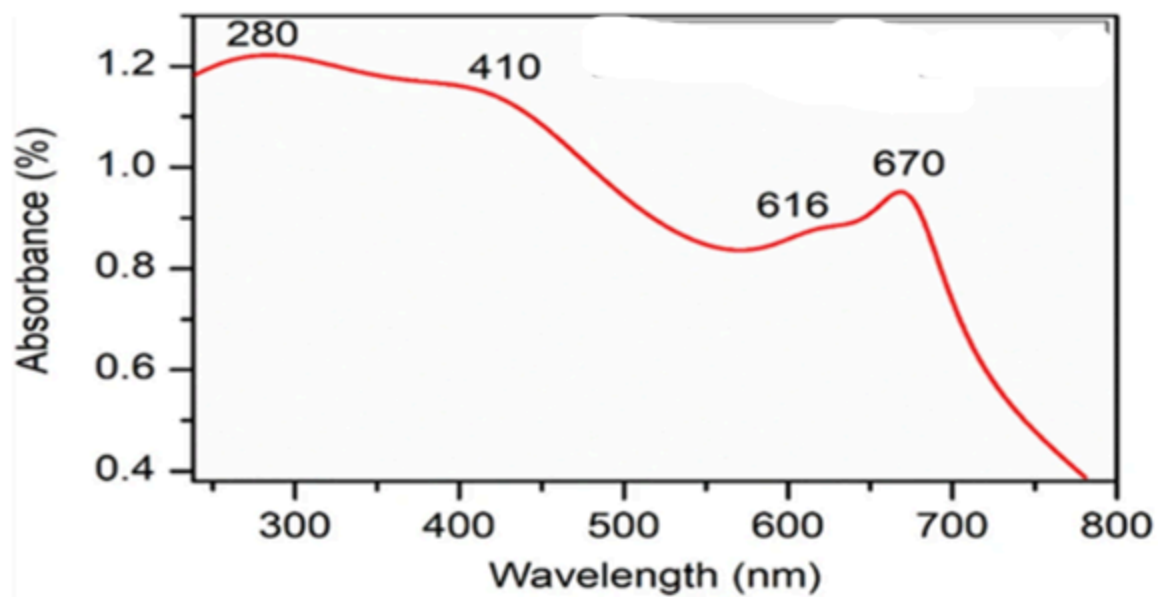


Figure 9

Figure 8: UV–VIS spectrum of *Cannabis sativa* plants in the wavelength range 240–800 nm