Separation of Tetrahydrocannabinol Fraction from *Cannabis indica* Extracts by Chromatographic Method

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ABSTRACT

Tetrahydrocannabinol (Δ9-THC) from *Cannabis indica* leaves was extracted by using solvent extraction method applying condenser apparatus. The molecular and physical attributes of Δ9-THC, or tetrahydrocannabinol, were identified. The collected oil's content and a number of additional variables were studied. The optimum oil yield was predicted. Two samples were produced with multiple parameters. Sample (B) was made by soaking the leaves for seven days, whilst sample; (A) was made by using a condenser the instrument. The derived oil's content and multiple kinds of other factors were studied. For characterization of both samples TLC, GC-MS, IR and HPLC techniques were applied. Various physical and chemical characteristics for Tetrahydrocannabinol (Δ9-THC) oil was analyzed as different values such as acid value, specific gravity, saponification value, refractive Index by titration. Some physical tests were also performed for the detection of proteins, carbohydrates, Steroids, tennins, gums and mucilage. Both samples' results proved wide differences.

Keywords (Δ9-THC), *Cannabis indica*, Tetrahydrocannabinol, HPLC techniques

INTRODUCTION

It is estimated that almost 400,000 species of plants grow on land, 15% of which are yet to be explored[1]. The plants being used are called ethno botanicals and have been suggested by natural selection to be useful in therapeutic concerns with 520 varieties of ethno botanicals built[2]. As they can be found, such resources are important because there are low side effect drugs which can be found cheap, effective and safe to use[3]. Medicinal plants contain Secondary Metabolites which are found in parts such as leaves, stems, barks and roots and they help in the treatment and preparation of drugs[4]. There is a chance that the pharmaceutical industry started with traditional healing use of plants instead because active compounds are extracted and then those compounds are made into drugs[5]. Secondary metabolites are produced by plants to fight specific stresses such as antioxidants, UV absorbing pigments and protection against bacteria and viruses[6]. Flavonoids are a class of secondary metabolites associated with plant structures that provide health benefits, and are critical in the plant's growth, metabolic activities and

thermal tolerance. (Hussain et al., 2018)[7]. Alkaloids, amino acids, and protonal alkaloids are classes of chemicals with grave effects on the gastrointestinal excavation os both man and animals[8]. They are used for provisions for health euphoriants and other purposes. Extractions of the plant materials are carried out with the aid of solvents, temperature, and pressure in order to recover the bioactive components[9]. Hydro distillation is one of the affordable methods of phytochemicals extraction. Certain Green methods such as soxhlet extraction, supercritical fluids extraction, extraction using electromagnetic radiation, and ultrasound-assisted extraction divide water-soluble substances from plant extracts[10]. Others include TLC, HPLC, and affinity chromatography, which are some of the purification techniques used in cannabis with 480 known compounds with some of them having medicinal values[11]. Cannabis sativa, a psychotropic fex exalting plant[12].

REVIEW OF THE LITERATURE

The study of Gupta, Michalski, Vasilakis, and Domb (2018) the plant Cannabis sativa or C. Sativa for short has two subspecies; Cannabis sativa, wide species found mostly across Europe and America including Cannabis Indica, a strains which originated most probably from Afghanistan. Gaoni and Mechoulam (1964) Further analysis of the research explains that Tetrahydrocannabinol also known as THC (D'Souza et al., 2004) has many physiological and psychological effects such as feelings of elation or happiness, anxiety, impairment in verbal working memory, and so on. Studies on the medical use of cannabis in animals and humans have proved the anti-inflammatory, neuroprotective, anxiolytic and antipsychotic effects of the mainly Δ9-tetrahydrocannabinol. Radwan Chandra, Gul and ElSohly (2021) Gaoni and Mechoulam were the first ones to use sophisticated techniques such as IR and NMR spectroscopic techniques, fractional distillation, and sophisticated multiple stationary phases to increase the amounts of psychoactive compound delta 9 THC from the cannabis plant. Schwier (2015) During the 1970s, the United States grabbed cannabis also called Hashish or Indian cannabis from Afghanistan, the first crossbreeding Hybrid, and its origins. (Gloss 2015). These are short and bushy plants that generally occur in regions with sandy loam soils of moderate elevation as indicated by their broad leaves and a thick concentration of THC as opposed to CBD, they are meant for use at night. Flemming, Muntendam, Steup and Kayser (2007) THC, the hallucinogenic element of Cannabis indica, exhibits antinausea, anticancer, and other therapeutic properties for the treatment of glaucoma, pain, multiple sclerosis, and spinal cord injury. Lewis-Bakker, Yang, Vyawahare, and Kotra (2019). A conclusive component for the use of marijuana for medical purposes is Δ9-THC since it is an extract from the plant that is edible to promote the establishment of new industries like the edible film and infused oil industries. Muller-

Vahl, Prevedel, Theloe, Kolbe, Emrich, and Schneider (2003) showed practical examples of medicinal uses of marijuana *(Cannabis indica).*

RESEARCH DESIGN& METHODOLOGY

To begin with the extraction, the cannabis indica leaves were harvested, sorted, dried, and ground with a lot of attention. The quality indices were measured with the help of analytical grade reagents and solvents. The aqueous method of extraction was employed for the tetrahydrocannabinol (THC oil, Δ9-THC) and in this case treatment was done through a lot of filtration and boiling in order to get better yields.

Fig 1. Cannabis plant harvested , sorted and dried

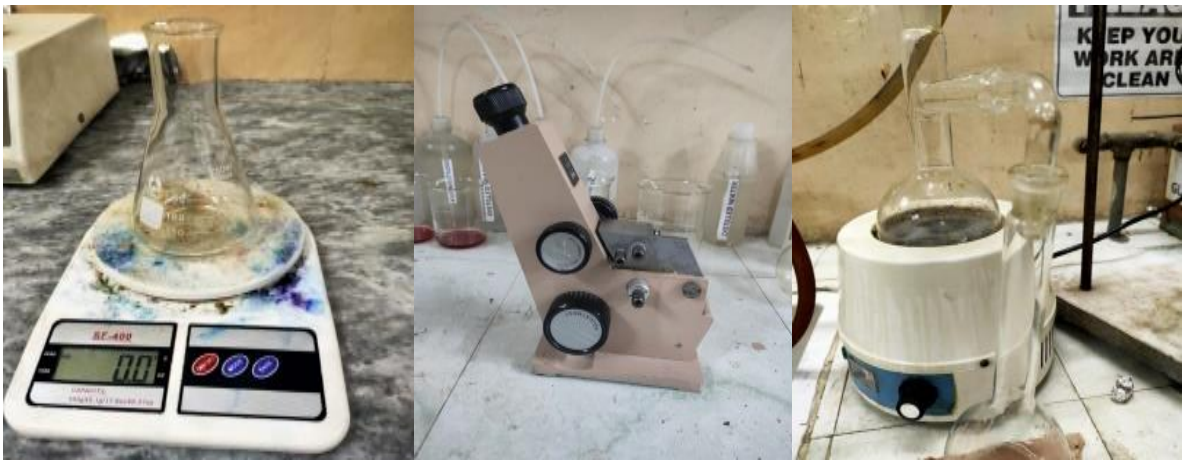

Fig 2 . Aqueous method of extraction

Nevertheless, this approach was considered methodologically incorrect due to poor yield. The later approach worked out in the decompressing and condensing the oil after crushing up the leaves. Where Samples of Cannabis Indica Leaves are sourced from and Processed i.e. cleaned air-dried. Breaking into smaller pieces, the leaves are crushed and methanol added as a solvent. Two forms of sample are gained which are the condensed and the soaked samples.Sample A is produced by adding 5 g of leaves into Duran flasks which covered with aluminum foil for about 7 days. Encore steps are taken to filter out the oil after boiling and drying the sample. Beaker one contains heating ethanol and diethyl ether mixed. The oil is then made to undergo a neutralizing process using ethanolic sodium

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hydroxide, phenolphthalein indicator and stopping the base inflow when dark pink is seen in the solution. The procedure also takes some extra steps in the attainment of the required amount for tetrahydrocannabinol.

Fig 3: Solvent heating of cannabis plant

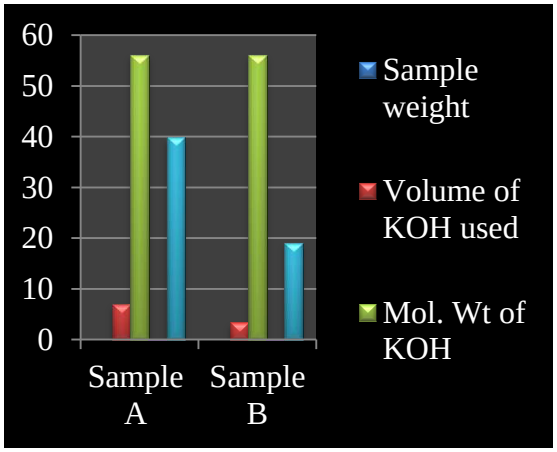
Fig4: Extrated Compound of Cannabis plant

The acid value was computed by use of the expression ($V°/W°$ 2.82.100) and two saponification assessment solutions were prepared. Then, the prepared sample solution was treated to 0.5N HCl solution whereas the control was compensated using the 0.5N HCl solution. The value of saponification was obtained using the Ws equation. The analysis involved the use of a refractometer whose cleaning and calibration involved the use of the previously described cross. The determination of the sample or the oil's density or specific gravity was done using a pycnometer. For the analysis of the liquid, a clean and dry viscosity metric was employed.The liquid portion was passed through multilayered grooved glass filtrate paper.

RESULTS DISCUSSION AND CONCLUSION

Cannabis indica leaf oil was extracted using solvent extraction, with two samples prepared: soaked leaves oil (A) and condensed leaves oil (B). Physical and chemical i

properties were analyzed, including acid value, saponification value, refractive index, and specific gravity for both samples. Acid Value of Sample "A" and sample "B" was titrated with 7.1ml KOH, resulting in acid values of 39.83g and 19.07g respectively, based on the molecule weight of KOH. After that the spherification value of sample A was determined using a 5g sample with a specific gravity of 123.42, while sample B's value was determined using a similar method. At last sample A's specific gravity was determined using empty pycnometer, while sample B's was determined using empty pycnometer, with specific gravity values of 7.85g, 38.39g, and 40.82g, respectively, resulting in12.4. The refractometer lenses were cleaned, methanol and cotton were applied, and 3-4 drops of condensed sample "A" and soaked sample "B" were added, resulting in the refractive index value. Physio-chemical Tests for Tetrahydrocannabinol Oil was shown in Table 4.1.6. Shows the positivity for various tests that were recorded during oil extraction is explained. It indicates that THC oil produced from *Cannabis indica* contains proteins, reducing sugar, alkaloids, steroids, tannin, gums, and mucilage.



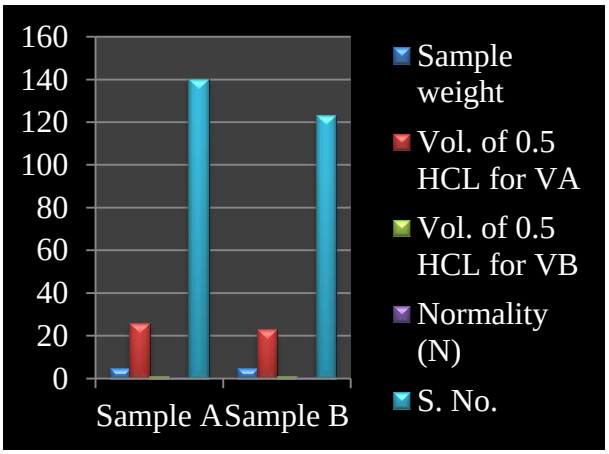
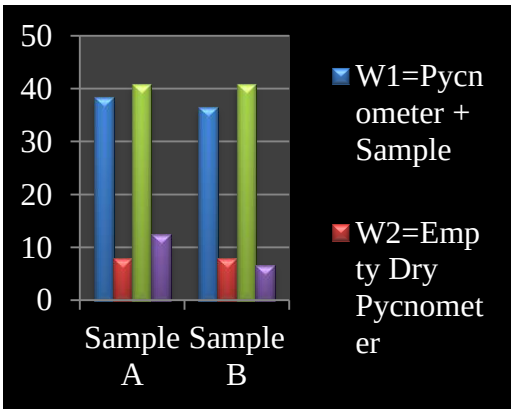


Table (A) Determination of Acid Value of Oil Table (B) Determination of Sponification Value



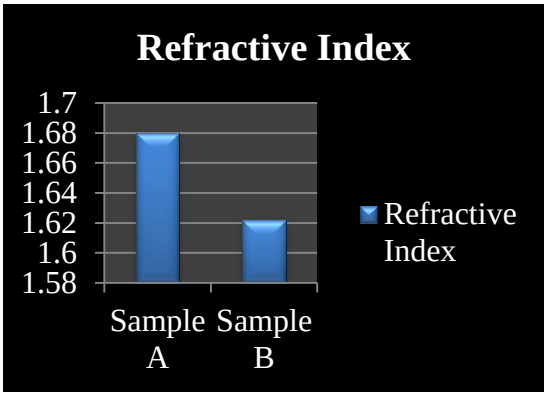


Table. Determination of Specific Gravity Determination of Refractive Index

Physical Tests	Sample A	Sample B
Molish test **(For Reducing Sugar)**	(Violet ring formed) Positive	(Violet ring formed) Positive
Benedict test **(For Reducing Sugars**)	(Reddish orange colored precipitates) Positive	(Reddish orange colored precipitates) Positive
Biuret test **(For Proteins)**	(Cream color) Positive	(Cream color) Positive
Millon's test **(For Proteins)**	(Pink color end point) Positive	(No pink color end point) Negative
Hager's test **(For Alkaloids)**	(Appearance of yellow color) Positive	(Appearance of yellow color) Positive
Feric chloride test **(Test for Tennins)**	(White colored precipitates) Positive	(White colored precipitates) *Positive*
Saponins test **(Test for Alkaloids)**	(Formation of foam) *Positive*	(Formation of foam) Positive
(Test for gums andmucilage)	(Swelling of filtrate) Positive	(Swelling of filtrate) Positive

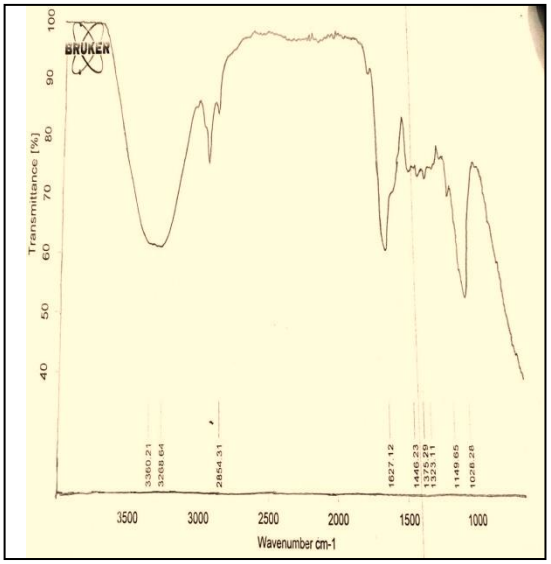
Table. Physio-chemical Tests for Tetrahydrocannabinol Oi

Characterization of Tetrahydrocannabinol Oil

Infra-Red Spectroscopy

The IR chromatogram of "B" sample is illustrated in Fig. 6. A free alcohol functional group is represented by peak 3292.34 cm-1, by intramolecular OH group at 2952.34 cm-1, by alkanes at 2843.11 cm-1, and 1637.06 cm-1 at disubstituted alkenes. Fingerprint region peaks include 1400.91cm-1 which shows an alcoholic group, a secondary alcohol 1102.72cm-1 and a fluoro compound at 1014.98cm-1. Likewise, Haldhar et al., (2021) reported those peaks. Infra-red spectroscopy has been done in order to find out these different functional groups, which are ascribed to sample A. The IR chromatogram of the

sample A is shown in Fig. 4.1. Fig. 1: assigned peak as 3360.21 free alcohol group; 3268.64cm-1-intramolecular- hydroxyl group; 2854.31cm-1-alkane; and 1627.12cm-1 were shown by alkene substituted. Fingerprint region peaks include 1446.23cm-1, 1375.29cm-1, 1323.11cm-1, 1149.65cm-1 and 1028.28cm-1 showed by methyl group, phenol, aliphatic ether and amine group respectively.



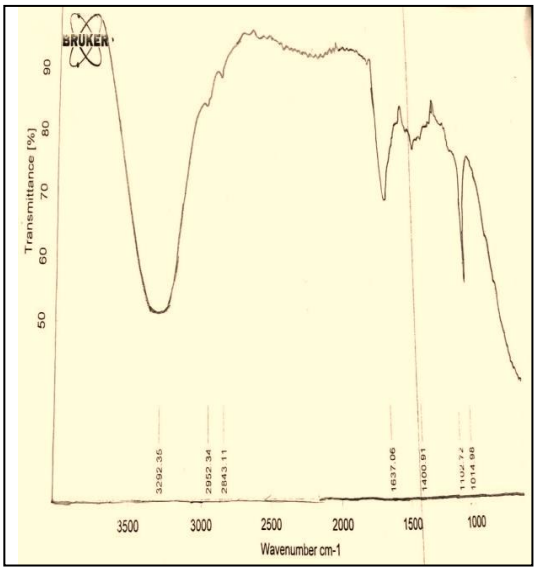
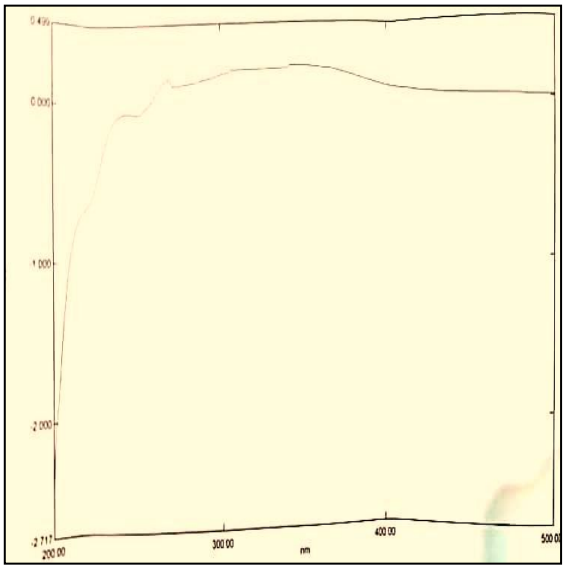


Fig.1. IR-Chromatogram of Sample "A

Fig.2. IR-Chromatogram of Sample "B"

Ultraviolet Spectroscopy

UV spectroscopy was performed to find lambda max of different components present in sample. The UV-chromatogram of sample "A" showed maximum absorbance at 275 nm which matched to literature. The CBD was shown maximum absorbance at 209nm, 256nm and 275nm which showed by Ryu et.al,. (2021). The THC showed maximum absorbance at 276nm and also matched by Peschel and P oliti (2015). The UV-chromatogram of sample "B" showed maximum absorbance at340nm that matched to literature.



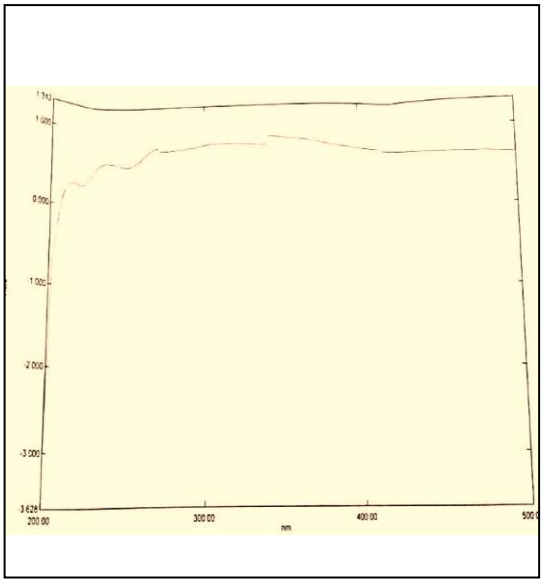


Fig.3. UV-Chromatogram of Sample "A"

Fig.4. UV-chromatogram of sample "B"

Gas Chromatography Mass Spectroscopy (GC-MS)

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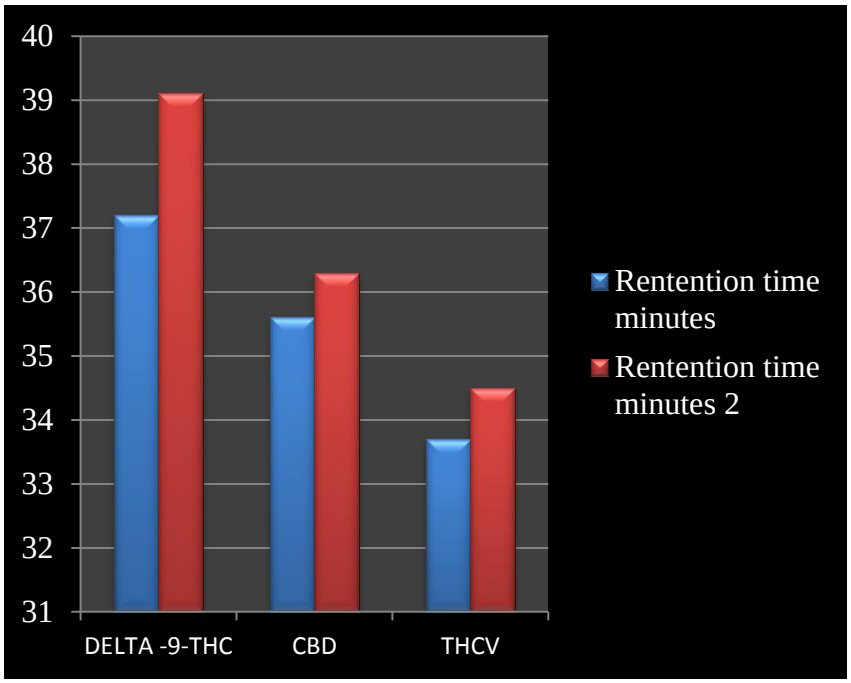
Gas chromatography mass spectroscopy was used to analyze Cannabis extracts, revealing cannabinoids as the most abundant compound with the strongest peaks, along with terpenoids like limonene and caryophyllene.

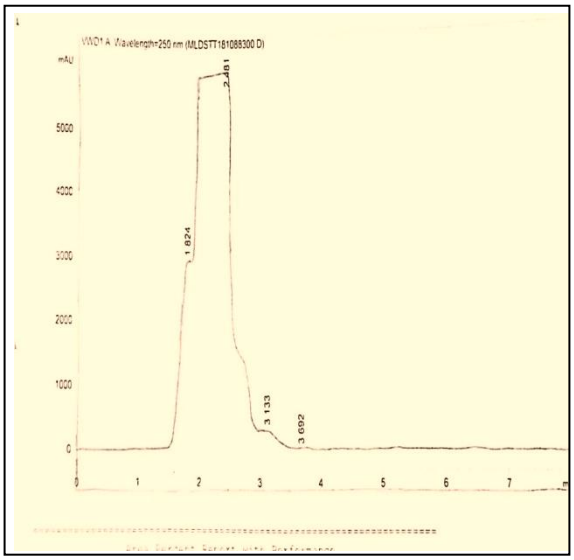
Table . Retention Time for GC-MS



The sample of *Cannabis indica* comprises of numerous phytochemicals but the but its foremost components are THC, CBD and THCV demonstrating the retention time for GC 37.7-39.5 minutes, 35.6-36.3 and 33.7-34.5 minutes. (Brenneisen, 1988) also showed same results.

High Performance Liquid Chromatography (HPLC)

HPLC was performed to qualitative and quantitative analysis of different components present in *Cannabis indica* oil. THC and CBD content in *cannabis indica* leaves were analyzed. The CBD content was 0.92% and THC content was 0.10%.



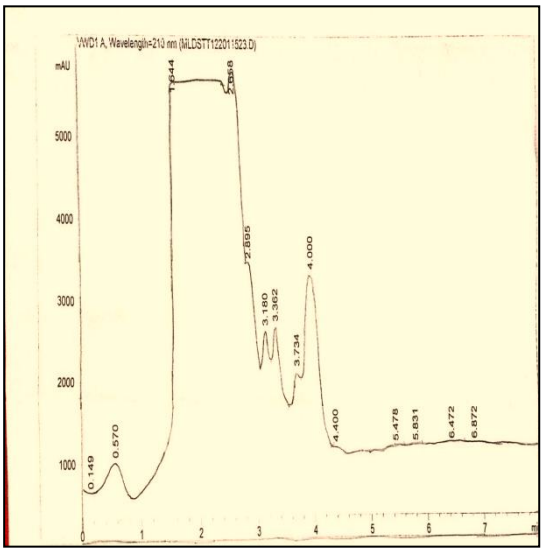


Fig.5. HPLC-chromatogram of sample "A Fig.6. HPLC - Chromatogram of Sample "A"

The different cannabis constituents THC, CBD,THCV and CBDV showed different rentention time as 6.48, 3.51, 3.79, and 2.37 at resolution of 1.89, 1.2, 2.1 and 1.69 respectively and this was the results which resembles shown by Galettis, Williams, Gordon and Martin(2021).

Thin layer chromatography

The results of the thin-layer chromatography revealed six (6) clearly pigmented spots. The R_f values of different cannabinoids is given below in the table.
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The R_f values of different cannabinoids is given below in the table.

Cannabinoids	Rf values	Spot Color
Tetrahydrocannabinol	o.61	Light red
Cannabidiol	0.63	Yellow
Tetrahydrocannabivarin	0.49	Dark red
Cannabinol	0.54	Purple

Discussion

Tetrahydrocannabinol (Δ9-THC) oil was obtained through a solvent extraction procedure that demonstrated its acidic nature. Following alteration, changes in acid and specific gravity were noted. In order to improve the oxidative stability, elementary iodine was incorporated. The highest yield of the oil, 36.77%, was obtained by the solvent extraction method while the aqueous extraction method gave the lowest yield. In addition, the fatty acid profile of the oil was investigated.

Conclusion

A solvent extraction method was employed for the purpose of recovering tetrahydrocannabinol (Δ9-THC) oil from the dried leaves of the plant. Oil yield was found to be approximately 36.77%. The physical and chemical characteristics of tetrahydrocannabinol (Δ9-THC) were assessed. Tetrahydrocannabinol (Δ9-THC) oil was extracted both with and without modifications which included measuring the pH, acid, and saponification values, determining the refractive index, specific gravity, viscosity, and Iodine value of the oil. The values of the mentioned parameters for oil extracted from source stage leaves of the soaked and condensed leaves were studied. 7.5 (PH), and the degree of unsaturation of the oil influences the oil's ability to resist oxidation.

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