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Qualitative Physiochemical and Phytochemical Analysis of Saintly Herb Indian Hemp (*Cannabis sativa L.)* with UV-VIS Spectrophotometer

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Abstract:- The Cannabaceae family includes Cannabis sativa. Carl Linneus made the initial discovery of the species in 1753. Native to Eastern Asia, Cannabis sativa is an annual herbaceous flowering plant that is now widely cultivated and distributed throughout the world. Its phytochemical byproducts, hashish and marijuana, are the most produced and commonly used illegal drugs in Europe and India, where they are also deeply ingrained in Indian culture and religious customs. Although this plant is most well-known for its narcotic qualities, pre-clinical research on hemp derivatives revealed potential anti-oxidative, anti-hypertensive, anti-inflammatory, anti-diabetic, anti-neuroinflammatory, anti-arthritic, anti-acne, and anti-microbial effects. This research presents a comprehensive overview of its phytoconstituents, antioxidants, antimicrobials, and therapeutic features. Considering the numerous significant new discoveries concerning this plant. This plant produces a broad variety of secondary metabolites that have been identified and show an extensive spectrum of biological activity. Cannabidiol (CBD) and delta-9 tetrahydrocannabinol (delta9-THC) are the main components of cannabis that give it its pharmacological effects. To sum up, Cannabis sativa is a researched plant with potential medical uses. With an eye toward the sociolegal context and potential directions for future research, this attempts to bring up to date the existing knowledge and data regarding the use of cannabis and its derivatives.

Keywords:- *Cannabis Sativa, Indian Hemp, Phytochemical, Saintly Herb, Marijuana.*

I. INTRODUCTION

Hemp, a fast-growing industrial strain of *Cannabis sativa*, was initially identified as Cannabis sativa. For thousands of years, people have been growing hemp, which is utilized for biofuel as well as textiles, food, fiber, building materials, paper, and medical applications. Particularly, hemp is produced to have THC contents of less than 0.3%. https://www.thewellnesssoldier.com. Dioecious, annual, blooming cannabis sativa is a herb. Typically, staminate plants are higher than pistillate plants, but they are weaker. Tall stems range in length from 0.2 to 2.0 meters. Nonetheless, most plants only grow to a height of 1-3 meters. (SG Gigliano). Humanity first found fire, which allowed us to burn herbs and incense in a regulated manner for inhalation. It was then that we learned the mysteries of cannabis. The term *Cannabis* (or marijuana) is used when describing a *Cannabis sativa* (HEMP) plant that is bred for its potent, resinous glands (known as trichomes), which contains high amount of THC. THC is an acronym for tetrahydro-cannabinol. (www.thewellnesssoldier.com).The indigenous plant of *Cannabis* are widely found everywhere in India, especially in Uttar Pradesh, West Bengal & Bihar. Hemp is prepared by drying the leaves of the male plants of *Cannabis* and the flowers of the female plants and the resinous substance deposited on the branches and leaves of *Cannabis* is called ‘charas’.

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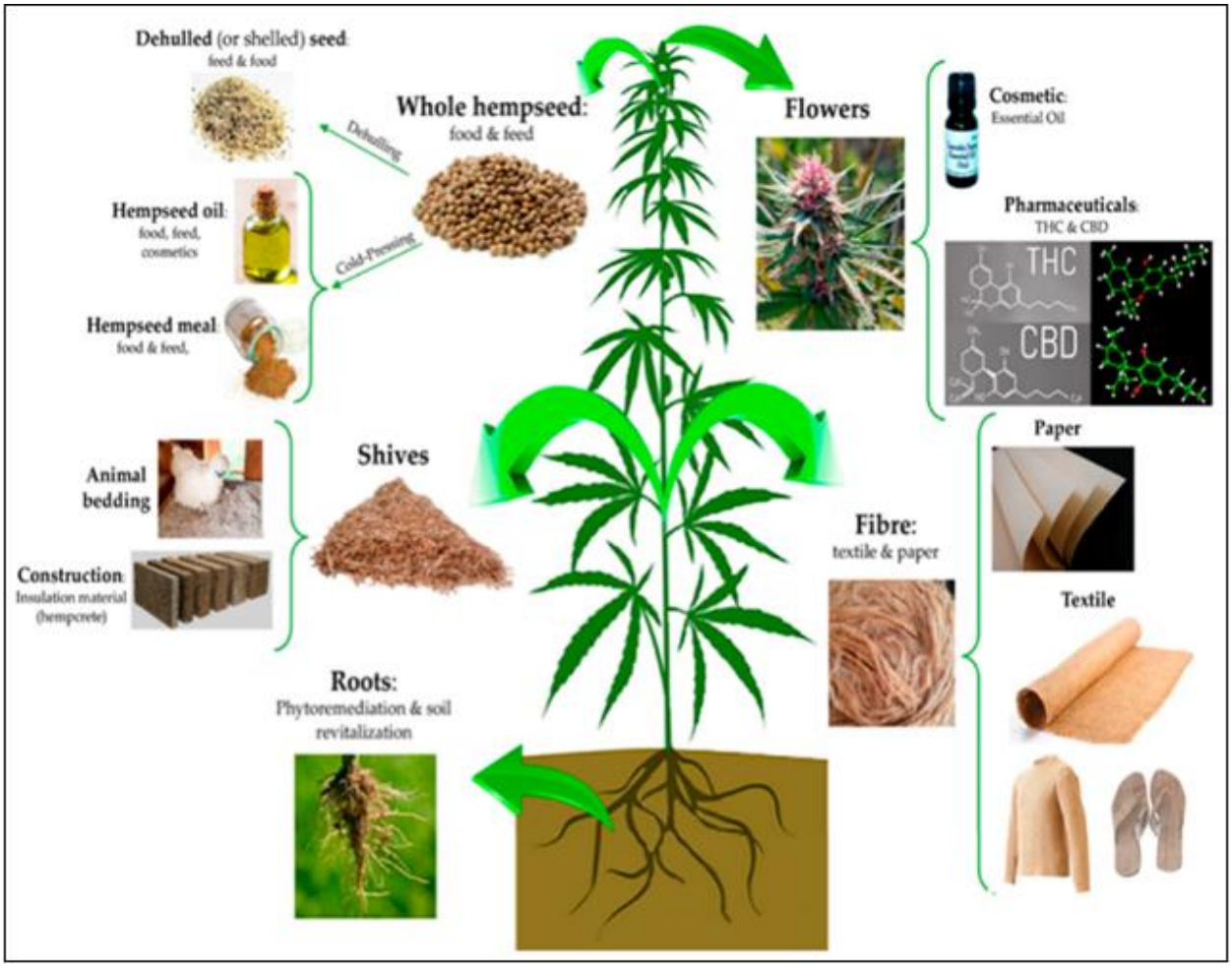

Fig 1 *Cannabis sativa L.* Plant Parts with Diversified Applications

Its fragrant, rosy, rain-fed blossoms reach heights of 3 to 8 feet. The leaves are grouped inversely, with 1-3 segments on the top leaves and 3–8 segments on the lower leaves. Lower leaflets are longer; tiny, greenish-conical, unisexual flowers are present. Autumn brings flowers and fruit, which are little granular fruits that contain flattened seeds. "Cannabinol" is the name of the soft, brown resin found in cannabis. The resin contains a red, viscous fluid that is thick. If left exposed to air, it turns resinous. Other ingredients included in Indian hemp include sugar, volatile oil, and calcium phosphate. It has phlegm-fighting, digestive, bile-stirring, anti-acute heat, sedative, and fire-enhancing properties. Its seeds have digesting properties that stop diarrhea and vomiting. Addiction, memory loss, paranoia, social anxiety disorder, heart damage, lung issues, low testosterone, irregular appetite, increased potency risk, making bad judgments, and hallucinations are some of the negative side effects of marijuana use.

II. MATERIALS AND METHODS

➢ *Preparation of Plant Extracts:*

6 grams of powdered cannabis sativa (leaves and fruits) were weighed. filled a round-bottom distillation flask with 120 ml of methanol. Using a rotary shaker, combine 6 grams of powder with 120 milliliters of methanol for 6-7 hours. After heating the crucible to 600 degrees Celsius in a hot air oven, we now weigh the empty crucible. After removing the sample from the rotary shaker, the extract was filtered using a Buchner funnel and Whatman No. 1 filter paper. Following filtering, the sample is placed in a crucible and placed in a water bath to evaporate completely at a temperature between 60^0 and 80^0C. The crude extract is still in the crucible after evaporation. Methanol was used to dilute the crude extract for analysis.

➢ *Preliminary Phytochemical Screening:*

Phytochemical analysis of all procedure of India Pharmacopoeia (1985). By this analysis the presence of several phytochemicals listed was tested for phytochemicals analysis as follows:

➢ *Qualitative Phytochemical Examination of Extracts:*

- *Detection of Alkaloids:*

Mayer's Reagent: Dissolve 1.36 g of Hgcl2, in 60ml of water and pour into a solution of 5g of KI in 10ml of H_2O, add distilled water to make the volume 100ml (White precipitate with most alkaloids in slightly acid solution). The formation of cream and white precipitate indicated the presence of respective alkaloids.

Wagner's Reagent: Dissolve 1.3g of Iodine and 3g of KI in 100ml distilled water. The appearance of colored precipitates indicated the presence of alkaloids. 2 ml of the

above mixture was treated with 2ml of wagner's reagent. The formation of brownish-red precipitate indicated the presence of respective alkaloids.

- *Detection of Saponins:*

2ml of crude extract was vigorously shaken with the 5ml of distilled water. If foam produced persists for 10 min, indicate the presence of saponins.

- *Detection of Carbohydrates:*

Extracts dissolved individually in 5 ml distilled water and filtered. The filtration was used to test for the presence of carbohydrates.

Fehling's test: Filtrates were hydrolysed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

- *Detection of Resins:*

Acetone- water test: Extracts were treated with acetone .Then small amount water was added and shaken. Appearance of turbidity indicates the presence of resins.

- *Phenol Test: (Mallikharjune LN et.al., 2007, Dey PM & Harbour JB, 1987, Evans WC, 1989):*

The crude extract was mixed with a few drops of 5% mixture of glacial acetic acid and 5% sodium nitrate solution. A muddy yellow, olive brown, Niger brown or deep chocolate colour indicated the presence of phenol.

- *Detection of Tannins:*

Gelatin test: (Mace and Gorbach, 1963) Crude extract was mixed with 2ml of 2% solution of fecl3, a blue green or black coloration indicated the presence of tannins.

- *Detection of Diterpenes:*

Copper-acetate test: The small amount of copper acetate was added in water. Then the extract was treated with few drops of this solution. Appearance of emerald green colour indicates the presence of diterpenes.

- *Detection of fixed oil and fats:*

Stain test: Small quantities of extracts were pressed between two filter paper. An oily stain on filter paper indicates the presence of fixed oil.

- *Qualitative Physiochemical Examination of Powdered Drug of Cannabis Sativa:*

Physiochemical test of powdered drug of *Cannabis sativa* leaves for the presence of secondary metabolites and colour observation showing the following results, when treated with different reagents.

➢ *Thin Layer Chromatography (TLC):*

- *Instrumentation and Experimental Procedures:*

This plant contains phytochemicals, according to the phytochemical analysis. To validate the presence of important groups such as alkaloids, flavonoids, saponins, etc. in the extract, this extract was subsequently submitted to TLC. RF value was used to sort out individual compounds. Dried extracts were redissolved in methanol after the solvent evaporated. The fraction was examined using TLC on a Merck Silica Gel 60 glass plate with various effluents. UV/VIS observations of the chromatograms were made both prior to and following the spraying agent processing. By comparing the flavonoids and phytochemicals to co-chromatographed standards and information from the literature, they were identified (Mabry, T.J. et al 1970). Solution system Water: Methanol: Butanol: Chloroform (10:10:1:6). Light and vapors of iodine. The sample was placed on the plate and allowed to dry for a short while. Then the solvent system was prepared and allowed to stabilize for 10 min. Then the plate was dipped in the solvent chamber and allowed to run up to three forth of the plate. Then it was removed and was air dried. The plate was examined visually.

➢ *Standardization:*

- *Macroscopic Examination:*

✓ Size: A graduated ruler in millimetres was used for measurement of the length, width of crude materials.
✓ Colour: Untreated sample was examined under diffused daylight.
✓ Surface characteristics, texture: The material was touched to determine if it is soft or hard; bended and ruptured to obtain information on brittleness and the plant material were fractured to observe whether material is fibrous, smooth, rough, and granular.
✓ Odour: The material was powdered and the strength of the odour was determined whether (weak, distinct, strong) and then sensation of odour whether (aromatic, fruity, musty, mouldy, rancid etc) was observed.
✓ Taste: The small amount of both plant materials was tasted and observation was taken.

➢ *Microscopic Examination:*

- *Microscopy of the Leaf & Stem:*

Trichomes, stomata, and epidermal cells are crucial leaf-identifying features. The precise nature of the leaf cannot be explored in the transverse slice. Therefore, surface/epidermal exposure becomes crucial for the detailed microscopical analysis. Fresh stem microscopy was investigated. A transverse piece of the stem was obtained and stained with saffranin for microscopy. Sections were captured on photomicrographs. The sections that were manually cut and stained using various reagents were subjected to histochemical examination. The stem was first cleaned with a chloral hydrate solution, then stained for five to ten minutes in 1% saffranin, and finally mounted in 50% glycerine. (K. Nilesh *et al.*, 2011).

- *Qualitative Phytochemical Screening with UV-VIS Spectrophotometer:*

Using a spectrophotometer, the Systronics UV-VIS Spectrophotometer 119, the qualitative analysis of various phytochemicals was performed. The analysis was based on the UV spectra obtained from the absorption maxima at each

wavelength of the phytochemical in question. Pure methanol was used for the calibration. A spectrophotometer was used to screen newly prepared samples after they were placed in a cuvette (Hoover et al., 1987). Samples of Cannabis sativa were subjected to an absorption spectrum scan in the 260–440 nm wavelength range. Based on the phytochemicals' λ-max, the absorbance of the current phytochemicals was noted.

III. RESULT & DISCUSSION

The results of a variety of research were very positive and showed that *Cannabis sativa* has a wide range of therapeutic and antisciatica properties. The different test results that support the phytochemical features are listed below. Currently available are alkaloids, flavonoids, saponins, tannins, and carbohydrates from the preliminary phytochemical investigation. However, anthraquinone is not present in them. Display these secondary metabolites that are concentrated in the plant's leaves.

Shaking and cold extraction-In the beginning, an extraction using water was carried out, using 6.00g of powdered Cannabis sativa leaves and 120ml of methanol. Next, use filter paper to filter. After transferring the filtrate to a crucible, it evaporated, and the final weight was determined.

Table 1 Nature and Percentage Yield of Extracts of *Cannabis sativa*.

Sr. no.	Name of the extract	Nature	Color	%Yield (w/w)
1.	Methanolic extract with Shaking	Shade	Green	17.6%

- Firstly extracts of *Cannabis sativa* were made, which was observed and found that 17.6% methanolic extract from shaking method and dark green in colour.
- STANDARIZATION-Macroscopic Examination

Table 2 Macroscopic Examination-Leaves Powder

Sr. no	Organoleptic Characterization	*Cannabis sativa*
a)	Size	5-10 cm
b)	Surface Characteristics, texture	Opposite arranged, Thick
c)	Taste	Bitter
d)	Color	Green
e)	Odour	Strong odour

Cannabis sativa leaves were analyzed macroscopically to determine their size, odor, texture, taste, color, and surface properties. It was discovered that Cannabis sativa had a thick, rough surface that was arranged in opposition. The leaves were 5–10 cm wide, green in color, and tasted unpleasant.

➢ *Microscopic Analysis:*

A fresh *Cannabis sativa* leaf was examined under a microscope. There were lignified phloem fibers, pitted xylem arteries, simple covering unicellular trichomes on the leaves, and wavy epidermal cells with anomocytic stomata. A microscopy of a fresh Cannabis sativa stem was performed. Its results from the microscopy investigation may help to differentiate it from adulterants and replacements. Microscopic analysis permits a more thorough analysis of unrefined and makes it possible to recognize structured structural elements like the epidermis, starch grains in endosperm, parenchymatous cells.

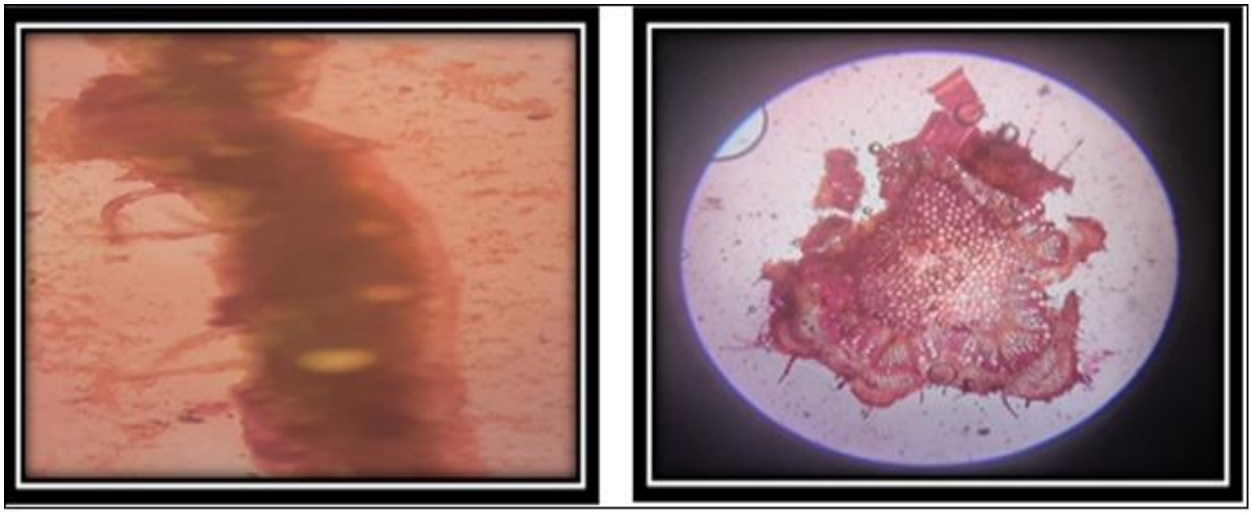

Fig 5 T.S. of *Cannabis sativa* Leaf & Stem

➢ *Preliminary Phytochemical Screening:*
Qualitative Phytochemical Analysis of *Cannabis sativa*

Fig 6 Phytochemical Analysis of Various Phytocomponents of *Cannabis sativa*

Table 3 Details of the Qualitative Phytochemicals Tests

S. No.	Phytochemical Name	Reach or reagents test	Observation	Test Result
1.	**Alkaloids**	Mayer's reagent	Yellow Colour Precipitates	-
2.	**Alkaloids**	Wagner's test	Red Yellow colour	-
3.	**Saponins**	Foam test	Foam produced	+
4.	**Carbohydrate**	Fehling's test	Red precipitates	++
5.	**Resins**	Acetone-water test	Appearance of turbidity	-
6.	**Phenol**	Ferric chloride test	Appearance of the muddy yellow colour	+++
7.	**Tannin**	Gelatin test	Dark black precipitate	+++
8.	**Diterpenes**	Copper-acetate test	Appearance of Emerald green colour	+++
9.	**Oil and fats**	Stain test	Appearance of the oil	+++

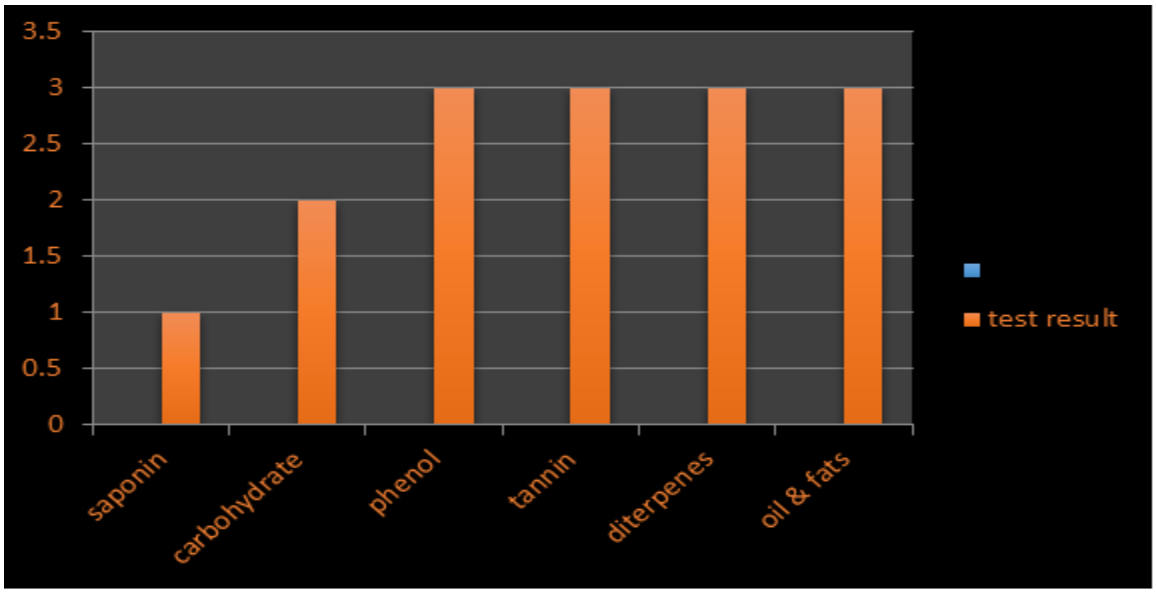

Fig 7 Phytochemical Analysis of Various Phytocomponents of *Cannabis sativa*

➢ *Details of the Qualitative Physiochemical Tests:*

Table 8 Qualitative Physiochemical Analysis of *Cannabis sativa* is as follows:

Sr.no.	Reagents	Color observed	Result
1.	Powder + conc.Hcl	Brownish black	-
2.	Powder + conc.HNO3	Light brownish	+
3.	Powder + conc.H2SO4	Light brick red	-
4.	Powder + Glacial acetic acid	Light brownish	-
5.	Powder + 5% NaOH	Light brownish	-
6.	Powder + 5% KOH	Light brownish	-
7.	Powder + 5% Ferric chloride	Yellow brownish	+
8.	Powder + Picric acid (Aq. Solution)	Yellowish	+
9.	Powder + Ammonia solution	Light brownish	-

(Absent = - , Present = +)

Table 9 TLC with Solvent System Chloroform: Methanol: n Butanol: Water (10:10:1:6)

Sr.no.	Plant species	Extract	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	Colour	R_f value
1.	*Cannabis sativa*	Methanol	0.5	5	Light Orange	0.1
			1.5	5	Light green	0.3
			2.5	5	Light brown	0.5
			4.5	5	Dark brown	0.9

Following the visualization process in a TLC examination using a solvent system, four spots with corresponding RF values of 0.1, 0.3, 0.5, and 0.9 were seen.Cannabis sativa, commonly known as "marijuana," is a plant that is used globally to cure a variety of illnesses. Cannabis sativa is recommended for a number of ailments in Ayurveda. Gather the Cannabis sativa leaves, then dry and ground them. First, decide which study will be conducted using this sample, macroscopic and microscopic. The qualitative phytochemical screening of plant extracts often provides the necessary information about the chemical ingredients for the pharmacological discovery of new medicinal plants. Ascorbic acid, reducing sugar, and proteins were absent from the extracts. In this example, saponins were consistently detected. Only by examining the RF values of components in various solvent systems can the best solvent system for a given plant extract be chosen. Currently, TLC profiling of caudex and leaf plant extracts in various solvent systems revealed the existence of a variety of phytochemical types in these plants. Variations in the compound's RF values also give insight into their polarity. This information will be useful in choosing the right solvent system to further separate the compounds from these extracts of plants.

Table 10 Qualitative Phytochemical Screening with UV-VIS Spectrophotometer

S. No.	Wavelength	Absorbance
1.	260	0.029
2.	280	0.162
3.	300	0.197
4.	320	0.284
5.	340	0.505
6.	360	0.717
7.	380	0.413
8.	400	0.239
9.	420	0.145
10.	440	0.038

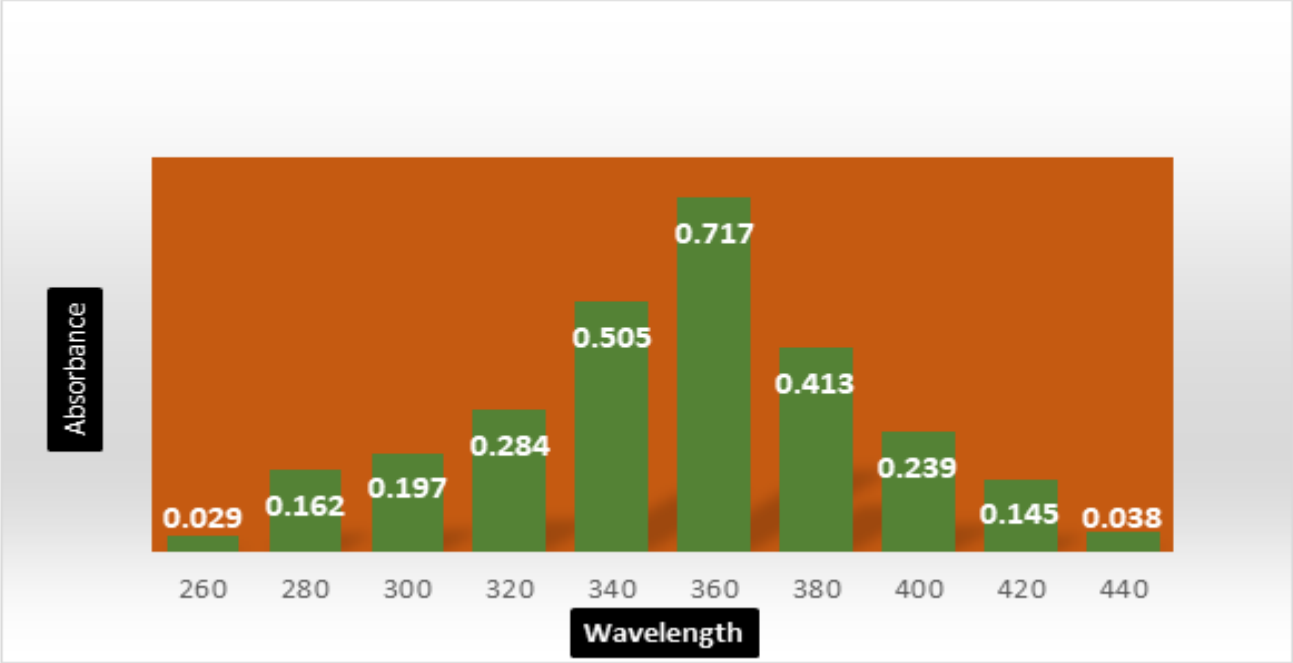

Fig 9 Qualitative Phytochemical Screening of UV-VIS

IV. CONCLUSION

The plant species *Cannabis sativa,* commonly referred to as "hemp," is a member of the cannabinoid family and is used globally to treat a variety of illnesses. Patients receiving treatment with cannabis or cannabinoids had a higher chance of seeing a noticeable improvement in their pain levels. Improved reported complaints by persons with muscle spasms connected to multiple sclerosis have been linked to oral cannabis. Oral cannabis are effective in treating and avoiding nausea and vomiting brought on by chemotherapy. A qualitative screening process was used to look for the following patterns in the samples: phenol, tannin, diterpenes, oil and fats, carbohydrate, and saponins. The results showed a trend towards these patterns. Four spots were seen in the TLC analysis with the solvent system following the visualization step. Whose relative RF values are 0.1, 0.3, 0.5, and 0.9. We looked through online data that indicates a variety of phytochemicals at various wavelengths are present. Saponins, alkaloids, and tocopherol are present in plant extracts' UV-VIS spectra at wavelengths of 260, 280, and 300 nm, with absorbance values of 0.029, 0.162, and 0.197, respectively. In addition, we screened at wavelengths of 340, 360, and 420 nm, where absorbance values of 0.505, 0.717, and 0.145, respectively, indicate the presence of rutin, flavonoids, and chlorophyll. When considering the likelihood and severity of harm associated with many other psychoactive substances that are often used, both legal and illicit, such as cocaine, heroin, amphetamines, alcohol, and tobacco, the harm caused by heavy cannabis users is negligible. Cannabis users who drive under the influence are likely to cause harm to other people. Measuring tools are now available to establish whether a driver is under the influence of cannabis and regulations and enforcement to deter this behaviour should be broadly implemented.

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