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RESEARCH ARTICLE

Phyto-history of Vijaya extracts for its potential Phytocomponents.

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Abstract

Phytochemicals found in extracts of Cannabis sativa indicates their potential as a source of therapeutic constituents that may supply novel medicines. The menthol: acetic acid-based extraction developed here is therefore efficient; and owing to its biodegradability and pharmaceutically acceptable toxicity, it is a safer, more sustainable and greener alternative to common organic solvents for phytocannabinoid extraction. Cannabis species were differentiated on the basis of genetic, morphological and chemotaxonomic variations. The terpenoid content will also differ in different species which is the major phytoconstituent which has immense medicinal properties. Cannabis sativa maximum phytochemicals are soluble in organic solvent. Maximum solubility obtained in Acetic acid, Benzene, Dicholoromethane, Based upon the results of this in vitro investigation, it is referred that Cannabis sativa may considered to be safe for use in humans after other confirmative parameters. It is proposed that deeper investigations must be performed to access the safety and use of Cannabis sativa leave extracts.

PhytochemicalsCannabis sativaterpenoidsolubilityleave extracts

INTRODUCTION

Cannabis is a genus of flowering plants in the family Cannabaceae. The three species of this plant are known which includes Cannabis sativa, Cannabis indica and Cannabis ruderalis. Cannabis sativa may be accepted as single individual species, all the three may be treated as subspecies of a single species, Cannabis sativa (1). The genus is widely accepted as being indigenous to and originating from Central Asia, some consider its origin as South Asia (2). Cannabis is an annual, dioecious, flowering herb. The leaves are palmately compound with serrate leaflets. The plants have imperfect flowers, with staminate "male" and pistillate "female" and flowers occurring on separate plants. Many monoecious varieties have also been described, in which individual plants bear both male and female flowers. There are 157 Cannabis species were differentiated on the basis of genetic, morphological and chemotaxonomic variations. The terpenoid content will also differ in different species which is the major phytoconstituent which has immense medicinal properties. The genus Cannabis was first classified using the "modern" system of taxonomic nomenclature by Carl Linnaeus in **1753** (3, 4).

Historical background: The plant Cannabis, was extensively utilized for its medicinal properties till 19th century after then there was a decline in its medicinal usage due to its emergence as an illegal recreational drug. The Cannabis plant has a history of medicinal use dating back thousands of years across many cultures and it is first referred in Hindu Vedas between 2000 and 1400 BCE, in the Atharvaveda. The plant is used by Sufis and Korakkar Mooli in the Tamil language, meaning Korakkar's herb. Cannabis use eventually became a ritual part of the Hindu festival of Holi. New advances in science in 2013, globally 60,400 kilograms of Cannabis was produced legally (6). In 2014 there were an estimated 182.5 million Cannabis users (3.8% of the population aged 15–64) (7).

Govt. Policies: Now, the narcotics department within the Union Finance Ministry have a research and development

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project on compounds such as Cannabidiol (CBD) and tetrahydro Cannabinol (THC) found in Cannabis, commonly known as ganja. The narcotics department's sanction means that Cannabis will be grown in a monitored way, at the Central Institute of Medicinal and Aromatic Plants (CIMAP) in Uttar Pradesh and Uttarakhand. Under the Narcotic Drugs and Psychotropic Substances Act, 1985, India doesn't allow the cultivation of Cannabis. Cannabis, coca and opium are prohibited for any general cultivation, consumption or possession. But, the Act has always allowed Cannabis to be used only for medical and scientific purposes (8).

Indogenix Biosciences (IBS): We are a Pharmaceutical Company mainly focusing on research and development of Phytochemicals present in Medicinal Plants, and its relation with human endocannabinoid system, with (Drug Controller License no. MP/25D/19/664, Excise License no. BPL/ND/28574/HD-1A/2020 & BPL/ND/34231/HD-1A/2021), "We are using leaves of Cannabis (for medical use) in order to obtain cannabinoids, which are micro-molecules stored by the plant Vijaya. In order to assess the quality of our products, we use an accurate third party testing system that can provide us a clear insight of cannabinoid potency, levels of active components and variety". The present study deals with its different crude phytoconstituents as a potent anticancer agent.

Material & Methods

CHEMICALS: Double Distilled water (Arkray Healthcare Pvt.Ltd), Silica Gel 100-400 mesh, 200-400 mesh & Reactor grade (Swambe Chemicals),Ethyl alcohol (Analytical), n-Hapten (Qualigens), DMSO (Himedia), KCl (Himedia), NaCl (Himedia), Acetic acid (Rankem), Methanol (Fisher Scientific), Edible Ethanol, n-Hexane (Fisher Scientific), Chloroform (Rankem), Dimethyl Sulphoxide (Fisher Scientific), Toluene (Himedia), Ethyl acetate (Himedia), Ammonia ((Fisher Scientific) , B enzene (Qualigens), D iethyl e ther (Fisher Scientific),, 1N Hcl (Qualigens), N.Saline (Jedux), Acetone (Himedia), Isopropyl alcohol (Himedia), Dichloromethane (Himedia), Pet Ether (Fisher Scientific), M ayer's r eagent, Wagner'sreagent (Sunchem), Dragendorff's reagent (Sunchem), Borntrager's reagent (Sunchem), Ferric chloride (CNH), Lead acetate (Qualigens), NaoH (Himedia), H_2SO_4 (Himedia), Molisch's reagent (Sunchem), Acetonitrile (Spectrochem), 1,4-Dioxan (Oxford).

INSTRUMENTS REQUIRED:

Laminar Air flow (Jindal), Hot air oven (Scintech), Electronic balance (Precision Series), Hot plate (Scintech), Deep freezer -20 (BOSCH- R600), Water bath (Scintech), Micropipettes of different variance & Multi channel (Himedia), RotaVap (Thermo Scientific™), Savant™ SpeedVac™.

GLASSWARE:

Glass bottles 50ml,100ml, 250ml, 500ml & 1000ml (Borosil), Test tubes 15ml (Borosil), Beakers 20ml, 50 ml, 250ml, 500ml, 1000ml, 5000 ml (Borosil), Conical flask 250, 500, 1000ml(Borosil), Petri plates of different size (Borosil), Separating funnels 250ml, 500ml,1000ml (Borosil), Measuring cylinders 100 ml, 1000ml (Borosil), TLC Chamber with lids (Borosil), Chromatography Clomuns 600X40 mm (Borosil), Watchglass (Borosil). Etc.

Other requirements:

Disposable gloves (Ansell, Nytrile), Disposable syringes (Dispovan), Surgical face mask & Head cover (Medical use), Micropipette with tips (0-100μl & 100-1000μl) (Thermo Scientific), TLC silica gel 60 F245 Plates (Merck).

EXTRACTION:

Extraction techniques are used to separate components of Cannabis and remove them from the plant matrix. With Cannabis, extraction techniques are often used to isolate specific desirable compounds, and Cannabis contains at least 1123 cannabinoids including cannabidiol (CBD) and tetrahydrocannabinol (THC) (9).

Following steps are involved in the extraction of components from Cannabis leaves:

Collection of Cannabis leaves by Indogenix Biosciences: The Cannabis leaves will be procured for medicinal preparations from Madhya Pradesh State Excise Department under the following Indogenix Bioscience LLP; Excise License no. BPL/ND/34231/HD-1A/2021) and (Drug Controller License no. MP/25D/19/664)

Various methods can be used for this:

1. Cannabis alcohol extraction: Several common forms of Cannabis rely on solvent such as alcohol. In brief, the Cannabis soaks in alcohol **(Edible Ethanol),** the plant material is then removed, the liquid filtered, and the alcohol is removed with some form of evaporation like using Rota Vap (Thermo Scientific) and Speed Vac™, extracted by Indogenix Bioscience LLP, extracted crude (VIJAYA) **50** grams received with given code (Laboratory code: INDO-DRUG for Indogenix internal code: IPV4000).

2. Extraction of cannabinoids from Cannabis sativa leaves is performed by solvent extraction method. (This Solvent process is validated & approved by M. P Excise department & Indian Govt. approved Laboratories for Indogenix Biosciences). Briefly **100** grams of dried leaves (Biomass) of Cannabis sativa was course powered by grinding in a mixer then Macerated with inorganic solvents i.e.- **(Acetic Acid: Methanol)**at molar ratio (1:1)

in a well closed separating funnel and kept this funnel for seven days at ambient temperature, during this period shaking is done occasionally. After that extract solution was collected and concentrated using water bath. Then collected extract was air dried at room temperature for complete solvent evaporation and final extract given names are (Laboratory code: JNCH-RAW & INDO-RAW & Indogenix internal code: XRV4000). The percentage yield was calculated of all collected extract (Table No: 01).

3. All of the residues collected from the above method are suspended again with same solvent system and aged it for 6 months in a well closed separating funnel and kept this funnel for 6 months at ambient temperature, during this period shaking is done occasionally. After that extract solution was collected and concentrated using water bath and final crude extract given name was JNCH-AGED.

SOLUBILITY STUDY:
Solubility study of crude extracts (Lab code: INDO-DRUG, JNCH-RAW, INDO-RAW & JNCH-AGED) was tested with various solvents. It was determined by taking definite quantity (5mg) of extract in 10 ml of solvent at room temperature in test tubes and well solubilizes by shaking. The solubility was observed by visual inspection (Table No: 02).

PHYTOCHEMICAL SCREENING OF CANNABIS SATIVA:
The extracts are tested for the presence of bioactive compounds by using the following standard methods and there results are given in (Table No: 03).

Test for Alkaloids:

Mayer's reagent: A portion of extract was treated with few drop of diluted hydrochloric acid and 0.5 ml of Mayer's reagent. If white precipitate observed it indicates the presence of alkaloid.
Wagner's Reagents: Few drops of diluted HCl and 0.5 ml Wagner's reagents has added to a portion of extract. A brown flocculent precipitate indicates the presence of alkaloids
Test for Steroids:
Salkowski Test: To 2 ml of extract, add 2 ml of chloroform and 2 ml of conc. H2SO4.Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
Libermann-Burchard reagent: Another test was performed by mixing the crude extract with 2 ml of chloroform. Then 2 ml of concentrated H2SO4 and acetic acid was poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for Terpenoids:
Salkowski Test: Crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 3 ml of concentrated H_2SO_4 was added and heated for about 2 min. A gray color indicated the presence of terpenoids.

Test for Flavonoids:
Lead Acetate Test: To small quantity of extract (50 mg), add lead acetate solution. Yellow colored precipitate is formed indicate the presence of flavonoids.
Alkaline Test: 2ml of extract with addition of increasing amount of NaOH to the residue show yellow coloration which decolorizes after addition of HCL. Indicates the presence of flavonoids.

Test for Saponins:
Foam test: To 1 ml of extract was dissolved in 5 ml of Distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth with honeycomb structure indicates the presence of Saponins.

Haemolytic Test: Add small quantity of extract to one drop of blood, placed on glass slide. Presence of haemolytic indicates the presence of Saponins.

Test for Tannins and Phenol Compounds:
Ferric Chloride: Small quantity of extract (50 mg) was mixed with water and heated on water bath. The mixture was filtered and ferric chloride (FeCl3) was added into the filtrate. A dark green solution indicates the presence of Tannins.

Lead Acetate: The extract (50 mg) was dissolved in Distilled water and added 3 ml of 10% lead acetate solution. If bulky white precipitate observed this is indicated the presence of phenol compound.

Test for Lipids:
Solubility Test: Oils are soluble in ether, benzene and chloroform, but insoluble in 90% ethanol and in water.
Spot Test: A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicted the presence of fixed oil.

Test for carbohydrate:

Molisch reagent test: To 2 ml of extract add 2 drops of molisch reagents and mix it properly. Then added slowly conc. H2SO4 in sloping the test tube by its aside without mixing. A purple ring at the interface between the acid and test extract layer which confirm the presence of carbohydrate.

THIN LAYER CHROMATOGRAPHIC ANALYSIS

TLC analysis was done for separation and identification of component from a mixture of component. So for this we used readymade TLC Silica gel 60 F254 Plates (Merck Chemicals Pvt. Ltd. Mumbai). Plates were loaded by extract with the help of capillary tube. The plates were dried and placed into a TLC chamber containing different combination of mobile phase. Chamber was pre saturated with solvent system. In this study we used four different solvent system (S1) Acetic Acid: Methanol (1:1), (S2) Hexane: Dioxane (90:10), (S3) Toluene: Chloroform: Methanol (10:1:1), and one more solvent system (S4) Ethyl Acetate: Methanol: Ammonia (5:2:3). TLC plates are exposed in chamber and when the solvent reached up to 2/3 of plate, these plates were removed from chamber and the Rf value was calculated, and compared with reference cannabinoids Rf value (10) and the calculated Rf values are given in (Table No: 04).

COLUMN CHROMATOGRAPHY

For the separation of phytoconstituents from the mixture of component was done by using Column chromatography with column size

Weight of crude extract obtain: **8.34** grams. (600X40 mm). Silica gel with (100-400 mesh) was used as a stationary phase and mobile phase was selected on the basis of nature of stationary phase.

The slurry of silica gel was made with a relevant solvent by using motor pestle and placed in a column that is plugged at the bottom by glass filter. The extract to be separated was dissolved in a solvent (100 mg/ml) and introduced at the top of the column and allowed it to pass through the column. The different fractions of components were collected separately by adding more solvent at the top of column. To evaporate the solvent from the different fractions collected separately details are given in (Table No: 05).

OBSERVATION TABLE:

PERCENTAGE YIELD: After cleaning and processing of 100 grams of *Cannabis sativa* leaves, percentage yield of extract was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of dried leaves}} x100$$

Calculation:

Weight dry leaves: **100** grams.

Weight of coursed powder of leaves: **96** grams.

TABLE NO.1: PERCENTAGE YIELD

Solvent system used	Sample Code	Weight of powdered leaves	Weight of crude extract obtained	% Yield Crystals
Acetic Acid: Methanol (1:1)	JNCH-RAW	48 gms	2.70 gms	5.62%
	INDO-RAW	48 gms	2.19 gms	4.56%
	JNCH-AGED Re- suspended Marc material of JNCH-RAW & INDO-RAW	-do- 96 grams 'Marc' material obtained from above groups was aged for 6 months in same solvent system.	3.45 gms	3.59%

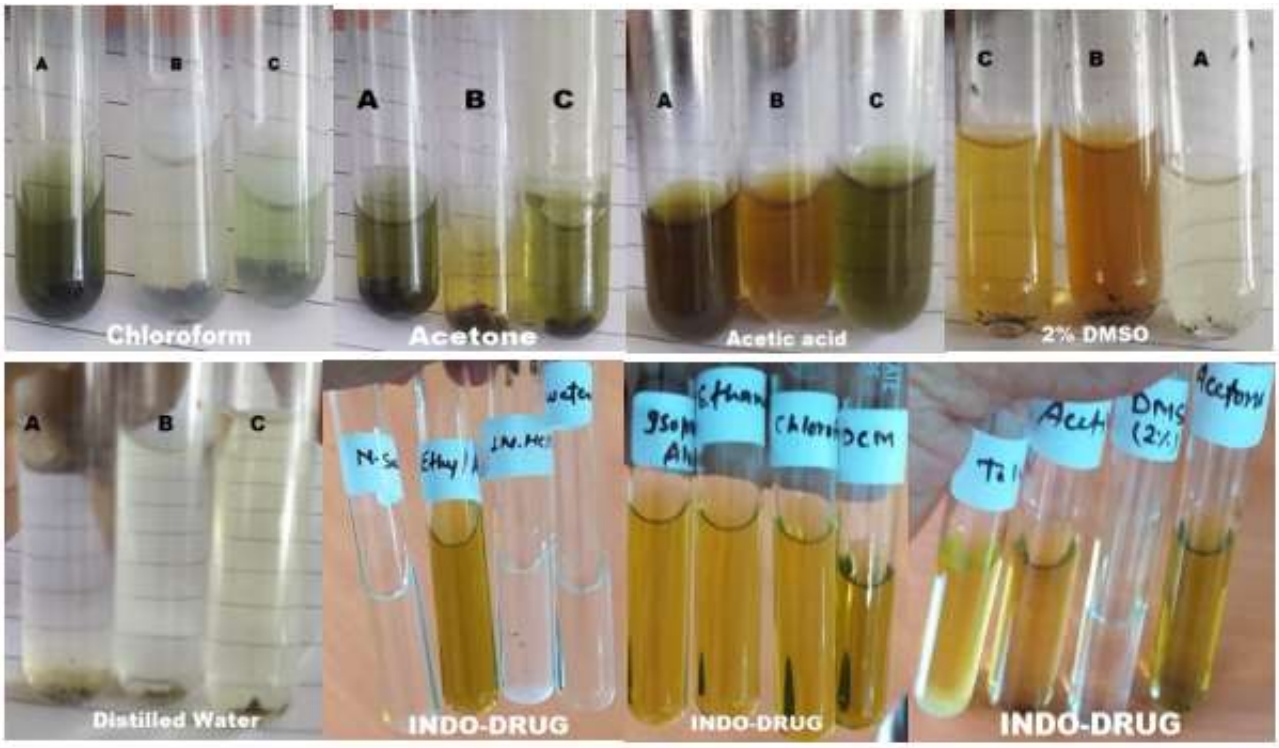
Note: Percentage yield of *Cannabis Sativa* leaves obtained after processing of 100 grams leave is **8.68** % crude extract as crystal form is obtained.

TABLE NO. 2: Solubility test result of crude extract crystals by using following solvents-

S.no.	Chemicals used	JNCH AGED	JNCH RAW	INDO RAW	INDO DRUG
1.	Benzene	++++	+++	+++	++++
2.	n-Hexane	++++	--	+++	+++
3.	Methanol	+++	+++	++++	++
4.	Acetic acid	++++	++++	++++	++
5.	2% DMSO	--	++	++	--
6.	Chloroform	+++	--	++	+++
7.	Distilled water	+	+	+	--
8.	Diethyl ether	++++	--	+++	+
9.	Ethyl acetate	++	--	+	++++
10.	1N HCL	--	++	++	--
11.	50%Edible Ethanol	++	+++	+++	++++
12.	N. Saline	--	++	++	--
13.	Acetone	+++	++	++++	++++
14.	Isopropyl alcohol	++	++	+++	+++
15.	Dichloromethane	++++	+++	++	++++
16.	Toluene	++++	+++	++	+++
17.	N-heptane	++	++	++	++
18.	Pet-ether	+	--	++	+++

Note: Following symbols are used to indicate the solubility:Freely soluble + + + +

- Soluble + + +
- Sparingly soluble ++
- Slightly soluble +
- Insoluble --



Images No: 01

Photograph showing solubility of following samples- **A**: JNCH-RAW, **B**: JNCH-AGED, **C**: INDO-RAW and **INDO-DRUG**, in Chloroform, Acetone, Acetic acid, 2% DMSO, Distilled water and other solvents shown in (Table No: 02).

TABLE NO. 3: PHYTOCHEMICAL SCREENING DATA

PHYTOCONSTITUENT	TEST PERFORMED	INDO DRUG	JNCH AGED	JNCH RAW	INDO RAW
		Edible Ethanol	Acetic acid + Methanol Extracted		
Alkaloids	**Mayer's reagent test**	+	+	+	+
	Wagner's reagent test	+	+	+	+
Lipids	Solubility test	-	+	-	+
	Spot test	-	-	-	-

Tannin and Phenol	Ferric chloride	+	-	-	+
	Lead acetate	+	-	-	+
Flavonoids	Alkaline test	+	-	+	+
	Lead acetate	+	-	+	+
Steroids	Salkowski test	-	+	-	-
Saponins	Foam test	-	+	+	+
	Hemolytic test	-	+	+	+
Terpenoids	**Salkowski test**	+	+	+	+
Carbohydrate	Molisch test	-	-	-	-

Following symbols are used: Positive test : + sign, Negative test : - sign

Note: Qualitative screening of phytochemical present in *C. sativa* leaf extract revealed the presence of Phenols, Tannins, Flavonoids, Saponins, Steroids, Terpenoids, and Alkaloids.

Images No: 02

Showing Biochemical test results of samples (Lab Code: INDO-DRUG, JNCH-RAW, INDO-RAW & JNCH-AGED) for Flavonoids, Saponins, Steroids and Tannins with other test.

TABLE NO. 4: Observed TLC with calculated Rf values

Table: 4.1, With solvent system Acetic acid: Methanol

Solvent System: 01	**Acetic Acid: Methanol (1:1)**		
	RF. Value B-1	**RF. Value B-2**	**RF. Value B-3**
JNCH RAW	0.828	0.89	0.968
JNCH AGED	0.781	0.875	0.968
INDO RAW	0.833	0.916	-
INDO DRUG	0.625	0.833	0.937

Table: 4.2, With solvent system Hexane: Dioxane (9:1)

Solvent System: 02	Hexane: Dioxane (90:10)			
	RF. Value B-1	RF. Value B-2	RF. Value B-3	RF.Value B-4
JNCH RAW	0.114	0.142	0.285	-
JNCH AGED	0.142	0.228	0.314	-
INDO RAW	0.176	0.228	0.342	0.457
INDO DRUG	0.194	0.222	0.361	0.416

Table: 4.3, With solvent system Toluene:Chloroform:Methanol (10:1:1)

Solvent System: 03	Toluene: Chloroform: Methanol (10:1:1)			
	RF. Value B-1	RF. Value B-2	RF. Value B-3	RF.Value B-4
JNCH RAW	0.691	0.932	0.186	-
JNCH AGED	0.691	0.162	-	-
INDO RAW	0.217	0.482	0.162	-
INDO DRUG	0.452	0.921	0.113	0.227

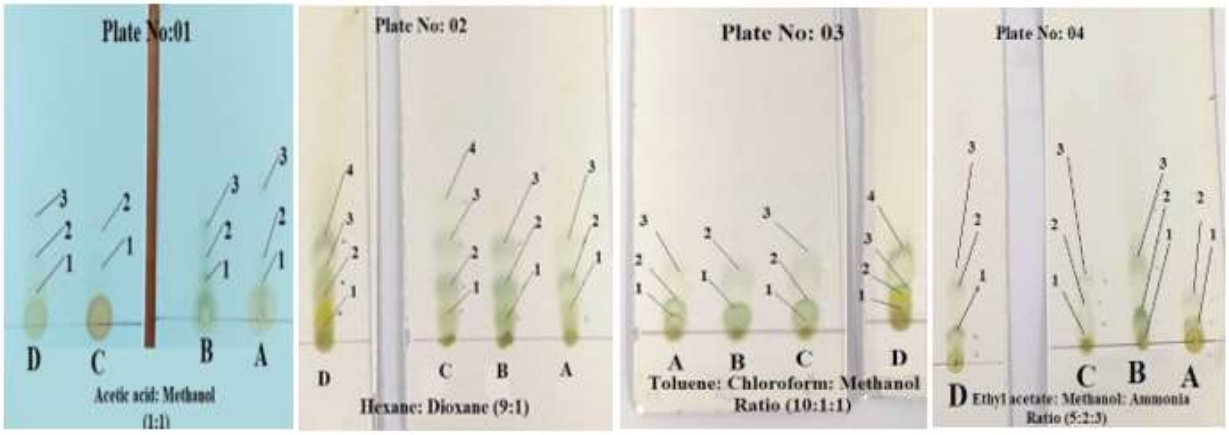
Table: 4.4, With solvent system Ethyl Acetate: Methanol: Ammonia (5:2:3

Solvent System: 04	**Ethyl Acetate: Methanol : Ammonia (5:2:3)**		
	RF. Value B-1	**RF. Value B-2**	**RF. Value B-3**
JNCH RAW	0.305	0.855	0.982
JNCH AGED	0.31	0.843	-
INDO RAW	0.354	0.981	0.984
INDO DRUG	0.826	0.845	0.421

Table No.4.5: Standard Rf values of different Cannabinoids by using Hexane: Acetone (87:13)

S No.	Cannabinoids	Average RF	(+/- Standard Deviation)
1	8-THC	0.390	(+/- 0.014)
2	9-THC	0.336	(+/- 0.017)
3	CBD	0.305	(+/- 0.012)
4	THCV	0.297	(+/- 0.013)
5	CBC	0.271	(+/- 0.015)
6	CBDV	0.257	(+/- 0.008)
7	CBN	0.254	(+/- 0.013)
8	CBG	0.151	(+/- 0.008)

Note: Furthermore calculation of Rf values concluded that the chances of change in Rf value for Cannabinoids with different solvent system, Fine separated bands are obtained with above given Rf values indicating that we have around same Rf values as compare to the published data Rf value shown by (10)



Images No: 03 Showing TLC silica gel plates 60 F245

Plate No: 01- Samples A,B,C & D (Lab code: JNCH-RAW, JNCH-AGED, INDO-RAW & INDO-DRUG respectively) Sample: A,B & D shows 3 separated bands & sample C have 2 fine bands.
Plate No: 02- Samples A,B,C & D (Lab code: JNCH-RAW, JNCH-AGED, INDO-RAW & INDO-DRUG respectively) Sample: A & B shows 3 separated bands & sample C & D have 4 fine bands.
Plate No: 03- Samples A,B,C & D (Lab code: JNCH-RAW, JNCH-AGED, INDO-RAW & INDO-DRUG respectively) Sample: A & C shows 3 separated bands & sample B have only 2 bands, sample D have 4 fine bands.
Plate No: 04- Samples A,B,C & D (Lab code: JNCH-RAW, JNCH-AGED, INDO-RAW & INDO-DRUG respectively) Sample: A shows only 2 separated bands & sample B, C & D have 4 fine bands.

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Table No.5: COLUMN CHROMATOGRAPHY DATA

COLUMN NO.	SAMPLE	FRACTIONS OBTAINED						
01	**INDO RAW**	**F1**, 150 ml, Bottle green	**F2**, 100 ml, Brown	**F3**, 60 ml, Off brown	**F4**, 80 ml, Pinkish brown	**F5**, 110 ml, Baby pink	**F6**, 65 ml Bottle green	
02	**INDO DRUG**	**F1**, 100 ml, Brown	**F2**, 220 ml, Dark green,	**F3**, 200 ml, Bottle green	**F4**, 50 ml, Light green	**F5**, 100 ml, Off green	**F6**, 56 ml Pinkish green	**F7**, 70 ml light green
03	**JNCH RAW**	**F1**, 100 ml Dark green	**F2**, 150 ml Bottle green/ Brown	**F3**, 100 ml Brownish/red	**F4**, 100 ml Light green	**F5**, 80 ml, yellow	**F6**, 45 ml Pinkish green	
04	**JNCH AGED**	**F1**, 100 ml, Dark Bluish green	**F2**, 75 ml, Bottle green	**F3**, 90 ml, Dark green	**F4**, 55 ml, Bottle green	**F5**, 60 ml light yellow		

Note: Column chromatography with glass column size (600X40 mm). Silica gel with (100-400 mesh) used for the separation of crude extracts given in (Table No.5). The elution was fractionated on the basis of color and evaporated at ambient temperature, the final extract was stored at 4°C for identification from methods like GC, GC-MS, HPLC for expanded result and further parameters.

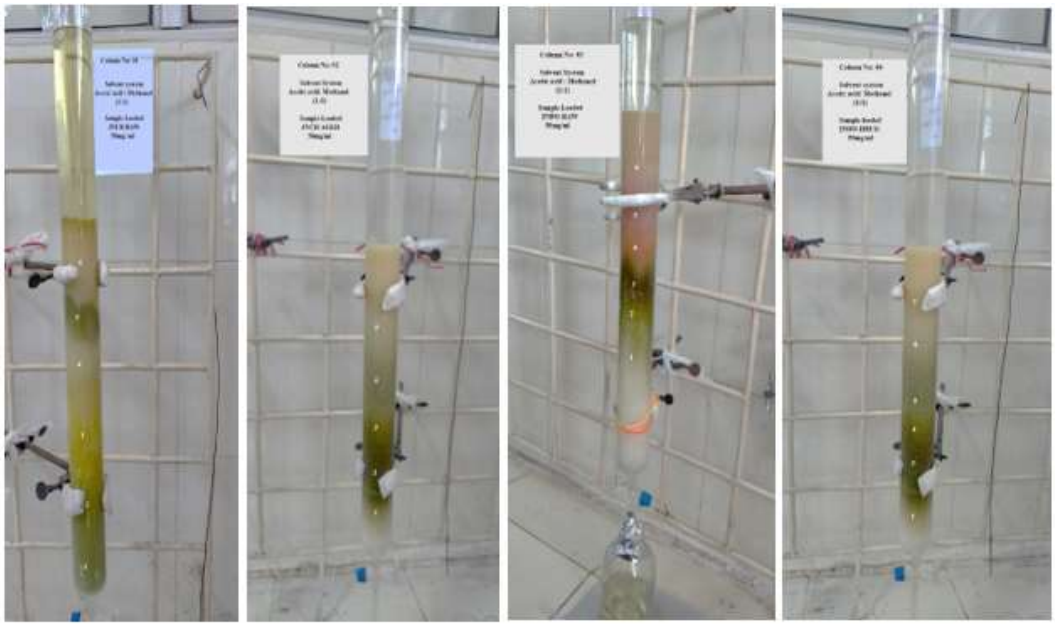

Image No 4 : Showing fine separated fractions by Column Chromatography of different samples:

- Column No: 01- Lab code: JNCH-RAW with six separated fractions.
- Column No: 02- Lab code: JNCH-AGED with five separated fractions.
- Column No: 03- Lab code: INDO-RAW with six separated fractions.
- Column No: 04- Lab code: INDO-DRUG with seven separated fractions.

RESULTS AND DISCUSSION:

PERCENTAGE YIELD:

Percentage yield of *Cannabis Sativa* leaves obtained (Table No: 01) after processing of 100 grams leave is **8.34** % crude extract with solvent system (Acetic acid and Methanol 1:1) as crystal form is obtained.

SOLUBILITY TEST:

Result obtained from the solubility study (Table No.02) shows that the **JNCH AGED** extract was freely soluble in Benzene, Acetic acid, Diethyl ether, Dichloromethane, Toluene. **JNCH RAW** was freely soluble in Acetic acid and **INDO RAW** was freely soluble in Acetic acid, Acetone, and **INDO DRUG** was freely soluble in Ethyl Acetate, Edible ethanol, acetone, and Dichloro-methane. As **INDO DRUG** sample have freely soluble in Edible ethanol as it is one of the best consumable form used in medicinal preparations.

PHYTOCHEMICAL SCREENING:

Qualitative screening of phytochemical of *C. sativa* leaf extract, (Table No: 03) i.e. **(INDO-DRUG, JNCH-AGED, JNCH RAW, & INDO RAW)**. Revealed the presence of phytochemicals such as Phenols, Tannins, Flavonoids, Saponins, Steroids, Terpenoids, and Alkaloids. In humans, it was presented that there is a correlation between dietary phenolic compound intake and a reduced frequency of chronic diseases such as cancers, cardiovascular and neurodegenerative diseases (11), the phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. Terpenes are lipophilic compounds that easily cross membranes and the blood-brain barrier in particular. They present a wide-array of pharmacological properties, which have recently been described in several.

CHROMATOGRAPHY: TLC & COLUMN

TLC:

Results obtained from chromatographic examination by calculating the Rf values with different solvent system (Table no.04) for consecutive samples i.e. **(JNCH RAW, JNCH-AGED INDO RAW and INDO-DRUG).** As values are indicating the similar Rf value with slight variation this may be due to difference in solvent system used. Rf values are calculated upto three decimal to validate the results. On the basis of separated band & color its Rf values confirm the presence of cannabinoids. Sample (**JNCH RAW)** band no:1 with solvent system Ethyle acetate, Methanol and Ammonia (5:2:3) gives same Rf as compared to standard **CBD:** Rf 0.305 value (10). **INDO RAW** band no:1 with solvent system Hexane, Dioxane (9:1) shows 0.342 Rf as compared to standard **9-THC**: Rf 0.336 value. **JNCH-RAW** band no:03 shows Rf 0.285 as compared to **CBC:** Rf 0.271 value. **INDO-DRUG** band no:3 with Rf 0.113 shows some similarities with compare to standard CBG: Rf 0.151value.

Furthermore calculation of Rf values concluded that the chances of change in Rf value for Cannabinoids with different solvent system, Fine separated bands are obtained with above given Rf values indicating that we have around same Rf values as compare to the published data Rf value shown by (10). Several other methods as **GC, GC-MS, HPLC**, is proposed to confirm the actual identity of the isolates for expanded result.

COLUMN CHROMATOGRAPHY:

Column chromatography with separated fractions of crude extracts given in (Table No: 5). The elution was fractionated on the basis of color and evaporated at ambient temperature, The standardized solvent system (Acetic acid: Methanol) gives excellent result with good separated fractions of different color with different loading samples **(JNCH RAW, JNCH-AGED INDO RAW and INDO-DRUG)** There are seven fractions isolated from INDO-DRUG. Six fractions from JNCH-RAW & INDO-RAW, while JNCH-AGED have less fractions with 5 isolates, the final extract was stored at 4℃ for identification from methods like GC, GC-MS, HPLC for expanded result and further parameters.

CONCLUSION:

It can be concluded from results that in case of *Cannabis sativa* maximum phytochemicals are soluble in organic solvent. Maximum solubility obtained in Acetic acid, Benzene, Dicholoromethane, as compared to published data by (Madane AN. *et al.*, **2013**) that Chloroform and acetone are best solvent for phytochemical screening and further studies. The results demonstrated that crude extracts **(Lab code: JNCH RAW, JNCH-AGED INDO RAW and INDO-DRUG)** obtained by using (Acetic acid: Methanol) solvents from *Cannabis sativa* possess biological compounds, such as terpenoids, glycosides, alkaloids, flavonoids, flavones, steroids, tannins, phenols and saponins. Phytochemicals found in extracts of *Cannabis sativa* indicates their potential as a source of therapeutic constituents that may supply novel medicines. The menthol: acetic acid-based extraction developed here is therefore efficient; and owing to its biodegradability and pharmaceutically acceptable toxicity, it is a safer, more sustainable and greener alternative to common organic solvents for phytocannabinoid extraction. (Tomas Krizek, *et.al.*, **2018**). Triplicate analyses of four different TLC mobile phase systems for the analysis of cannabinoid. Furthermore statistical calculation of their Rf values concluded that the chances of error in Toluene: Chloroform: Methanol for Cannabinol (CBN) is lesser while more in Hexane: Dioxane. Likewise, similar pattern of separation observed in Acetic acid: Methanol as it is a safer, more sustainable organic solvents for phytocannabinoid separation shows similarities of Rf values as compared to the published values by (Wise A. *et al.*,**2019**). More investigations must be performed

to access the safety and use of *Cannabis sativa* leave extracts. As (Ali Sharif. *et al.*, **2017**) proposed for other plant for same purpose. This work revealed that after further research cannabis can be useful in certain types of medical conditions, led to the development of various herbal medical cannabis products.

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