Indian J. Applied & Pure Bio. Vol. 35(2), 195-204 (2020). **ISSN: 0970-2091**

Phytoconstituents characterization of extricates prepared from seeds of Hallucinogenic plants

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Abstract

The present study deals with the phytochemical examination of Phytochemical profiling and therapeutic importance of *Cannabis sativa* L., *Cannabis indica* Land *Salvia divinorum* L. an important hallucinogenic plant. Qualitative phytochemical analysis of the extracts prepared from *seeds showed* the presence of alkaloids, Anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, Saponins, steroids, sugar, glycosides, tannins, and xanthoproteins. The FT-IR spectrum confirmed the presence of alkyl methyl, and alcohol, ethers, and estergroup, Carboxylic acid, and anhydrides were also present. The bioactive components of the ethanol extract of *Cannabis sativa L.,* and *Salvia divinorum* L seeds were investigated using Perkin-Elmer Gas Chromatography-Mass Spectrometry (GC-MS), while the mass spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology (NIST) library.

The therapeutic activities of plants novel to specific plant species or their groups are reliable with the idea that the presence of secondary metabolite in a specific plant is exclusive or extensively particular[6,23] Screening dynamic mixes from plants needs to prompt the creation of new restorative medications which have proficient therapeutic and preventive jobs against different infections including malignant growth and Alzheimer's sickness[17,29] Plants stay a crucial wellspring of medications, and these days much accentuation has been given to Nutraceuticals. An enormous number of restorative plants and their filtered constituents have demonstrated useful helpful possibilities[12,17]. To advance the utilization of therapeutic plants as potential clusters of antimicrobial mixes, it is critical to completely explore their organization and action and in this manner approve their utilization[20]. A few phytochemicals created by plants have antimicrobial movement and utilized for the advancement of new antimicrobial medications[21]. It has been indicated that in-vitro screening techniques could give the required fundamental perceptions to choose

rough plant extricates with possibly helpful properties for additional compound and pharmacological examinations[22]. The assurance of phyto-constituents is to a great extent performed by generally costly and frequently relentless methods, for example, gas (GC) and fluid (LC) chromatography joined with explicit recognition plans[4,32]. Investigation of modest quantities of synthetic substances has become simpler and more practical attributable to the improvement of hyphenated chromatographic methods, for example, GC or LC-MS. GC-MS examination can distinguish unadulterated mixes present at under 1gm[14]. Notwithstanding, straightforward, savvy, and fast tests for identifying phyto parts are essential. Spectroscopic (FTIR) strategies together or separate can be utilized right now well as regular techniques.

Salvia divinorum (*Hojas de Maria, Yerba Maria, Hierba de la pastora, Ska Maria pastora, Magic Mint, Diviner's wise*) is an enduring plant having a place with the Lamiaceae family, local to Sierra Mazatec area of Mexico with a long history as a divinatory hallucinogenic compound. It was customarily taken by biting, drinking or smoking by Mazated clans as a strong psychedelic drug. It has been remained moderately obscure as entheogen until the mid-1990s when Siebert.firstly reported his self-test consumption. It quickly turned out to be effectively accessible online where it is as of now sold as dried leaves, removes or live plants. It is generally ingested as a mixture, smoked or bit its crisp leaves[2].

The hemp plant, *Cannabis sativa,* wellspring of the opiate, is a local of Central Asia and is presently developed in numerous pieces of the world. It is a herbaceous yearly that develops to a stature of four to eight feet or more. The leaves are long, thin and serrated and have around five to seven projections emerging from a similar point, rather like the fingers of a hand spread fanwise. Male and female blossoms develop on independent plants. The seed is hard and hard. The plant is secured with glandulose hairs wealthy in a resinous exudate. The tar contains the majority of the dynamic element of the hemp, however the seeds additionally contain a modest quantity. Customarily the blossoming highest points of the female plant have been viewed as the most extravagant wellspring of gum, however this isn't presently commonly acknowledged. Opiate intensity fluctuates with the heredity of the plant and with the atmosphere a hot dry air will in general increment the yield of tar and a few people feel this is on the grounds that the sap has a defensive capacity[30].

The seeds of *Cannabis sativa, Cannabis indica* and *Salvia divinorum* were gathered from Siwan region of Bihar. The plant was related to the assistance of nearby verdure and validated in Botanical Survey of India, NBRI, and Lucknow.

Preparation of Extracts for Phytochemical Screening :

Freshly gathered seeds of *Cannabis sativa* L., *Cannabis indica* L. and *Salvia divinorum* L. tests of were dried in the shade, and afterward coarsely powdered independently in a grinder (crushed by hands first), The coarse powder (100g) was separated progressively with oil ether, benzene, ethyl

acetic acid derivation, methanol and ethanol, each 250 ml in a Soxhlet contraption for 24 h. All the concentrates were sifted through Whatman no. 41 channel paper. All the concentrates (Ethanol and Methanol) were exposed to Qualitative tests for the distinguishing proof of different phytochemical constituents according to standard methods[24,27,31].

FT-IR Analysis:

A little powder of plant example was blended in with KBr salt, utilizing a mortar and pestle, and compacted into a slight pellet. Infrared spectra were recorded as KBr pellets on a Brukers Tensor 207, between 4000-400 cm^{-1}. [21]

Extraction to perform GC-MS:

Stem and leaf of *Cannabis sativa* L., *Cannabis indica* L. and *Salvia divinorum* L, were cleaned, conceal dried and pummeled to powder in a homogenizers. The necessary amount of powder was gauged and moved to extractor and treated with ethanol until the powder is completely inundated. The jar was shaken each hour for the initial six hours, and afterward it was kept aside and again shaken after 24hours. This procedure was again processed for three days, and afterward the concentrate was separated. The concentrate was gathered and dissipated to dryness by utilizing vacuum refining unit. The ethanol separates therefore acquired was utilized for GC-MS examination[19].

GC-MS Analysis:

The GC-MS examination of stem and leaf extricate were performed by utilizing a Perkin-Elmer GC Clarus 500 framework and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) furnished with an Elite-I, intertwined silica hair like segment (30mm × 0.25mm 1D × 1 µMdf, made out of 100% Dimethylpolysiloxane). For GC-MS location, an electron ionization framework with ionizing vitality of 70 eV was utilized. Helium gas (99.999%) was utilized as the transporter gas at steady stream rate 1ml/min, and an infusion volume of 2 µl was utilized (part proportion of 10:1); Injector temperature 250 °C; Ion-source temperature 280 °C. The stove temperature was modified from 110 °C (isothermal for 2 min.), with an expansion of 10 °C/min, to 200 °C, at that point 5 °C/min to 280 °C, finishing with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a filtering interim of 0.5 sec and parts from 45 to 450 Da. Complete GC running time was 36 min[18]. The relative % measure of every segment was determined by contrasting its normal pinnacle territory with the absolute regions, programming adjusted to deal with mass spectra, and chromatograms was a Turbo mass.

Distinguishing proof of Compounds:

Interpretation of mass range GC-MS was directed utilizing the database of National Institute Standard and Technology (NIST) having in excess of 62,000 standards. The range of the obscure segment was contrasted and the range of the obtained parts were coming the NIST library. The Name, Molecular weight, and structure of the segments of the test materials were obtained by their resemblance to the content.

Table 1-1. Preliminary Phytochemical screening of the chosen plants were performed in order to attain the information about secondary metabolites of *Cannabis sativa* L., *Cannabis indica* L. and *Salvia divinorum* L.

Bioactive Components	*C. sativa* L.			*C. indica* L.			*Salvia divinorum* L.		
	Methanol	Ethanol	Petroleum Ether	Methanol	Ethanol	Petroleum Ether	Methanol	Ethanol	Petroleum Ether
Alkaloids	+	+	-	-	+	+	+	+	-
Anthraquinones	-	-	+	+	-	-	-	-	-
Catachins	+	-	+	-	+	-	+	+	+
Coumarins	+	+	-	+	-	+	-	+	-
Flavanoids	-	-	+	-	+	-	+	-	+
Phenols	+	+	+	+	-	-	-	-	-
Quinones	+	-	-	-	+	-	-	+	-
Saponins	-	-	+	+	+	-	+	-	+
Steroids	+	+	-	-	-	-	-	+	-
Tannins	-	-	+	+	-	+	+	-	+
Terpenoids	+	+	-	-	+	-	-	+	-
Reducing Sugar	-	-	+	-	-	+	-	-	+
Glycosides	-	+	-	+	+	-	+	+	-
Xanthoproteins	+	-	+	-	-	+	-	-	-
Fixed oils	-	+	+	+	+	-	+	+	+

Table-2. Result interpretation for *Cannabis sativa* For FTIR.

Peak Value	Functional group	Compound Class
3330.41	O-H Stretching	Alcohol
2956.24	N-H Stretching	Amine salts
2927.13	O-H Stretching	Carboxylic acid
1462.70	C-H bending	Alkane
1374.30	C-O stretching	Alcohol
1215.47	C-O stretching	Vinyl Ether
1167.74	C-O stretching	Tertiary Alcohol
1123.53	CO-O-CO Strecthing	Secondary Alcohol
1054.03	C=C Bending	Alkene
1009.03	S= O Strecthing	Sulfoxide
967.8	C=C Bending	Alkene
834.48	C=C Bending	Alkene

FTIR Spectrum Data for Ethanolic extracts of *Cannabis sativa* L., *Cannabis indica* L., *Salvia divinorum* L.

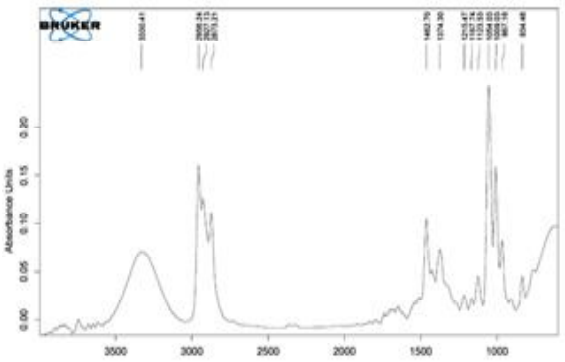

Figure 1: FTIR Spectrum of *Cannabis sativa*

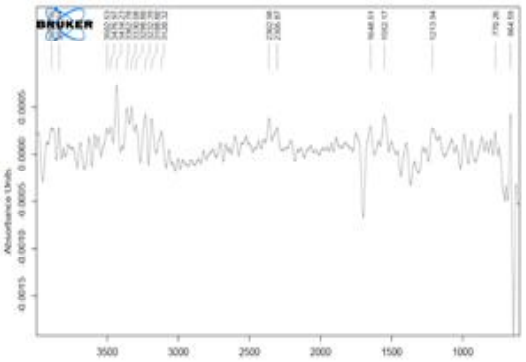

Figure 2: *FTIR Spectrum of Cannabis indica*

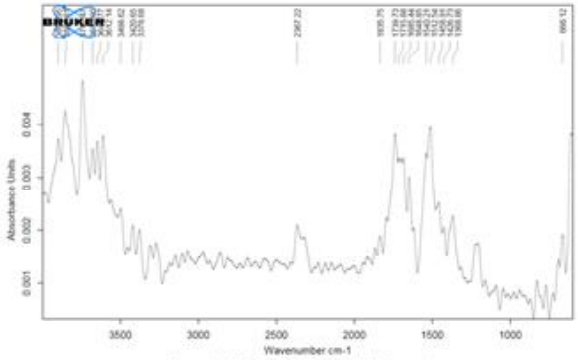

Figure 3 : FTIR Spectrum of *Salvia divinorum* L.

Table-3. Result interpretation for *Cannabis indica* L for FTIR

Peak Value	Functional group	Compound Class
3896.16	O-H Stretching	Alcohol
3502.53	N-H Stretching	Primary amine
3476.97	N-H Stretching	Primary amine
2367.22	O=C=O Stretching	Carbon di oxide
1835.75	C=O Stretching	*Conjugated acid halide*
1715.88	C=O Stretching	Conjugated halide
1540.21	N-O Stretching	Nitro Compound
1368.86	C-H Stretching	Alkane
667.8	C-Br Stretching	Halo Compound

Table-3. Result interpretation for *Salvia divinorum* L for FTIR.

Peak Value	Functional Group	Compound Class
1707.90	C=O Stretching	Conjugated aldehyde
1423.51	O-H Bending	Alkane
1359.71	C-N Stretching	Amine
1221.47	C-O Stretching	Vinyl ether
1092.59	C-O Stretching	Aliphatic ether
903.46	C=C Bending	Alkene

Later to FTIR Spectrum analysis of the extracts were further evaluated for their Hallucinogenic Components, The below mentioned are the results of these mass spectrometry.

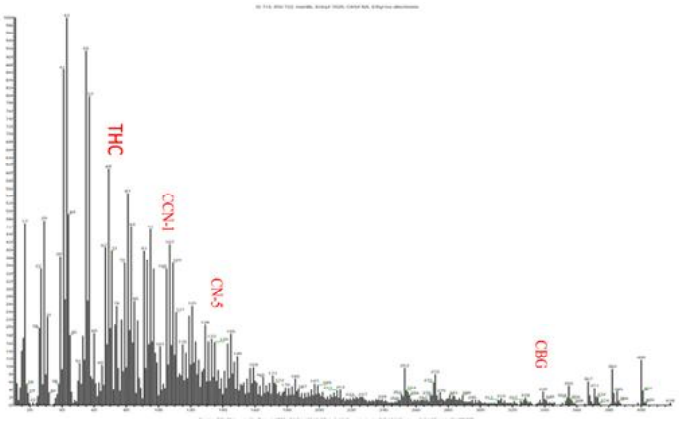

Figure 4: The above figure represents GC Chromatogram of *Cannabis sativa* L. The plot is made between Intensity and RT.

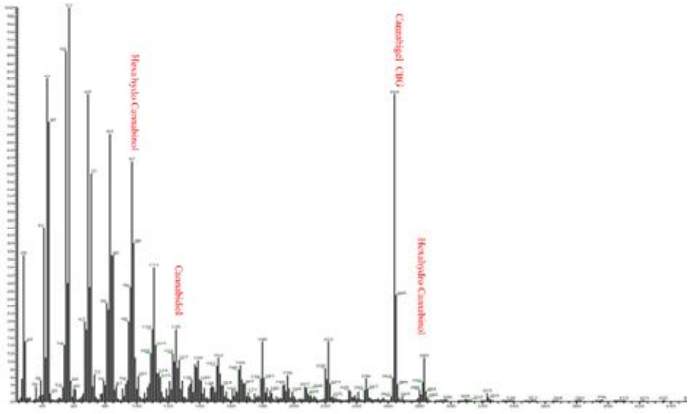

Figure 5: The above figure represents GC Chromatogram of *Cannabis sativa* L. The plot is made between Intensity and RT.

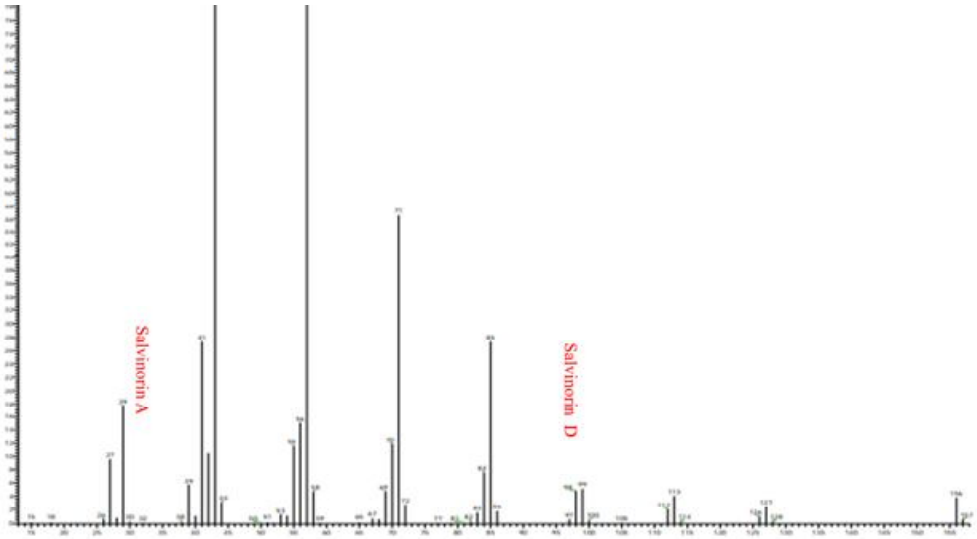

Figure 6: Represents GC Chromatogram of L. The plot is made between Intensity and RT

The prevailing compounds were THC (14.9%), Squalene (11.45%), CN-1 (9.5%), Vitamin E (8.3%), CN-5(13%) in the extricate of *Cannabis sativa* L., and in *Cannabis indica* L. Heptadecane, 2,6-dimethyl- (7.9%), Cannabigeel (7.0%), 11- Hydroxy THC (6.6%), 9,12,Octadecadienoic acid (Z,Z) (6.6%), 5α-androstan-16-one, cyclic ethylene mercaptole (5.4%), 9,12-Octadecadienoic acid, methyl ester, (5.0%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (4.5%), Vinyl ethers, ethyl ester (4.1%), CBD (4.1%) and Cyclopentaneundecanoic acid, methyl ester (8.9%). *Salvia divinorum L*. Salvinorin A (49%), Salvinorin C (32%), Salvonirin D (13%), the major components were detected in the ethanolic extract.

The plants are known as therapeutic wealthy in optional metabolites, which incorporate alkaloids, glycosides, flavonoids, bug sprays, steroids, and related dynamic metabolites. They are of incredible restorative esteem and have been widely utilized in the medication and pharmaceutical industry. As of late, a few investigations have been accounted for on the Phytochemistry of plants over the world. In the current examination, the subjective phytochemical examination uncovered that the methanol and ethanol separates contained some Phytoconstituents[26]. Alkaloid, Anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, sugar, glycosides and xanthoproteins are found in both the concentrates. Alkaloids have calming, antiasthmatic, and antianaphylaxis properties with results of changed immunological status in-vivo. Moreover, an alkaloid, which is one of the biggest phytochemical bunches in plants amazingly affects people, and this has prompted the advancement of ground-breaking painkiller drugs[28].

Flavonoids, the significant accumulation of phenolic mixes which are accounted for their antimicrobial, antiviral, and spasmolytic action. Flavonoids can rummage hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which high lights a large number of the flavonoids wellbeing advancing capacities in these seeds taken under research. They are significant for the counteraction of infections related with oxidative harm of layer, proteins, and DNA. Flavonoids, then again, are intense water-solvent cancer prevention agents and hardly any extreme foragers, which forestall oxidative cell harm and have solid anticancer action[18]. Flavonoids have been alluded to as nature's organic reaction modifiers due to solid trial proof of their innate capacity to change the body's response to allergen, infection, and cancer-causing agents[11]. They show hostile to unfavorably susceptible, calming and anticancer exercises[38]. Phenolics have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and mitigating exercises[1]. Plants determined normal items, for example, flavonoids, terpenoids, and steroids and so on have gotten extensive consideration lately because of their different pharmacological properties including cancer prevention agent and antitumor action[9,10]. Numerous tannins containing drugs are utilized in medication as astringent. They are utilized in the treatment of consumes as they encourage the proteins of presented tissues to frame a defensive covering. They are likewise restoratively utilized as mending specialists in irritation, leucorrhoea, gonorrhea, consumes, and heaps and as a cure. Tannins have been found to have antiviral, antibacterial, antiparasitic impacts, calming, antiulcer and cancer prevention agent property for conceivable restorative applications. Tannins are known to have general antimicrobial and cancer prevention agent exercises. Ongoing reports show that tannins may have potential incentive as cytotoxic antineoplastic operators[25]. It was likewise announced that specific tannins had the option to repress HIV replication specifically and was additionally utilized as diuretic[8]. Coumarin has been utilized as anticoagulant drugs and to treat lymphedema (Online). Plant steroids are known to be significant for their cardiotonic exercises, gang's insecticidal and antimicrobial properties.

These perceptions referred to on phytochemical mixes bolster the current discoveries on the handiness of seeds of *Cannabis sativa* L.in different medicaments. It proposes that the *Cannabis indica* L. plant can be utilized as antimicrobial movement, cell reinforcement, and antiallergic, mitigating, and antidiabetic, anti -carcinogenic, anticancer operators later on.

From the FT-IR spectrum showed the presence of C=O, C-H, C=C, C-O, and O-H functional groups. These holding structures are answerable for the nearness of alkyl gathering, methyl gathering, Alcohol accumulations, ethers, esters, carboxylic corrosive, and anhydrides. Also some peaks demonstrate the nearness of amino acids, alkanes, ethers, natural halogen mixes and starches in plants. Carboxylic acids present in the therapeutic plant fills in as a primary pharmaceutical item in used in restoration of ulcers, jaundice, cerebral pain, stomatitis, hemicranias, fever, torment in the liver, treatment of edema, and rheumatic joint agonies. Amines, amides, and

amino acids are the principle gatherings of protein blend, and herbs fill in as herb oil and hair tonic.

THC (14.9%), Squalene (11.45%), CN-1 (9.5%), Vitamin E (8.3%), CN-5(13%) in the extricate of *Cannabis sativa* L., and in *Cannabis indica* L. Heptad cane, 2,6-dimethyl- (7.9%), Cannabigeel (7.0%) property of mitigating and against joint as detailed by before laborers[5,13,15]. Phytol is one of the fourteen mixes of the current investigation. Phytol was seen to have antibacterial exercises against Staphylococcus aureus by making harm cell films accordingly there is a spillage of potassium particles from bacterial cells. Phytol is key non-cyclic diterpene liquor that is an antecedent for Vitamins E and K1. It is utilized alongside straightforward or corn syrup as a hardener in confections.

As a matter of fact these metabolites have chemo preventive action against colon carcinogenesis, as well all above mentioned therapeutic importance viz. anti- inflammatory, anti- arthritic. The outcomes show that, responsive oxygen species-promising novel class of pharmaceutical for the treatment of rheumatic joint pain and conceivably other ceaseless provocative infections. Consequently, this sort of GC-MS examination is the initial move towards understanding the idea of dynamic standards right now, and this kind of study will be supportive for additional point by point study. Further examination concerning the pharmacological significance of *Cannabis sativa* L., *Cannabis indica* L. and *Salvia divinorum* L. their assorted variety and subsequent photochemistry may add new information to the data in the customary therapeutic frameworks.

This examination was bolstered by Department of Botany, Gopeshwar College (Hathwa, Bihar). We want to thank my Principal, Gopeshwar College (Hathwa, Bihar) for his remarkable help in availability of stuffs and significant recommendations in progress of this paper. Additionally, we want to acknowledge co-activity of research scholars of the division.

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