ISSN : 2249- 5746
International Journal of Ayurvedic and Herbal Medicine 14:4 (2024) 4435-4444
Journal homepage: http://www.interscience.org.uk
DOI: 10.47191/ijahm/v14i4.04
Impact Factor: 7.734

# HPTLC, Pharmacognostic standardisation and Biological activity of Cannabis sativa

**Danapur Vijay*[1], Iddalagi Sana[2]**
[1,2] Vriksha Vijnan Private Limited, 31/2, SSB Complex, Subramanyapura Main Road Bengaluru, Karnataka, India

**ABSTRACT:** *Cannabis sativa* belongs to the family Cannabinaceae and has notable medicinal properties as it is used as for psychoactive effects. Cannabis which contains CBD and THC exhibits a range of potential therapeutic benefits, including anti-inflammatory, analgesic, anxiolytic and neuroprotective properties. Methanolic extracts of *Cannabis sativa* were obtained through reflux extraction to perform further experiments. Various experimental tests were carried out to understand the physicochemical, fluorescence properties of Cannabis sativa. HPTLC and phytochemical profiles were also obtained. Preliminary phytochemical analysis showed the presence of alkaloids, flavonoids, Tannins, Phenols and steroids. Microscopy of the sample powder showed the presence of starch, stigma shaped papillae and Calcium oxalate crystals. HPTLC profiling showed 9 bands of a pattern unique to *Cannabis sativa*. The *Cannabis sativa* showed antidiabetic and anti oxidant activity when bioautography was done.

**KEYWORDS:** Cannabis sativa, Physicochemical evaluation, Phytochemical HPTLC profiling, Microscopical evaluation Bioautography

## INTRODUCTION

*Cannabis sativa* L., commonly known as cannabis or hemp, is an erect herb that belongs to the family Cannabinaceae and is well known for its traditional, medicinal, and recreational uses. It is an annual herbaceous plant with various morphological features. It typically grows tall, reaching heights of up to 3 meters. Its aromatic nature and distinctive leaves often arranged oppositely or alternately, contribute to its recognizable presence. Notably, cannabis exhibits dioecious flowers, a trait distinguishing male and female reproductive organs [1] and [9]. Thriving in nitrogen-rich environments, particularly in well-drained, sunny locales near water sources, wild Cannabis sativa demonstrates vigorous growth and adaptability [9].

Within its complex chemical composition lie over 560 compounds, among which 204 are cannabinoids, including the well-studied Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) [2]. These cannabinoids engage with the endogenous cannabinoid system (ECS), comprising lipid ligands and enzymes, to elicit a spectrum of psychopharmacological effects, offering therapeutic relief for an array of ailments [3]. The therapeutic potential of cannabis sativa extends beyond conventional medical applications, encompassing skincare treatments through modulation of the ECS. Dysregulation of the ECS is implicated in various skin diseases, with the cannabinoid receptors CB1 and CB2 serving as key regulators of inflammation and pain perception [4]. Additionally, orphan G protein-coupled receptors such as GPR55 and GPR18 respond to cannabinoid ligands, unveiling new avenues for dermatological interventions [5].

The resurgence of hemp cultivation heralds a paradigm shift towards eco-conscious practices, leveraging its therapeutic, nontoxic, and biodegradable qualities to address pressing environmental concerns [4].

At the heart of Cannabis sativa's pharmacognosy lies a rich array of bioactive compounds, prominently including cannabinoids, terpenes, and flavonoids. Among these, cannabinoids are of particular interest due to their profound effects on the human body's endocannabinoid system (ECS). THC (Δ-9-tetrahydrocannabinol) and CBD (cannabidiol) are two of the most extensively studied cannabinoids, with THC responsible for the plant's psychoactive effects and CBD exhibiting a range of potential therapeutic benefits, including anti-inflammatory, analgesic, anxiolytic, and neuroprotective properties [6].

The physiological effects of Cannabis sativa are primarily mediated by the endocannabinoid system (ECS), which comprises endogenous cannabinoids such as anandamide and 2-arachidonoylglycerol, as well as cannabinoid receptors (CB1 and CB2).CB1 receptors are primarily located in the central nervous system, while CB2 receptors are predominantly found in peripheral tissues, particularly in immune cells. Activation of these receptors by cannabinoids modulates neurotransmission, immune function, pain perception, appetite regulation, and mood [7]. Pharmacognostic studies of Cannabis sativa have further elucidated the plant's chemical composition, aiding in the identification, isolation, and characterization of its bioactive constituents. Terpenes, aromatic compounds responsible for the plant's distinct aroma and flavor, contribute synergistically to the pharmacological effects of cannabinoids through the entourage effect. Additionally, flavonoids, plant pigments with antioxidant properties, confer potential therapeutic benefits, including anti-inflammatory, anti-cancer, and neuroprotective effects [8]. *Cannabis sativa* is used for Agnimandya (for acidity), Anidra (for sleeplessness), Atisara (for loose motion) etc., [10].

## MATERIALS AND METHODS

### Collection of samples:

Leaf parts of *Cannabis sativa* L. are collected in and around Bangalore. The plant thus collected is identified and authenticated using "Flora of presidency of Madras". The plant material was screened for the presence of admixtures, foreign matters (sand, glass particles, and dirt), mold, or signs of decay. The plant material was later made into powder using mortar and pestle and the samples were stored in airtight containers for further use [1].

### Organoleptic evaluation:

Organoleptic evaluation is a qualitative method where we use sense organs to study characteristic features of plant samples. It involves the study of both physical and sensory characteristics like color, smell, taste, sight and touch, etc [11] [Table 1].

### Physico-Chemical Evaluation

### Moisture

2gms of powdered sample was measured and taken in a Petri dish, where the weight of both the empty Petri dish and the Petri dish with the powdered sample was noted and kept for drying in the oven for about one hour and allowed to cool down in desiccator and weight of it was measured taken.[11] [Table 2]

$$\text{Moisture (\%)} = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

Where, $W_1$ = weight of empty Petri dish, $W_2$ = weight of sample + Petri dish, $W_3$ = weight of sample after drying

### Determination of total ash:

Two grams of powdered drug was incinerated in a sintered silica crucible by gradually increasing the heat to 500-600°C until the drug was free from carbon and then cooled. This was kept in a desiccator for 15-20 min weighed using electronic balance and noted down the readings [12] [Table 2].

$$\text{Total Ash (\%)} = \frac{(B-C)}{(A)} \times 100$$

Where, A- Sample weight in grams; B-Weight of dish+ contents after drying; C-Weight of the empty dish in grams

**Determination of Acid insoluble ash:**

The total ash obtained was boiled for 15 min in 25 ml of hydrochloric acid filtered to collect the insoluble matter on Whatman filter paper and ignited in a sintered crucible. It was allowed to cool and then kept in a desiccator for 15 min. The residue was weighed in the electronic balance and the acid-soluble ash was calculated using the formula [13] [Table 2]

$$\text{Acid insoluble ash (\%)} = \frac{(B-C)}{(A)} \times 100$$

Where, A = Sample weight in grams B = Weight of dish + content after drying in grams
C = Weight of empty dish in grams

**Determination of Extractive values:**

Two grams of powdered plant material from both plants' understudy were extracted with ethanol and water. Thus obtained extracts were allowed to dry to room temperature. After complete evaporation, the weight, nature, and color of the extracts were recorded [13] [Table 2].

$$\text{Extractive value (\%)} = \frac{(B-C)}{(A)} \times 4 \times 100$$

Where, A = Sample weight in grams B = Weight of dish + content after drying in grams
C = Weight of empty dish in grams

**Powder Microscopy Studies:**

The powdered plant material was soaked in 10% Nitric acid overnight. The sample is washed with distilled water the following day. Slides are prepared by staining the soaked plant material with saffranine and observed under a microscope and the images were captured [14] [Fig 3].

**Fluorescence Studies:**

The fluorescent examination evaluating the behavior of the powder samples with various chemical regents such as methanol, ethanol, water, hexane, chloroform, concentrated HCl, $H_2SO_4$, and $HNO_3$ were observed in daylight as well as under UV radiation. Fluorescent analyses of all the plant powders were carried out according to the methods of Chase and Pratt [15][Table 3].

**Preliminary phytochemical tests:**

Extract of *Cannabis sativa* L. was subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as Alkaloids, Tannins, Phenols, Flavonoids, Steroids, and Saponins. This examination was done following the methods of Gibbs [16], Peach, and Tracey [17] [Table 4]

**HPTLC Studies:**

Place a sufficient quantity of suitable mobile phase into the chromatographic/glass chamber. Close the chamber and allow it to stand for at least 30 minutes at constant room temperature for complete saturation. The silica plate was placed on the spraying chamber of the HPTLC the amount of concentration of the sample to be sprayed on the plate was given as input through the software ISHAAN after the process of spraying the plate was air dried at room temperature, then in the already saturated solvent chamber i.e., Hexane: Dioxane

(9:1) and then the solvent is made to run up to the specified distance on the silica plate. After that, the plate is air-dried and visualized under visible light, UV light at 254nm, and long UV at 366nm. Then the derivatization was carried out with the anisaldehyde-sulphuric acid solution. Then the graphical presentation of the RF values is designed through the software named JustTLC [18] [19] [Chromatograph 1] [Graph 2] [Fig 4].

**Bioautography Method:**

**Antioxidant and Anti-Diabetic Activity:**

A solvent system of toluene: ethyl acetate (8:2) was taken to develop the chromatogram. Air-dry the chromatogram for the complete removal of solvents. Later spray the chromatogram with a solution of 0.2% DPPH in Methanol/Ethanol to observe antioxidant activity and with an iodine solution for anti-diabetic activity. Chromatograms were examined in visible light [20] [Fig 5&6].

## RESULTS

The plant under study *Cannabis sativa* was identified and authenticated as per the study requirements. The organoleptic and physicochemical studies conform to the limits mentioned in the API.

The preliminary phytochemical studies of methanolic extract of *Cannabis sativa* showed varied results where except saponins, all other group of chemicals viz., alkaloids, flavonoids, tannins, phenols and steroids were present. As far as HPTLC fingerprinting profile is concerned, 9 bands were observed in 10 µL after derivatization with Anisaldehyde Sulphuric acid at RF values 0.98, 0.91, 0.87, 0.77, 0.45, 0.35, 0.22, 0.16, 0.14 and it can be used as quality standard method for identification and authentication.

The fluorescence studies of the powder of *C.sativa* on treating with various solvents indicated different colors when observed under visible and UV light (366 nm).

Powder microscopy studies show starch granules, part of a stigma from papillae, covering trichomes, calcium oxalate crystals, elongated parenchyma of the perigone.

In the bioautography studies, the plant Cannabis sativa indicated the presence of anti-oxidant and antidiabetic activity. The anti-oxidant activity of this is determined by the using the DPPH agent. The formation of the creamish patches on the purple back ground confirms the anti-oxidant activity at RF values 0.98, 0.96, 0.88, 0.81, 0.57, 0.5, 0.36, 0.17. The anti-diabetic activity of this is determined by using the Iodine solution spray. The appearance of the white patches on yellow or brown background confirms the plant has anti-diabetic property at RF values 0.98, 0.66, 0.58, 0.39, 0.24, 0.18.

## CONCLUSION

In conclusion, the research conducted on *Cannabis sativa* encompasses a comprehensive analysis of its pharmacognostic standardization, phytochemical profile and biological activity. Through meticulous experimentation, the study has elucidated the physicochemical properties, fluorescence characteristics, and microscopic features of *Cannabis sativa*. Furthermore, HPTLC profiling provided valuable insights into the unique fingerprint profile of *Cannabis sativa*, offering a reliable method for identification and authentication. The preliminary phytochemical analysis revealed the presence of various bioactive compounds such as alkaloids, flavonoids, tannins, phenols, and steroids, underscoring the rich pharmacological potential of the plant. Notably, bioautography studies demonstrated the anti-diabetic and antioxidant activities of *Cannabis sativa*, further substantiating its therapeutic relevance.

Overall, this research contributes to the body of knowledge surrounding *Cannabis sativa*, shedding light on its multifaceted medicinal properties and paving the way for further exploration and utilization of this versatile plant in pharmaceutical and therapeutic applications. Moving forward continued research and development in this area hold promise for harnessing the full potential of *Cannabis sativa* in improving human health and well-being.

## ACKNOWLEDGEMENT

We thank **Dr. Gandhi P. C. Kaza**. Advisor to the Government of West Bengal and J&K. Former Advisor, IGP & Director, APFSL, Chairman, Truth Labs for discussing about conceptualizing fingerprint profile for Cannabis sativa.

## REFERENCES

1. J.S.Gamble. *Cannabis sativa Linn.* Flora of Presidency of Madras (1928); Vol. III: pp. 1350.
2. Ravindra B. Malabadi [1] *, Kiran P. Kolkar [2], Raju K. Chalannavar [1] and Himansu Baijnath [3]. "*Cannabis sativa*: Difference between Medical Cannabis (Marijuana or drug type) and Industrial hemp". GSC Biological and Pharmaceutical Sciences 2023; 24(03): 377–381.
3. Mnekin L, Ripoll L. Topical Use of *Cannabis sativa* L. Biochemicals. *Cosmetics* 2021; 8(3):85. https://doi.org/10.3390/cosmetics8030085
4. Tahseen KARCHE, Manager Rajdeo SINGH. "The application of hemp (Cannabis sativa L.) for a green economy: a review". *Turkish Journal of Botany* 2019; pp.43: 710-723, Irakli, M. Tsaliki, E, Kalivas, A, Kleisiaris, F, Sarrou, E, Cook C.M. "Effect of Genotype and Growing Year on the Nutritional, Phytochemical, and Antioxidant Properties of Industrial Hemp (*Cannabis sativa* L.) Seeds". *Antioxidants* 2019; *8*:491.
5. Pisanti, S., Malfitano, A. M., Ciaglia, E., Lamberti, A., Ranieri, R., Cuomo, G & Bifulco, M. "Cannabidiol: State of the art and new challenges for therapeutic applications". Pharmacology & Therapeutics 2017; 175: 133-150.
6. Di Marzo, V., & Piscitelli, F. "The endocannabinoid system and its modulation by phytocannabinoids". Neurotherapeutics 2015; Vol 12(4): pp 692-698.
7. Russo EB. Taming. "THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects". *British Journal of Pharmacology* 2011; Vol 163(7):1344-64. doi: 10.1111/j.1476-5381.2011.01238.x. PMID: 21749363; PMCID: PMC3165946.
8. Go MK, et al., "Cannabinoid Biosynthesis Using Noncanonical Cannabinoid Synthases.". International Journal of Molecular Science. 2023 Jan 9;24(2):1259. doi: 10.3390/ijms24021259. PMID: 36674774; PMCID: PMC9862763.
9. Anonymous, Vijaya (*Cannabis sativa*), Ayurvedic Pharmacopoeia of India, Pharmacopoeia Commission for Indian Medicine & Homeopathy, Ministry of Ayush, Govt. Of India, Vol. I, pp no. 166-167, 1986.
10. Jiyun Yang, Jeehyun Lee, "Application of Sensory Descriptive Analysis and Consumer Studies to Investigate Traditional and Authentic Foods: A Review". National Library of Medicine. 2019; Vol. 8(2): pp no. 54. Doi: 10.3390/foods8020054 (organoleptic evaluation).
11. Khare, C. Cannabis sativa Linn. In: Khare, C. (eds) Indian Medicinal Plants. Springer, New York, 2007. https://doi.org/10.1007/978-0-387-70638-2_277
12. Quality control methods for medicinal plant materials.World Health Organization Geneva,1998
13. Betty P Jackson, Derek W Snowdon. *Cannabis sativa L. "*Atlas of microscopy of medicinal plants, culinary herbs, and spices", pp. 34-37, 1992.Chase Jr, C.R., Pratt R, "Fluorescence of powdered vegetable drugs with particular reference to the development of a system of identification", Journal of the American Pharmaceutical Association. 1949; 38(6): pp 324- 331.
14. Gibbs RD: Chemotaxonomy of flowering plants, Mcgill Queen's University Press, Montreal 1974; Vol 1: 523-619.

15. Peach K and Tracey MV: Modern methods of plant analysis. By Springer-Verlag Ohg, Berlin. Heidelberg, New York, Published by Narosa Publishing House, New Delhi. Vol. II. 467-474, Vol. III, 1959: 586-589.
16. Yifan Liu [a], Thomas A. Brettell [a], Justin Victoria [b], Matthew R. Wood [b], Marianne E. Staretz [a,] "High-performance thin-layer chromatography (HPTLC) analysis of cannabinoids in cannabis extracts". Forensic Chemistry. June 2020; Vol 19:100249.
17. Stahl E. Thin layer chromatography, *Springer International Student Edition New York*, 1965.10.
18. Stasiłowicz-Krzemień A, Sip S, Szulc P, Cielecka-Piontek J. Determining Antioxidant Activity of Cannabis Leaves Extracts from Different Varieties—Unveiling Nature's Treasure Trove. *Antioxidants*. 2023; 12(7):1390. https://doi.org/10.3390/antiox12071390
19. https://upload.wikimedia.org/wikipedia/commons/8/81/Cannabis_ruderalis_male_plant.jpg
20. Vlad RA, Hancu G, Ciurba A, Antonoaea P, Rédai EM, Todoran N, Silasi O, Muntean DL. "Cannabidiol - therapeutic and legal aspects". Pharmazie. 2020 Oct 1;75(10):463-469. doi: 10.1691/ph.2020.0076. PMID: 33305718.
21. Indian drug Manufacturers, Indian herbal pharmacopoeia, Indian drug Manufacturers' Mumbai, 2002.
22. Government of Canada, "The Biology of *Cannabis sativa* L. ( Cannabis, hemp, marijuana)"
23. Nakea M Pennant[1], Cimona. V. Hinton[2], "The evolution of cannabinoid receptors in cancer", 2023.
24. Antonella Casiraghi[1], Gabriella Roda[2], et.al, " Extraction Method and analysis of Cannabinoids in Cannabis Olive Oil preparations", Natural Product Chemistry and Analytical Studies, 2017.
25. Priyanka Kumar[1], Charles Mpofu[1], Dianne Wepas[2, 3], "Analyzing the Perspectives of Health Professionals and Legal Cannabis users on the Treatment of Chronic Pain with Cannabidiol: Protocol for a scoping Review", JMIR Publications, 2023.
26. Duvall, C. S., "The African Roots of Marijuana", Durham: Duke University Press, 2019.
27. Small, E., Cannabis: a complete guide. CRC Press, Taylor & Francis Group, Boca Raton, USA, 2016.
28. [FNA] Flora of North America North of Mexico. 1993, New York and Oxford. 2020.
29. Kopustinskiene DM, Masteikova R, Lazauskas R, Bernatoniene J. "*Cannabis sativa* L. Bioactive Compounds and Their Protective Role in Oxidative Stress and Inflammation. Antioxidants (Basel)". 2022 Mar 29;11(4):660. doi: 10.3390/antiox11040660. PMID: 35453344; PMCID: PMC9030479.
30. E. J. Ajagun, T. A. Abdulsalam, A. C. Oni, F. Afolabi and L. A. Jayeoba, "Cannabis sativa l.: a review of the medicinal properties of this 'wonder plant' and an update on the Nigeria situation" *International journal of pharmaceutical sciences and research | IJPSR*, Jan. 31, 2024.
31. Patil N, Chandel V, Rana A, Jain M, Kaushik P. "Investigation of *Cannabis sativa* Phytochemicals as Anti-Alzheimer's Agents: An In Silico Study". *Plants*. 2023; 12(3):510. https://doi.org/10.3390/plants12030510

**Fig 1- *Cannabis sativa* plant [20]**

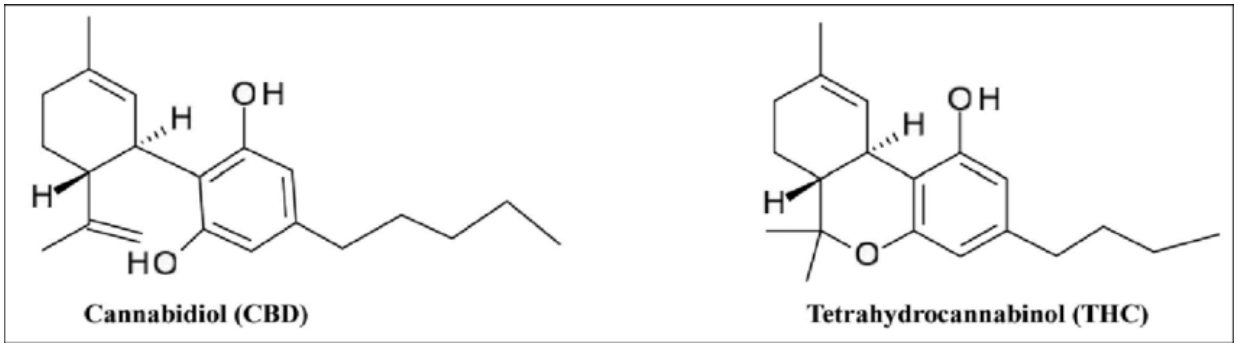



**Fig 2- Chemical structure of Cannabidiol (CBD) and Δ-9-Tetrahydrocannabinol (THC)[21]**

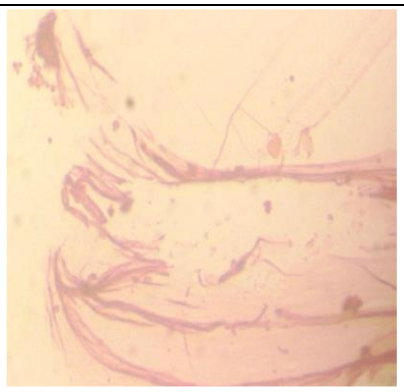
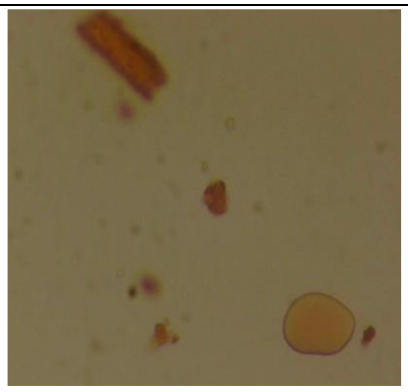
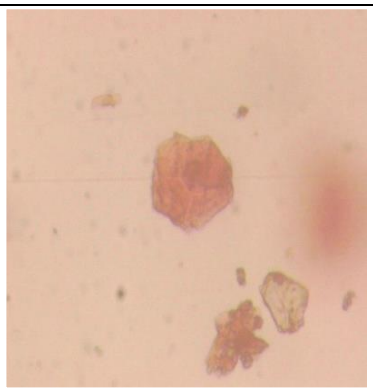
**Table 1- Organoleptic evaluation of Cannabis sativa plant**

| **Plant** | **Taste** | **Color** | **Odour** | **Texture** |
|---|---|---|---|---|
| *Cannabis sativa* | Characteristic | Light green | Characteristic | Coarse |

**Table 2- Physicochemical Evaluation**

| **Sample** | **Total ash (%)** | | **Acid insoluble ash (%)** | | **Alcohol soluble extracts value (%)** | | **Water soluble extracts value (%)** | | **Moisture (%)** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OV | LM (NMT) | OV | LM (NMT) | OV | LM (NLT) | OV | LM (NLT) | OV | LM |
| *Cannabis sativa* ( Powder) | 14.5 | 15 | 3.5 | 5 | 23 | 10 | 24.5 | 13 | 13.11 | NA |

OV – Obtained values; LM- Limit as prescribed by Ayurvedic Pharmacopeia of India
NMT – Not more than; NLT – Not less than

| | | |
|---|---|---|
|  |  |  |
| Part of a stigma showing papillae | Starch | Cluster crystals of calcium oxalate (enlarged) |

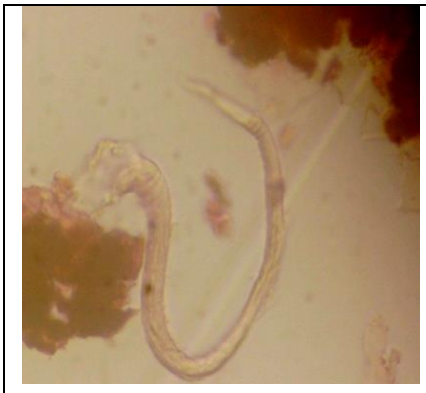
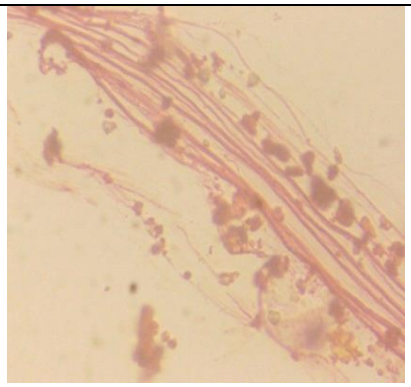
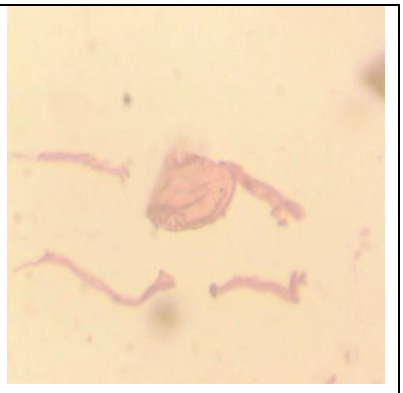
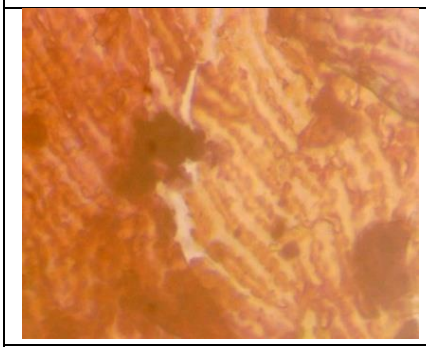
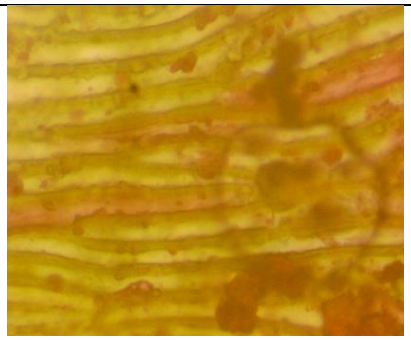
| | | |
|---|---|---|
|  |  |  |
| Covering trichomes, one warty-walled and containing a cystolith. | Part of a group of pericyclic fibers from the stem with associated parenchyma) | Part of a stigma showing papillae (enlarged) |
|  |  | |
| A sclerenchymatous layer of the pericarp in the surface | Elongated parenchyma of the perigone. | |

**Fig-3 Powder Microscopy studies**

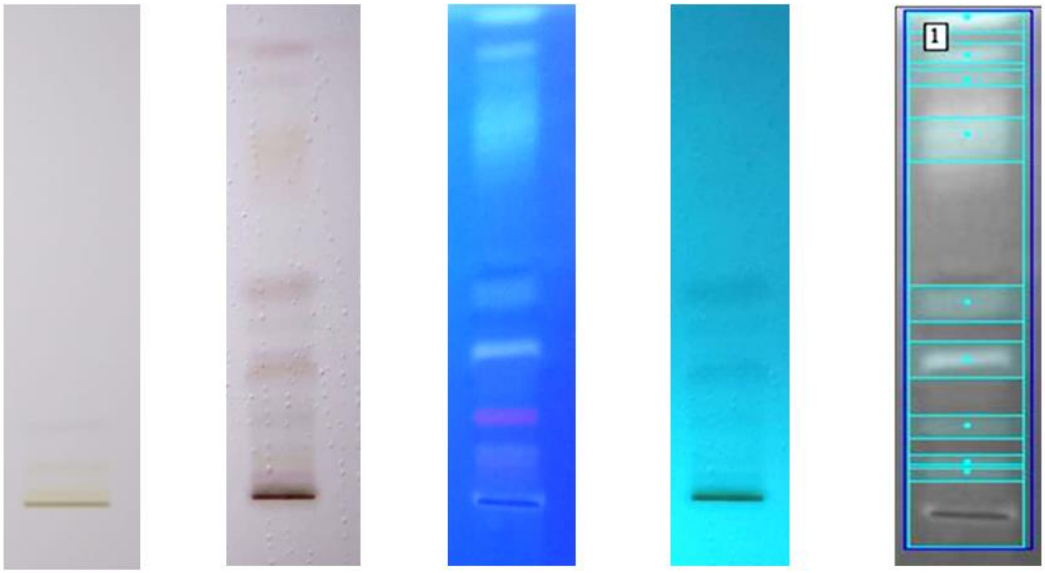
**Table 3 - Fluorescence Studies**

| Sl no. | Reagent added | Color |
|---|---|---|
| 1 | No reagent (raw sample) | Pink |
| 2 | Conc.$HNO_3$ | Yellowish brown |
| 3 | Conc. $H_2SO_4$ | Teal |
| 4 | Conc. HCl | Light pink |
| 5 | Methanol | Bright pink |
| 6 | Hexane | Bright pink |
| 7 | Chloroform | Light pink |
| 8 | Ethanol | Bright pink |
| 9 | Water | Light pink |

**Table 4 - Phytochemical Evaluation**

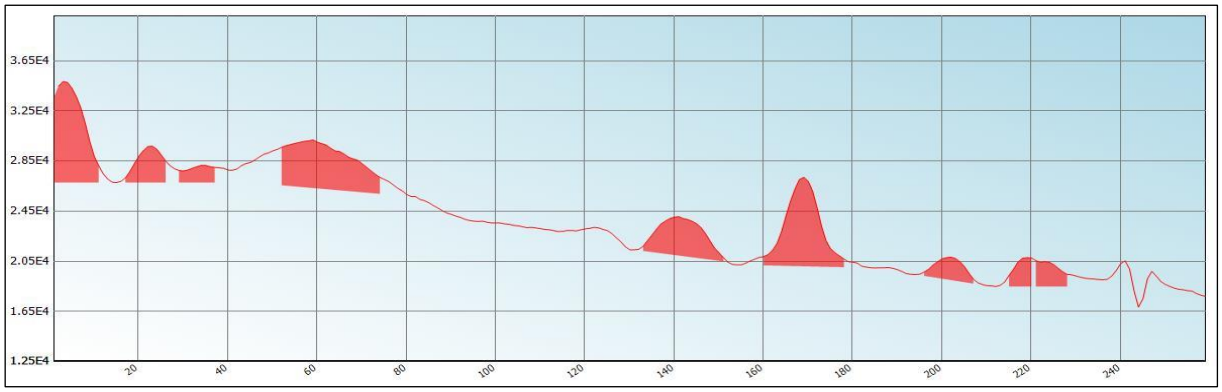
| Sl No | Test | Observation | Chemicals | Result |
|---|---|---|---|---|
| **Detection of alkaloids** | | | | |
| 1 | Mayer's test | Yellow cream ppt | Mayer's reagent | +ve |
| 2 | Dragendorff's test | Red ppt | Dragendorff's reagent | +ve |

| | | | | |
|---|---|---|---|---|
| **Detection of flavonoids** | | | | |
| 1 | Shinoda test | Yellowish brown color | NaOH and Conc. $H_2SO_4$ | +ve |
| **Determination of saponins** | | | | |
| 1 | Froth test | Emulsion forms | Water | -ve |
| **Detection of Tannins** | | | | |
| 1 | Braymer test | Precipitate formation | Water and Sodium chloride | +ve |
| **Detection of Phenols** | | | | |
| 1 | Ferric chloride test | Bluish black color | Ferric Chloride | +ve |
| **Detection of steroids** | | | | |
| 1 | Salkowski test | Red color | Chloroform and Conc. $H_2SO_4$ | +ve |

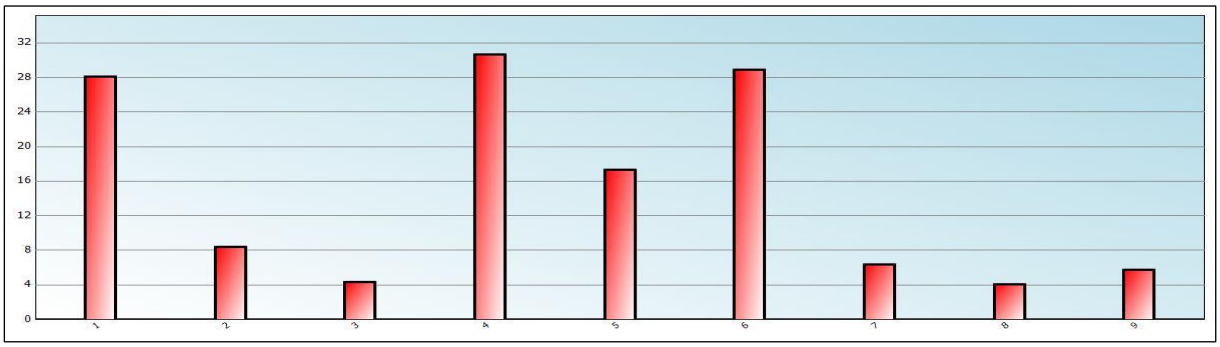



Under Visible light | After spraying Anisaldehyde $H_2SO_4$ | Under 366 nm | Under 254 nm | Bands assessed on plate with JustTLC

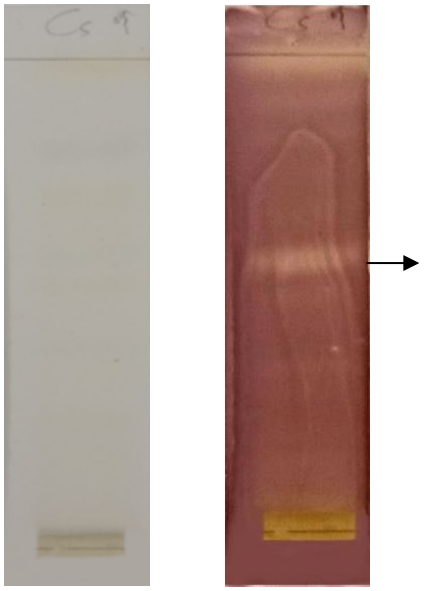
**Fig 4- HPTLC pictures**




Legend: ■ Lane 1 **Chromatograph 1 – Lane graph**




Legend: Lane 1 **Graph2 – Bar graph**

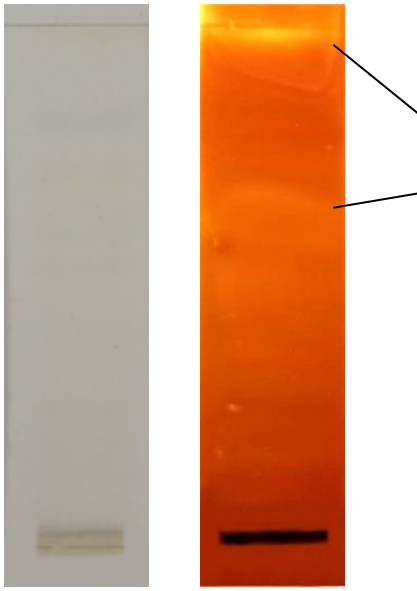


Creamish patches on a purple background

Before spraying

After spraying DPPH

**Fig 5 – Antioxidant activity**



White Patches on a yellow background

Before spraying

After spraying Iodine

**Fig 6- Antidiabetic activity**