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CannabinoidsRich Fraction From Cannabis Sativa For The Treatment of Diabetic Nephropathic Pain

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Abstract:

One of the most common complications of diabetes is diabetic nephropathy, which is a progressive kidney disease. Large and small sensory fibres are commonly affected by this condition, and it progresses gradually. A "glove and stocking" distribution is followed by the symptoms, which begin in the lower limbs, initially affecting the toes, and then spreading forward in the body. The inability to feel warmth, the inability to sense pain, and the increasing discomfort associated with nephropathy are some of the symptoms that are associated with this condition. Hyperglycemia is the primary source of diabetic nephropathy, according to the primary hypothesis. The condition known as nephropathic pain can be caused by damage to the peripheral nerve system, such as the painful diabetic nephropathy (PDN). Cannabinoids that have a beneficial effect on diabetic nephropathy can be extracted from the ethanolic extract of cannabis sativa. These cannabinoids target the CB1 receptor. When it comes to the treatment of diabetic nephropathic pain, two examples of cannabinoids that have been demonstrated to have therapeutic effects are tetrahydrocannabinol and cannabidol.

Keywords: Diabetic nephropathy, hyperglycemia, Cannabinoids,CB1 receptor and tetrahydrocannabinol.

1. INTRODUCTION

A unique, segmented clinical state characterised by partial or total loss of kidney function is known as diabetic nephropathy. Significant changes in the kidney, such as glomerular hypertrophy, mesangial enlargement, proliferation of fibroblasts, matrix deposition, glomerulosclerosis, and tubular necrosis, are associated with diabetic nephropathy. Globally, diabetic nephropathy is a significant health issue. It is a typical diabetic consequence. It typically affects both big and small sensory fibres and advances gradually. The symptoms follow a "glove and stocking" distribution, starting in the lower limbs, initially affecting the toes, and then moving upward. These symptoms include loss of feeling for warmth, loss of capacity to sense pain, and growing nephropathic discomfort. The endogenous cannabinoid system interacts with numerous different signalling systems that are crucial to the regulation of metabolism and is implicated in the mechanisms that control hunger. Both the brain and the peripheral nervous system contain cannabinoid type 1 (CB1) receptors. CB1 receptors have been found in brain circuits related to energy balance and reward.Hyperglycemic condition to be the main cause of diabetic nephropathy.

Grown all over the world, cannabis is an annual weedy plant with a global appeal. It may be taking 80 to 115 days to reach maturity, requires little fertilizer, is resistant to pests and crowding, is reasonably easy to grow, and is a good source of its incredibly valuable raw elements. It also performs well as an organic crop.Only the female plants are utilised for operation, and the preparation process and plant species utilized to extract the medication varies.The female cannabis plant's dry flowering tips and leaves, which are known as the drug-containing sections because they contain considerable amounts of the psychoactive ingredient tetra hydrocannabinol—the pure resin being its strongest form.The most prevalent and well-

studied phytocannabinoids are tetrahydrocannabinol (THC) and cannabidiol (CBD), which have different affinities and actions towards the widely expressed G-protein-couple cannabinoid receptors. The main ingredient in cannabis, Tetrahydrocannabinol, partial agonisticinteracts with CB1 and CB2 receptors. Cannabidol functions act as allosteric modulator of CB1 and has affinity for these receptors. Furthermore, Cannabidiol is nonintoxicating.Numerous genetically distinct cannabis chemovars have been produced through plant breeding, augmenting specific desired effects. Compared to recreational cannabis, medical cannabis typically has higher quantities of CBD, sometimes even surpassing the THC content. In actuality, THC dosages less than those required to produce psychotropic effects may still be able to relievesymptoms. Endogenous cannabinoids are generated from cell membrane phospholipids. Numerous tissues, including the kidney, have the endocannabinoids system. Research has indicated that this system affects renal blood flow (7–9), glomerular filtration rate (11–12), fibrosis (12–14), proteinuria (13–20), and tubular function (21–26). Because whole cannabis contains a variety of cannabinoid chemicals with varying affinities, it is difficult to estimate the potential renal effects and the anticipated cumulative effect on cannabinoids receptors.

ROLE OF ENDOCANNABINOIDS IN DIABETICNEPHROPATHY–

The model of Diabetic nephropathy that is clinically relevant and is created by cisplatin provides the first evidence of the involvement of CB1 receptors in Diabetic nephropathy(Fig-1). In mice with diabetes mellitus, the CB1 receptor was shown to have a more direct role in diabetic nephropathy. In the initial models, streptozotocin (STZ) was the inducer. Diabetic mice have higher levels of CB1 receptor expression in their kidneys, and the colonisation of CB1 receptor with nephrin indicates that podocytes are the primary site of expression for these receptors. The stimulation of CB1 receptors raised the amount of potein in the urine. involved, high glucose

induced dysfunction in podocyte, causes tubular damage, activating inflammatory mediators, and causing renal fibrosis has been identified as CB1 receptors. There is evidence from multiple lines of research that CB1 receptors protect the kidneys in diabetic renal disease. When exposed to high glucose, CB2 receptors were downregulated. However, in Streptozotocin induced diabetic mice and rats, CB2 receptor expression remained unaltered. Patients with severe diabetes have downregulated expression of it in their glomeruli.

Fig.1. Cannabinoid structure

2. Material And Methods

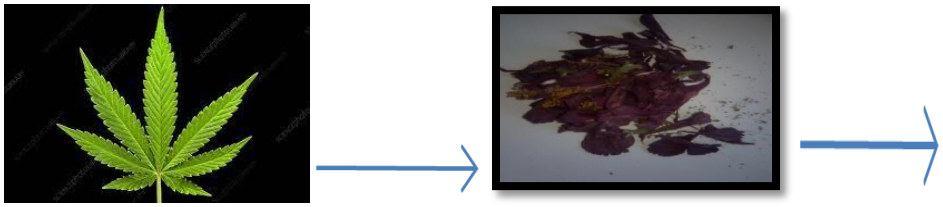
2.1 Plant resources and preparation – Cannabis sativa leaves were harvested from Indian plant species, verified, and dried at room temperature (25–30ºC) for several days. The 500g of leaves were then meticulously separated from the stem and seeds by hand-picking, and finally ground using an electronic grinder. The powdered extract was kept in sealed polyethylene bags for storage.

2.2 Extraction of Phytochemical / Phytoconstituentof Cannabis Sativa

The soxhlet apparatus extraction technique is used to extract the phytoconstituents found in cannabis sativa leaves(Fig.2). First, the crude powder of the leaves is packed into a thimble and

attached to a round-bottom flask. Next, methanol is added as a solvent from the top of the condenser, which is attached to the upper side of the thimble to a round-bottom flask and placed onto a heating mantle. Finally, the soxhlet extractor is attached above the flask, followed by a reflex condenser that has cold water entering at the bottom and exiting above the extractor.Using solvent ethanol,for extracting the phytochemicals/phytoconstituents.

Fig.2. Soxhlet Appratus



Cannabis Sativa desired leaves and powdered to desire size

Powdered drug was filled in Soxhlet apparatus for Pet. Ether extraction

Defatted extract was collected and dried ⟶ Marc was shade dried and

refilled in soxhlet for ethanolic extraction ⟶ Extract was collected

and dried by evaporating the solvent in dessicator ⟶ All extract were stored for further study

2.3 Phytochemical Analysis

The aim of the phytochemical analysis was to identify the alkaloids, glycosides, flavonoids,volatile oils, balsams, terpenes, , tannins, phenols, and resins that were found in the crude leaf extract of Cannabis sativa.

2.3.1 Test For Alkaloids (Mayer's Test)(Khandelwal,2017)

Alkaloids were identified by mixing two ml of ethanolic crude extract of cannabis sativa with a few drops of Mayer's reagent gives precipitate.

Test For Alkaloids (Dragendorff's Test)(Khandelwal,2017)

Alkaloids were identified by mixing two ml of ethanolic crude extract of cannabis sativa with a few drops of dragendorff's reagent gives orange brown precipitate.

2.3.2 Test ForSaponins (Foam test) (Khandelwal, 2017)

To determine saponins, shake the ethanolic extract of cannabis sativa with water.Persistent stable foam observed.

2.3.3 TestForTannins(FeCl3 test)(Khandelwal, 2017)

To measure tannins mix 2 ml of ethanolic crude leaf extract of cannabis sativa in a test tube it gives deep blue-black colour.

2.3.4Test For Flavonoids(Sulphuric acid test) (Khandelwal, 2010)

To detect flavonoids, 2 ml of ethanolic crude extract of cannabis sativa and add sulphuric acid it gives deep yellow solution.

2.3.5 Test For Cardiac glycosides(kellerkilliani test) (Khandelwal, 2017)

In a test tube, 2 ml of ethanolic crude leaf extract is mixed with 2 ml of glacial acetic acid and add 1 drop of 5% ferric chloride and conc. H_2SO_4. Reddish brown colour appears at the junction of two liquids layers and upper layer appears bluish green.

2.3.6 Test ForTerpenes (Salkowski test) (Khandelwal, 2017)

To detect terpenes, 5ml of ethanolic extract is mixed with 2 ml of chloroform and 3 ml of conc. $H_2SO_4$is added to form a layer. A reddish brown colouration of the interface is formed.

2.3.7 Test For Volatile Oil(Khandelwal, 2017)

Solubility test: Volatile oils are soluble in 90% alchohol.

2.3.8 Test For Resin –

To detect the presence of resin, add 2 ml of acetic anhydride with 2 ml of ethanolic leaf extract in an test tube. Add 3 drops of concentrated sulphuric acidand it gives a violet colour shift.

4. Result And Discussion (Table:1)

4.1 Phytochemical analysis

Table: 1- Phytochemical Screening of the Ethanolic Extract of Cannabis Sativa

S.No.	Phytochemical Constituent	Ethanolic Extract
1	Test for glycosides Keller Killani test	Positive
2	Test for Alkaloid Mayers's test Dragondroff's test	Positive
3	Test for volatile oils	Positive
4	Test for Steroids	Positive
5	Test for flavanoids Alkaline test	Positive
6	Test for Carbohydrates Molisch's test Benedict test Fehling test	 Positive Positive Positive
7	Test for Starch	Negative
8	Test for Saponins	Positive

4.2Characterization Of Leaf pigments (Thin Layer Chromatography)

TLC stands for Thin layer chromatography and is primarily based at the phenomena of adsorption. The cellular segment, which contains the dissolved solutes, travels across the stationary section's floor on this form of chromatography. Each solvent extract was subjected to skinny layer chromatography (TLC) the use of silica gel 60F254, 7X6 cm (Merck) cut the use of

not unusual household scissors, as according to the traditional one-dimensional ascending method. Soft pencil changed into used to make plate marks. Glass capillaries were utilised to identify the sample for TLC.The TLC chamber was developed using a mixture of Toluene: Ethyl acetate (6:4)For improvement, pre-saturation with mobile segment for 20 mins turned into employed. To discover the bands on the TLC plates, newly produced iodine reagents were hired after the run plates had been dried and sprayed. The retention aspect (Rf) became used to explicit the energetic compound's mobility, and values were decided for numerous samples

Detection and Calculation of Rf. Value

Once the chromatogram was developed the Rf Value (Fig. 4) (Table. 2) of the spot was calculated using the formula an

I. $R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$

Table: 2 Rf value of Mobile Phase

S.No.	Mobile Phase	Rf value
1.	Toluene:Ethylacetate (6:4) Dis.travelbymobile phase=4.5cm	0.8

Fig.3.Toluene:Ethylacetate(6:4)

4.3Characterization of Leaf pigments (Fourier transform infrared)

The FTIR-spectrophotometer is designedon the principle very much similar to an IR-spectroscopy in this monochromator is replaced by Michelson Interferometer. The interferometer makes use of a moving mirror so as to displaces segment of the radiation caused by the light source, thereby producing an interferogam that may be transformed by using an equation termed as the Fourier Transform so as to extract the desired critical spectrum from a cascade of overlapping frequencies. FTIR is an analytical technique to identify organic materials. FTIR method use infrared light to scan test samples and observe chemical properties.

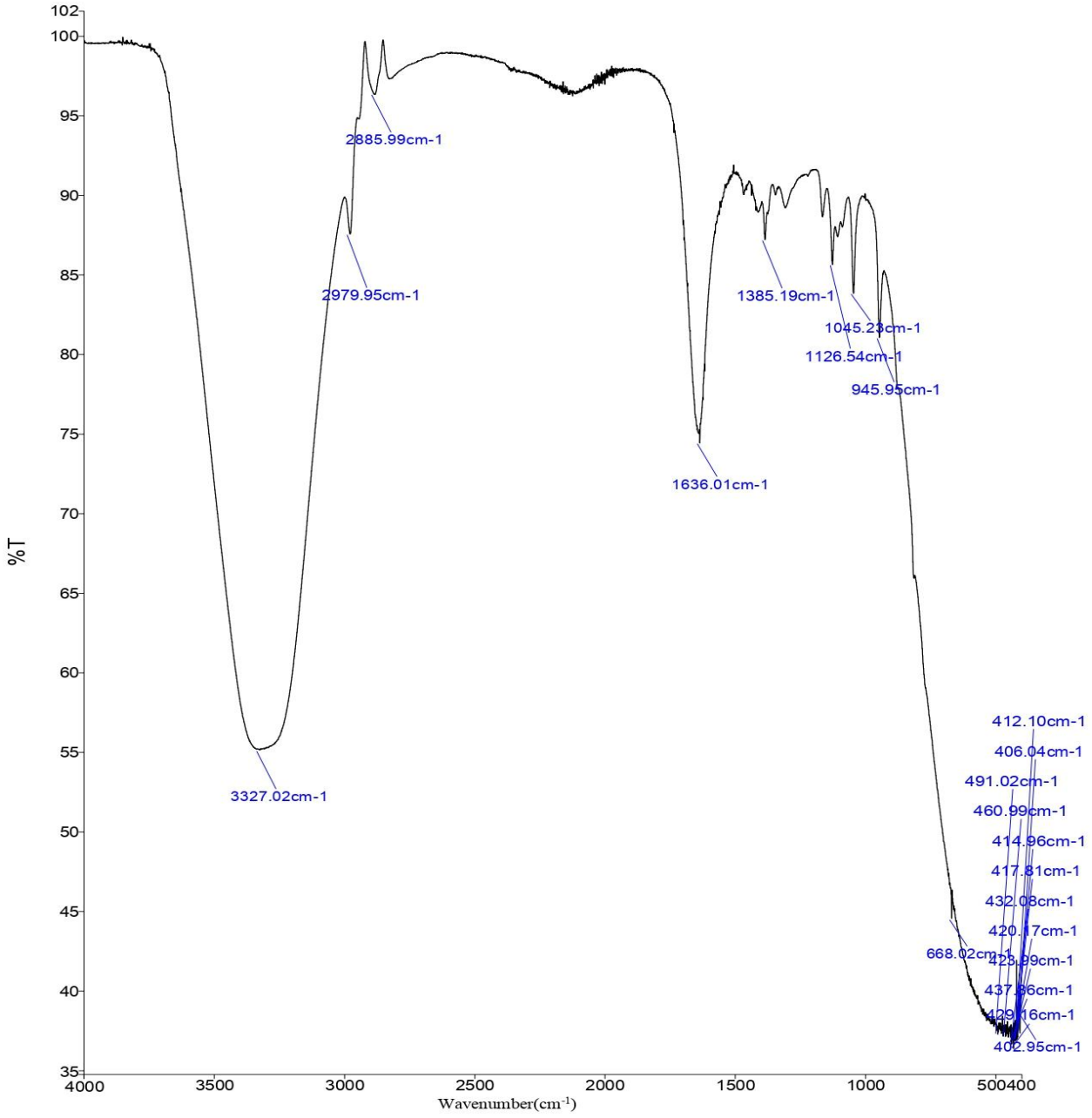


Fig.4. FTIR spectra of Cannabidol and Tetrahydrocannabinol

The FTIR spectrum of cannabidol and tetracannabino in figure-4 show, molecular vibration in the region of 3327 cm^{-1} corresponding to the aromatic (OH) stretching vibration, while the band at 2979 and 2885 cm^{-1} show CH_3- and -CH_2- (methylene) stretching, 1636 cm^{-1} show (C=C) stretching (phenyl ring) and show (C-O) at 1385 cm^{-1}.

Discussion

Cannabis sativa leaves of ethanolic extract were allowed to phytochemical screening, which identified the presence of alkaloids, terpenenes, cardiac glycoside, flavonoids, tannins, and saponin. It has been observed that rats treated to leaf extract of cannabis sativa had lower blood glucose levels due to cardiac glycoside and alkaloids. Diabetes-related nephropathic discomfort is lessened by tetrahydrocannabinol and cannabidiol. The FTIR spectrum of cannabidol and tetracannabinol in figure-4 show, molecular vibration in the region of 3327 cm^{-1} corresponding to the aromatic (OH) stretching vibration, while the band at 2979 and 2885 cm^{-1} show CH_3- and -CH_2- (methylene) stretching, 1636 cm^{-1} show (C=C) stretching (phenyl ring) and show (C-O) at 1385 cm^{-1}.The TLC column that was acquired during the matched the previous Perkin-Elmer Corp. findings. According to this, the formation of s bands is contingent upon chamber, chamber saturation, and environmental factors that may impact the growth of the cannabis sativa plant. Their findings indicate thattetrahydrocannabinol (THC) is the most widely travelled, closely followed by cannabidol (CBD).

Conclusion

In summary, the phytochemical analysis and characterization using TLC and FTIR techniques have identified two important bioactive compounds, namely tetrahydrocannabinol and cannabidol. These compounds have been found to possess the capability to alleviate nephropathic discomfort associated with diabetes. Thorough risk-benefit evaluations that take into account the significant potential positive effects of cannabis are required for the use of medical cannabis as a pharmacotherapy for treating diabetic nephropathic pain.

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