MICROBIAL BIOTRANSFORMATION OF CANNABINOIDS

A
Dissertation
Submitted in the partial fulfillment for the award of degree in
Master of Science in Biotechnology

Submitted by
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Dated: June, 2006 **Arshdeep Kaur Sarao**
Place: TIET, Patiala.

CERTIFICATE

This is to certify that the thesis entitled "***Microbial Biotransformation Of Cannabinoids"*** submitted by Ms. Arshdeep Kaur Sarao (roll number 3040003) in partial fulfillment of the requirements for the award of the degree of Masters of Science in Biotechnology submitted to Department of Biotechnology and Environmental Sciences, Thapar Institute of Engineering and Technology (Deemed University), Patiala, is a record of student's own work carried out under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other institution or university.

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LIST OF ABBREVIATIONS

ABS	Absorbance
CB1 & CB2	Cannabinoid Receptors 1&2
CBC	Cannabicyclol
CBCh	Cannabichromene
CBD	Cannabidiol
CBG	Cannabigerol
CBN	Cannabinol
CC	Column chromatography
FBB	Fast Blue B salt
GC	Gas Chromatography
LAF	Laminar Air Flow
N	Normal
nm	Nanometer
O	Ortho position
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
SDW	Single Distilled Water
rpm	Rotations Per Minute
THC	Tetrahydrocannabinol
THCV	Tetrahydrocannabivarin

TLC	Thin layer Chromatography
UV	Ultraviolet-Visible
Δ	Delta
λ	Lambda

CONTENTS

Executive Summary

The present work is focused on biotransforming the active compounds extracted from *Cannabis sativa*, also known as marijuana, bhang, pot and hemp etc. It has unique compounds called cannabinoids, which are responsible for the characteristic psychoactivity of the plant. These compounds are terpenophenolics and synthesized within the special trichomes (both staked and sessile). In the present work pathogenic fungi of *Cannabis sativa* has been evaluated for their potential to biotransform the cannabinoids. Tetrahydrocannabinol (THC) was isolated and purified by thin layer chromatography and was subjected to biotransformation by pathogenic fungi isolated from *Cannabis sativa*, namely *Aspergillus niger.* THC was found to be biotransformed to some related metabolite by this fungi as observed during the TLC analysis. Retention factor (Rf) of the new compound (biotransformed) was 0.16 as against pure THC having 0.91 when developed in a solvent system comprising of hexane: diethyl ether (4:1pure) as developing system. The λ_{max} of the biotransformed compound is 399 nm. Further characterization and evaluation of the biotransformed compound would be of immense potential for evaluating it as a pharmacophore. World over studies on biotransformed THC with least psychotropic effects is being currently looked at eversince Nabilone- a synthetic THc was approved by USA-FDA for its use as a drug in cancer chemotherapy.

INTRODUCTION

Biotransformations are chemical reactions catalyzed by live microorganisms or by preparations derived from biomass. Biotransformation leads to altered chemical structure and hence altered activity of the biotransformed compound, only if the activity and structure are positively correlated. Biotransformation can be carried out in many different ways, each method employing direct use of live organism or its preparation. Microorganisms are capable of catalyzing a vast number of reactions, which are essential for maintaining the life functions of the cell, including growth and reproduction because they harbor varied type of constitutive and inducible enzymes (Leuenberger, 1990).

Microorganisms employ such enzyme-catalyzed reactions, which are well organized in metabolic-pathways, for degradation or synthesis of a great variety of chemical compounds. Nutrients are degraded in catabolic pathway yielding energy and small molecules as building blocks for anabolic metabolism. More than 2000 enzymes have been catalogued till now each of them accepts a certain substrate and catalyzes a particular reaction, which usually represents a certain metabolic pathway. Besides their natural substrates many enzymes also accept foreign but structurally related compounds and thus catalyze unnatural reactions with the substrates supplied to the medium. Reaction products that are not further degraded are accumulated in the medium, from where they are isolated. Reaction catalyzed by microorganisms or their enzyme preparations are oxidations, reductions, hydrolysis, condensations and isomerizations.

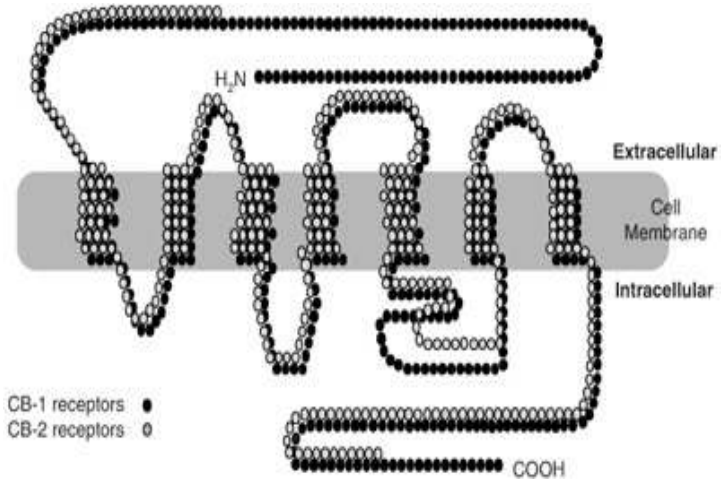
Biotransformation reaction system can be set up with growing culture/ previously grown cultures/ purified enzymes. The biotransformed products are isolated from the whole broth or the reaction medium after removing the biomass by filtration or centrifugation. After isolation of the product it can be further purified

with chromatographic techniques. Biotransformation studies using microbial systems or microbial biotransformations have been used as models to study the breakdown of drugs in human systems or conversion into congeners having a better therapeutic index then their parent compounds for use as pharmacophores (Clark and Hufford, 1991; Riva, 2001). *Curvularia lunata* (NRRL-2178) has been found to convert Lapachol into dehydro-α-lapachone3, which has been shown to possess antibacterial and anti-tumor activities whereas Lapachol itself does not have any anti-tumor activity (Otten and Rossazza, 1979). *Cunninghamella elegans* has been used as a model system in understanding the metabolism of drugs in animal and human systems (Hezari and Davis, 1993; Zhang *et.al*.1996; Rao and Davis, 1997). *Cunninghamella elegans* has been found to metabolize Mirtazapine antidepressant drug acting on α_2- adrenergic receptors via the same metabolic pathway as found in the human systems.

Cannabis sativa has pharmacologically active compounds called Cannabinoids (Terpenophenolics) responsible for the psychotropic activities of the plant and accumulated in considerable amount in glandular trichomes. Cannabinoids were initially thought to be a constituent of cannabis plant (*Cannabis sativa* L.) only, but recently some cannabinoid type bibenzyls have also been found in liverwort (*Radula perrottetii* and *Radula marginata*) (Toyota *et al*. 2002), the chemical structure of perrottetinenic acid in liverwort being similar to that of (-)-trans-∆-9-tetrahydrocannabinol (hereafter referred to only as THC,) in cannabis (Grotenhermen, 2004*).* Cannabinoids are considered analogs of parent compound – Cannabinol, a fusion product of terpene and substituted resorcinol.

Mechoulam *et al.* 1972 in Israel, achieved the complete synthesis of the pure compounds, established their molecular structures, and also began the study

of their structure-activity relationships. This work led to the synthesis of new cannabinoid derivatives and analogues that do not exist in nature. With these pure and potent chemicals, Devane *et. al.* (1992) identified specific binding sites (cannabinoid receptors) in the brain, and showed that the receptor-binding affinities of the different compounds paralleled their respective potencies of biological activity. Since cannabinoids themselves do not exist in the brain, the existence of the receptors implied that some other endogenous material in the brain normally binds to them. Devane *et. al.* (1992) later reported the isolation of Anandamide (arachidonyl-ethanolamine), a lipid material related to the prostaglandins that is formed locally in the brain and binds to the receptors, exerting actions similar to those of the cannabinoids but is less potent. Arachidonyl-glycerol and several other such materials have been identified subsequently. Two Cannabinoid receptors have been identified, the CB1 (cloned in 1990), and the CB2 receptor (cloned in 1993) (Pertwee, 1987), exhibiting 48% amino acid sequence identity. Besides their difference in amino acid sequence, they differ in signaling mechanisms, tissue distribution, and sensitivity to certain agonists and antagonists that show marked selectivity for one or the other receptor type. Both receptor types are linked to the inhibitory G protein, through which they act to inhibit adenylyl cyclase activity, preventing the activation of various Ca^{2+} channels in the cell membrane, while increasing K^{+} influx (Howlett, 2002).



Cannabinoid receptors and their endogenous ligands together constitute the "endogenous cannabinoid system," or the "endocannabinoid system" which has been found in mammals and many other species (De Petrocellis *et al*. 1999). THC, Anandamide, and other known cannabinoid receptor agonists bind to the extra cellular portion of the receptor, thereby activating the signal pathway inside the cell. Of the estimated 60 types of Cannabinoids majority of them have been neglected except THC which has been a centre of attraction through years due to its psychoactive properties (Gaoni & Mechoulam, 1964). Cannabidiol (CBD), a non-psychoactive component, has also been widely investigated due to its anti-inflammatory, anti-schizophrenic and antiepileptic properties (Pertwee, 2005).

Non-psychoactive dimethylheptyl homolog of cannabigerol (CBG-DMH) has hypotensive and vasorelaxant properties (Maor *et. al*., 2005). Another of the neglected plant cannabinoids, tetrahydrocannabivarin (THCV) (Thomas et.al. 2005), propyl homolog of THC (Gill *et.al.,* 1970) is a potent antagonist of WIN55212 (WIN) and of Anandamide. It is reported that it is about five times less active than THC (Gill *et.al*., 1970) and in producing a cataleptic effect in mouse and the time course of its action appears different. Assuming that its toxicity is low, as noted for most cannabinoids, can it serve as a drug in obesity or in nicotine dependence as Rimonabant (the generic name for SR141716) (Mechoulam, 2005). As viewed from the global market perspectives newer cannabinoids having same or enhanced medical potential but lesser psychotropic effects are in the current demand. *Efforts are being made to enhance the activity of cannabinoids accompanied with lesser side effects by altering their chemical structure.* This can be achieved both by chemical and biological means. Chemical methods involve a chemical reaction that would lead to an altered chemical structure due to

mechanisms employing some chemical reactions. Some of the synthetic Cannabinoids being produced are CP-55940 , HU-210, SR-144526, Nabilone and Levonantradol. Nabilone is used as an anti-emetic in chemotherapy and Levonantradol used as an anti-emetic and analgesic (Citron *et.al*., 1985; Dalzell *et.al*., 1986). Biological methods involve use of organism as whole or their extracts, usually enzyme preparations, to biotransform the compound.

Chemical modifications of Cannabinoids through microbial systems have has generated interest among microbiologists, phytochemists, medicinal chemists and pharmacologists for evaluating the biotransformed compounds or congeners as new drug candidates. Approval of USA-FDA for use of Nabilone a drug during cancer chemotherapy has augmented research in looking their potential applications in the field of medicine is going on within the whole scientific world.

Thus one can conclude that cannabis and cannabinoids have huge potential in medicine and pharmacology. Their global market prospects are increasing with the increased research in this field. Newer cannabinoids are being researched and produced in laboratories. Microbial biotransformations of the cannabinoids could lead to production of newer metabolites that might have desired pharmacological characteristics which would allow their more frequent use with least or no side effects i.e. lesser or no psychoactivity.

REVIEW OF LITERATURE

2.1 The Plant: *Cannabis sativa*

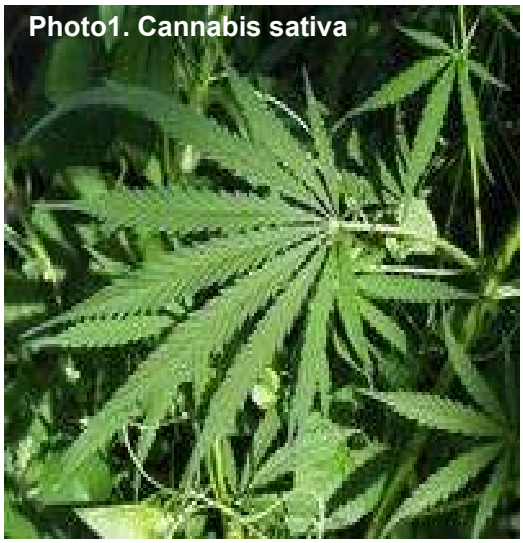


Photo1. Cannabis sativa

Cannabis sativa is annual herb, usually erect; stems variable, up to 5 m tall, basal leaves opposite, the upper leaves alternate, long petiolate, palmate, with 3-11, rarely single with serrate margins. The male plant is somewhat taller and more obviously flowered (photo 1). These flowers have five yellowish tepals, and five anthers that hang pendulously at maturity, dispersing their pollen to the wind.

The female plant exhibits a more robust appearance due to its shorter branches and dense growth of leaves and flower associated bracts. It has double-styled flower which possesses only a thin, closely adherent perianth, but is further protected by enclosure in a cuplike bracteole (Pate, 1994). *Cannabis* is also known by a variety of common names like ganja, Marijuana and Hashish.

Cannabis sativa is known to have around 60 cannabinoids; 20 nitrogen compounds; 18 amino acids; 34 known sugars and related compounds; 50 hydrocarbons; 7 simple alcohols; 12 simple aldehydes; 13 simple ketones; 20 Simple acids; 12 Fatty acids; 13 Simple esters and lactones; 11 Steroids; 103 Terpenes; 16 Non-cannabinoid phenols.

2.2 CANNABINOIDS

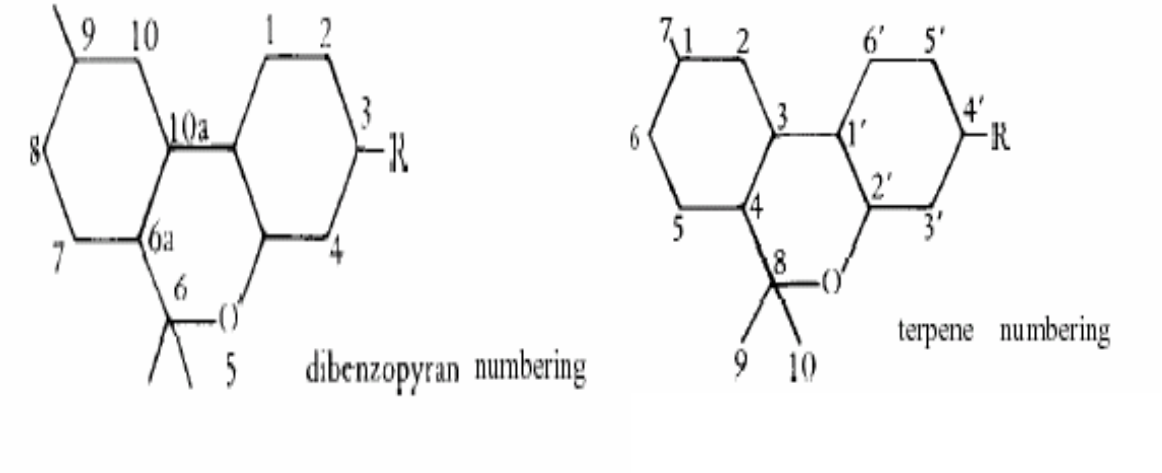
Mechoulam and Gaoni (1967) defined cannabinoids as a term used for the typical C_{21} group of compounds present in *Cannabis saliva L.* and include their analogs and transformation products. Cannabinoids are widely distributed in nature and belong to the chemical class of *terpeno-phenolics*. Cannabinoids are

unique among other psychotropic chemicals derived from plants in that they lack nitrogen and are non-alkaloids.

Cannabinol (CBN) was the first cannabinoid to be isolated from the resin of *Cannabis sativa* and was found to have carbon skeleton common to entire group of cannabinoids. It is 21-carbon system derived by fusion of a monoterpene and Olivetol (Rapaka and Makriyannis. 1998). Distinct numbering systems are employed are Dibenzopyran and Terpene numbering.

Dibenzopyran numbering: It considers cannabinoids as substituted dibenzopyrans, begins numbering from first unfused position of the aromatic ring.

Terpene Numbering: This method recognizes both *t*he terpene and aromatic nature of cannabinoids. Terpene ring is numbered in conventional way, i.e., starting from the carbon having branched methyl group. This branched if in turn numbered seven. Aromatic numbering is as usual.



2.2.1 THE CANNABINOID GROUP

The cannabinoid group can be distinctively divided into, Herbal/ Natural Cannabinoids; Endogenous Cannabinoids and Synthetic Cannabinoids (Fig.2).

2.2.2 Herbal/Natural Cannabinoids

Natural cannabinoids (also called herbal cannabinoids and classical cannabinoids) are nearly insoluble in water but soluble in lipids, alcohols, and other non-polar organic solvents. Natural cannabinoids occur naturally only in the cannabis plant, and are concentrated in a viscous resin that is produced in glandular structures known as trichomes. In addition to cannabinoids, the resin is rich in terpenes, which are largely responsible for the odor of the cannabis plant. Different cannabinoids present in plant are: *Delta*-9-Tetrahydrocannabinol (Δ^9-THC, THC) and D*elta*-8-tetrahydrocannabinol (Δ^8-THC), Tetrahydrocannabivarin (THCV), Cannabidiol (CBD, Cannabinol (CBN), Cannabichromene (CBC) and Cannabigerol (CBG). Of these CBC and CBG are non-psychoactive in nature.

2. 2. 3 Endogenous Cannabinoids

Endocannabinoids are naturally produced in the bodies of animals. After the discovery of cannabinoid receptors in 1980s, scientists began searching for natural compounds that activate these receptors. In the early 1990s, the first such compound was identified as arachidonyl ethanolamine and named Anandamide, a name derived from the *Sanskrit* word for bliss and amide. Anandamide is derived from the essential fatty acid-arachidonic acid. It has pharmacology similar to THC, although its chemical structure is different. Anandamide bonds primarily to the CB1 receptor, and is found in a wide range of animals. It is about half as potent as THC.

Another endocannabinoid, 2-arachidonyl glycerol, binds to both the CB1 and CB2 receptors, and is more abundant and less active than anandamide. About two other discovered endocannabinoids, palmitoyl ethanolamide and oleamide very little is known. To date five endocannabinoids have been identified (Grotenhermen, 2004). These are: (1) N-arachidonylethanolamide (anandamide)

(Devane *et. al.* 1992); (2) 2-arachidonylglycerol (2-AG) (Sugiura *et. al.* 1995); (3) 2-arachidonylglyceryl ether (noladin ether) (Hanus *et al*. 2001); (4) O-arachidonyl-ethanolamine (virodhamine) (Porter *et. al.* 2002), and (5) N-arachidonyl-dopamine (NADA) (Huang *et. al.* 2002).

2.2.4 Synthetic Cannabinoids

Historically, groups led by Adams and Mechoulam worked on laboratory synthesis of cannabinoids based on the structure of herbal cannabinoids and a large number of analogs have been produced and tested. Newer compounds are no longer related to natural cannabinoids or are based on the structure of the endogenous cannabinoids. Synthetic cannabinoids are particularly useful in experiments to determine the relationship between the structure and activity of cannabinoid compounds, by making systematic, incremental modifications of cannabinoid molecules. Some synthetic cannabinoids are CP-55940, HU-210, SR-144526, Nabilone and Levonantradol used as drug in cancer chemotherapy as analgesic and anti-emetic (Citron *et.al*., 1985; Dalzell *et.al*., 1986).

2.3 BIOSYNTHESIS OF CANNABINOIDS:

The first specific step in cannabinoid biosynthesis (Fig. 1) is the condensation reaction of geranylpyrophosphate (**GPP**) with olivetolic acid (**OA**), catalyzed by the enzyme *geranylpyrophosphate:olivetolate geranyltransferase* (**GOT**) (Fellermeier and Zenk 1998; Fellermeier, 2001). The resulting CBG is the direct precursor for CBD (Taura *et al.* 1996)) and CBC (Gaoni and Mechoulam1966).

Fig 1. The biosynthetic pathway of cannabinoids is shown (modified after Fellermeier *et al.* 2001). 1, geranylpyrophosphate; 2, olivetolic acid; 3, CBG(V); 4, CBC(V); 5, THC(V); 6, CBD(V); I, geranylpyrophosphate:olivetolate geranyltransferase (GOT); II, CBC(V) synthase; III, THC(V) synthase; IV, CBD(V) synthase. R_1 (= $-C_3H_7$) and R_2 (= $-C_5H_{11}$) indicate the propyl and pentyl forms of the different metabolites (Taura, F. *et.al*. 1995).

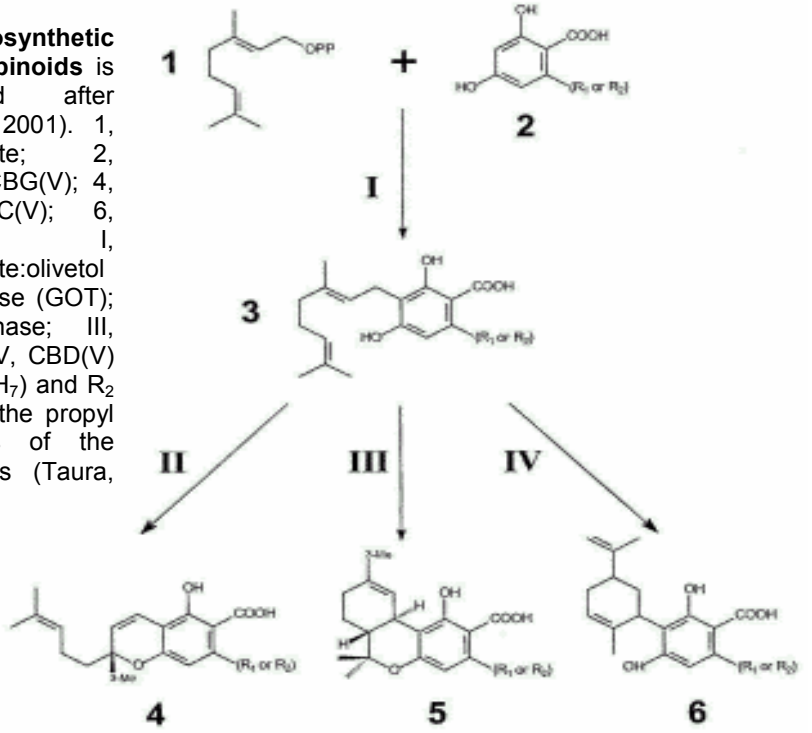
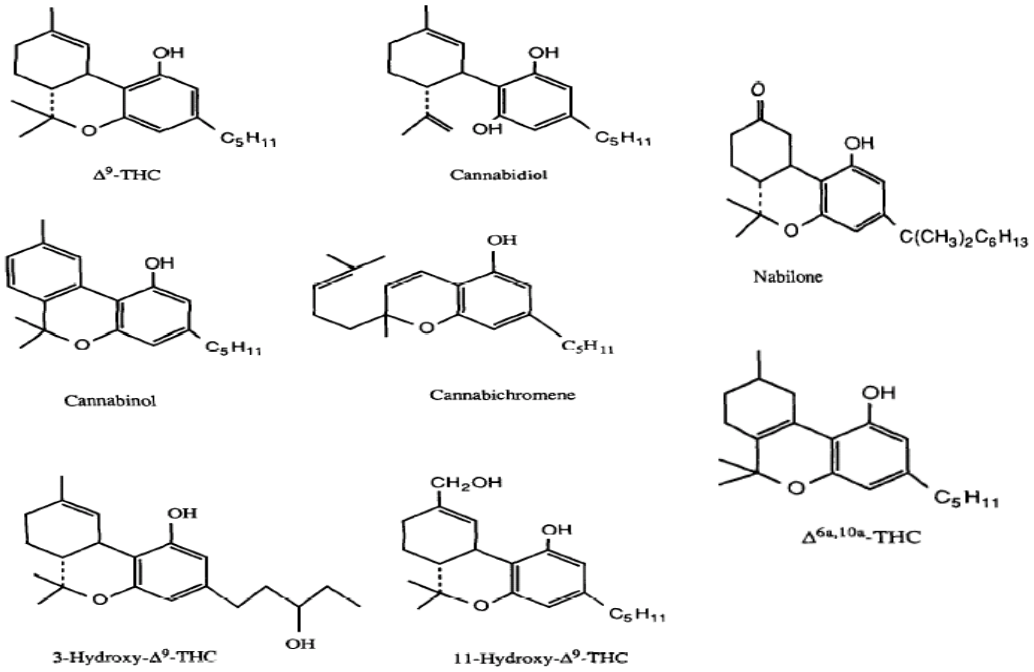


Fig 2. Chemical Structures of natural and synthetic Cannabinoids



2.4 PHARMACOLOGY OF CANNABINOIDS

Cannabis has a very long history of medicinal use, though it is illegal to grow in many countries since the leaves and other parts of the plant are widely used as a narcotic drug. The leaves and the resin that exudes from them are the parts mainly used, though all parts of the plant contain the active ingredients. The principal uses of the plant are as a painkiller, sleep-inducer and reliever of the nausea caused by chemotherapy, whilst it also has a soothing influence in nervous disorders (Brown, 1995). The active constituent isolated from *Cannabis*, which is used in pill, is THC. This pill contains pure ∆-9-tetrahydrocannabinol (THC) in sesame seed oil. It is now scheduled in Schedule II of the Controlled Substances Act. The research has shown that marijuana is more effective medicine than the THC pill (Chang, *et. al.* 1979). *Cannabis* as medicine has been proved effective in the treatment of Multiple Sclerosis (MS), people suffering with urination problem even when the bladder is empty and in treatment of glaucoma as a pain reliever (Chiej, 1984).

When given to patients suffering from AIDS**,** it helps them to put on weight (Abrams *et.al.* 2003). Since it strongly increases the desire for food it has been found of benefit in treating anorexia nervosa. It is used externally as a poultice for corns, sores, varicose veins, gout and rheumatism (Brown, 1995; Duke and Ayensu, 1985)*. It is not a narcotic and compared to many drugs, such as the opiates, barbiturates, etc., it does not produce physical dependence* (Adams and Martin, 1996)*.* Cannabinoid agonists inhibit gastrointestinal motility and gastric emptying in rats (Shook and Burks, 1989). In a study with humans, THC caused a

significant delay in gastric emptying (McCallum *et. al.* 1999). The evidence of existence of cannabinoid receptors at different sites like anterior eye, retina and corneal epithelium indicates that cannabinoids influence different physiological functions in the human eye (Pate, 2002). Vasodilation in the eye is observed as conjunctival reddening after THC exposure (Dewey, 1986). THC and some other cannabinoids decrease intraocular pressure. THC can inhibit DNA, RNA, and protein synthesis, and can influence the cell cycle under extremely high concentrations in vitro (Tahir *et. al.* 1992). It also interacts with the hypothalamic-pituitary adrenal axis influencing numerous hormonal processes (Murphy, 2002). Minor changes in human hormone levels due to acute cannabis or THC ingestion usually remain in the normal range (Hollister, 1986). Tolerance develops to these effects, however, and even regular cannabis users demonstrate normal hormone levels. After several weeks of daily smoking 8-10 cannabis cigarettes, a slight decrease in sperm count was observed in humans, with- out impairment of their function (Hembree *et. al.* 1978). In animal studies high doses of cannabinoids inhibited the acrosome reaction (Chang *et. al.* 1993). The seed of *Cannabis sativa* is anthelmintic, diuretic, laxative, narcotic and tonic (Duke. and Ayensu. 1985). It is used to treat constipation caused by debility or fluid retention (Brown, 1995). The seed is an important source of essential fatty acids and can be very helpful in the treatment of many nervous diseases. A high content of very active antibacterial and analgesic substances has been found in the plant. Antibacterial actions have been demonstrated for CBD, CBG and THC (Klingeren and Ham, 1976). Incubation with THC reduced the infectious potency of herpes simplex viruses (Lancz *et. al*. 2002). It has bactericidal effects on gram-positive microorganisms, in some cases up to a dilution of 1:150,000 (Chopra *et. al.* 1986).

Cannabis also has been used in the treatment of a wide range of conditions including alcohol withdrawal, anthrax, asthma, blood poisoning, bronchitis, burns, catarrh, childbirth, convulsions, coughs, cystitis, delirium, depression, diarrhea, dysentery, dysmenorrhoea, epilepsy, fever, gonorrhea, gout, inflammation, insomnia, jaundice, lockjaw, malaria, mania, menorrhagia, migraine, morphine withdrawal, neuralgia, palsy, rheumatism, scalds, snakebite, swellings, tetanus, toothache, uteral prolapse, and whooping cough (Duke,1985). Cannabinoids are also used in pain therapy (Adams and Martin, 1996). The side or undesirable effects produced by *Cannabis* includes sedation, intoxication, clumsiness, dizziness, dry mouth, lowered blood pressure or increased heart rate (Haney *et. al.* 1999). Research is needed to optimise dose and route of administration, quantify therapeutic and adverse effects, and examine interactions (Robson, 2001).

2.5 EXTRACTION OF CANNABIS

The extraction of cannabinoids was done following cold percolation method, employed by Mechoulam and Gaoni (1970). The word “cold” in this context means no heat is applied and extraction occurs at room temperature. The plant /herb material is allowed to steep in solvent after it has been dried and grounded as required before extracting the compound of interest. The cold percolation is carried out in the glass or stainless steel vessel. The plant material is placed in it and solvent is allowed to steep down through the vessel at normal room temperature and pressure. The extract is collected in sterile vessel.

2.6 ISOLATION METHOD OF DIFFERENT CANNABINOIDS

Mechoulam and Gaoni (1970) isolated Δ-1-THC from *Cannabis* extract and also isolated and purified other neutral cannabinoids from it using alumina column chromatography and Pentane: Ether as elution solvent systems. *Cannabis sativa*

1988). Debruyne (1994) used TLC, GC, and HPLC for identifying different cannabinoids.

2.6.1 Thin Layer Chromatography (TLC)

TLC is the simplest and cheapest method of detecting plant constituents because the method is easy to run, reproducible and requires little equipment. They play an important role as an analytical support in the work of phytochemists for the efficient localization and rapid characterization of natural products (Marston *et.al.* 1997). In TLC partition occurs between eluting solvent and the sample spots applied as row of spots on a layer of finely divided adsorbent (stationary phase) supported on glass plate. As the solvent ascends through the layer by capillary action, the sample is resolved into fractions.

Mechoulam and Gaoni (1970) achieved isolation and structure elucidation of THC, CBG, cannabichromene and cannabicyclol. They did qualitative analysis of cannabinoids by thin layer chromatography using silica gel as stationary phase and pentane: ether mobile phase. Potassium permanganate saturated in cupric acetate was used as visualizing agent. The Rf value of Δ^1-THC came out to be 0.51 and that of Δ^9-THC is 0.57. Cannabicyclol had Rf of 0.62. Maseda *et al*. (1983) developed the chromophoric labeling technique of Δ^9-THC and Cannabinol by making their dabsyl derivatives.

Table 1: FBB colors and Rf values of Cannabinoids

Cannabinoids	Color FBB	Rf Values
Δ-9-THC	Red	0.65
Δ-8-THC	Brown	0.65
CBD	Orange-Brown	0.64
CBN	Red-Brown	0.62
CBG	Purple	0.61
CBC	Red	0.58
THCA	Red	0.39
CBDA	Red	0.37
CBGA	Purple	0.31
CBCA	Purple	0.25

Verpoorte (2005) studied chromatographic and spectroscopic data of cannabinoids from *Cannabis sativa*

(Table 1). They used two different TLC systems (non-polar and polar system). For non-polar system silica gel plates were developed with methanol/5% acetic acid 19:1(v/v) while for the polar system, chloroform/methanol 19:1(v/v) was used as the eluent. After developing the plates were examined under UV 254 nm for the absorption of chromatographic spots for general visualization of compounds they sprayed anisaldehyde-sulphuric acid spray reagent and for selective visualization of cannabinoids, the TLC plate was sprayed with 0.5% fast blue B salt in water, followed by 0.1M NaOH. Galand *et.al.* (2004) has used a variety of planar chromatographic techniques for the separation and identification of Cannabis components from a variety of matrices.

2.6.2 Column Chromatography

Column chromatography is used for separation and purification of chemical compounds both solid and liquid from a mixture of compounds.It is technique in which the two phases are a solid (stationary phase - silica gel and alumina) and a liquid (moving phase - the eluent).

Mechoulam and Gaoni (1970) isolated and purified different cannabinoids of the *Cannabis* by column chromatography using acid-washed alumina as stationary phase and Pentane: Ether mobile phase. They charged 25 g of crude cannabinoids onto column and collected above 79 fractions of 150 ml each. Fraction 1-15 comprised non-cannabinoid material, fractions 16-29 had mainly cannabidiol, fraction 30-40 had cannabicyclol, ∆-1-THC, 47-62 comprised mainly ∆-1 THC and cannabinol, fractions 63-78 contained mainly of cannabinol, cannabichromene, and Cannabigerol. Fractions above 79 contained polar non-cannabinoid substances.

2.7 UV-VISIBLE SPECTROSCOPIC ANALYSIS

The measurement of ultraviolet and visible radiations provides a convenient means for the analysis of numerous organic and inorganic compounds. Ultraviolet-Visible detector is the universal detector used for quantitative and qualitative estimation and assessment of pure compounds. Every pure compound gives an absorption maximum at a particular wavelength called as $\lambda_{max.}$ Based on this absorption maximum that compound can be identified. At this wavelength compound is characterized by peak that gives an idea about its purity and concentration (Verpoorte, 2005).

2.8 FUNGAL PATHOGENS OF *Cannabis*

88 species of fungi attack *Cannabis* (McPartland, 1996) and more are being added every year (McPartland and Hughes 1994; McPartland and Cubeta 1996). Significant damage is caused by *Botrytis cinerea* in temperate regions with high humidity and cool to moderate temperatures and under which gray mold can reach epidemic proportions and completely destroy a *Cannabis* crop within a week. *B. cinerea* attacks many crop plants and weed species worldwide. *Fusarium oxysposrum f.sp. cannabis* has been found to kill cannabis (Tiourebaev *et.al.* , 2001). *Aschochyta*, *Phoma* and *Septoria* species have also been reported as pathogens of Cannabis (McPartland, 1995 a; 1995b)

2.9 ISOLATION OF PATHOGENIC FUNGI

The fungi are usually cultured on Potato dextrose agar (PDA) media. The surface sterilized source material is then inoculated on media plates in small pieces and kept at ambient temperature for growth of microorganisms their further isolation (Agarwal & Hasija, 1986). After isolation the microorganism is evaluated on Koch's postulates to see if it is really a pathogen or not (Saxena and Pandey, 2001).

Oxspring *et. al*. (2003) they tested pathogenesis of *Fusarium avenaceum* and *F. oxysporum*, on leek plant by seedling and disc assays and found the pathogen to grow very well on the plant seedlings and discs making the pathogen identifiable merely by its growth, conidia and colony color.

2.10 BIOTRANSFORMATION METHODS

Biotransformation means changing or altering structure of a compound using biological systems. This system can be a living organism or preparation of its biomass. Biotransformation studies have been carried out in various plant and animal based compounds. And majority of these have been successful at large scale.

Biotransformation methods generally include the addition of the substrate to be biotransformed to the mycelium of the fungus collected and dispersed in sterile distilled water and incubated for approximately 10-15 days at a defined temperature. Thereafter the broth is harvested and made cell free, pH adjusted in acidic range and extracted with suitable solvent for further analysis. Co-metabolization is also used for the biotransformation of compounds like Steviol have been done using *Aspergillus* and *Fusarium* species. (Oliveira *et.al*., 2005)

Binder and Popp (1980) reported the metabolic transformations of Δ^1-tetrahydrocannabinol (Δ^1-THC) by cultures of *Fusarium Nivale, Gibberella fujikuroi* (both *Ascomycetes*) and *Thamnidium elegans (Phycomycetes).* Robertson *et al.* (1975) reported biotransformation of Δ^9-tetrahydrocannabinol, Δ^8-tetrahydrocannabinol, cannabidiol and cannabinol by vegetative cultures of *Syncephalastrum racemosum.* Fukuda *et al* (1977) conducted screening program to find microorganisms that catalyze transformation reactions with cannabinoids.

They analyzed 358 cultures, consisting of 97 bacteria, 175 Actinomycetes, and 86 molds.

2.11 TLC STUDIES OF THE TRANSFORMED COMPOUNDS

TLC and TLC- bioautography is generally employed for assessment of the biotransformation of the compounds (Farooq *et.al.* 2002). Fukuda *et.al.* (1977) biotransformed cannabinoids with different microorganisms and analysed the transformed products with TLC eluted with either benzene, benzene: ethyl acetate (9:1) or in 7:3 ratio. The transformed products are observed by Fast Blue staining and under short UV as new spot.

AIM OF THE STUDY

This dissertation is aimed at studying the biotransforming capabilities of pathogenic fungi isolated from *Cannabis sativa* to modify the cannabinoids extracted from the plant's leaves. The objectives of the study are;

1. Isolation of pathogenic Fungi from *cannabis sativa* leaves.
2. Isolation and characterization of Tetrahydrocannabinol from *cannabis sativa* leaves.
3. *In vitro* biotransformation of cannabinoids.
4. Isolation and partial characterization of transformed products.

MATERIALS AND METHODS

4.1 EXTRACTION OF CANNABINOIDS

Cannabinoids were extracted from plant material by cold percolation method i.e., without using heat/high temperature during extraction. The cannabinoids were extracted using Petroleum benzine (purchased from Merck Co.) as extracting solvent. The leaves were collected from matured and healthy plant. Stalks and stems were separated from them These were then washed under running tap water for 15 mins to remove dirt and other particulate matter. Finally the leaves were dried in tray drier for 4 hrs at temperature not exceeding 40°C. The leaves dried were then grounded to powder and sieved through 2mm sieve. Petroleum benzine and powdered leaves were taken in the ratio of 10:1.The extraction vessel was sealed with paraffin film and placed at 120 rpm, 28°C for 5 days. After 5 days the extract was filtered through crude filter paper followed by Whatman filter paper 1.The filtrate was collected in pre-weighed beaker and the solvent was evaporated to dryness. The residue left was cannabis extract containing cannabinoids. The extract was dark brown and oily (Mechoulam and Gaoni, 1970).

4.2 ISOLATION OF CANNABINOIDS BY TLC AND COLUMN CHROMATOGRAPHY

4.2.1 TLC Analysis

TLC analysis of the extract was done using 20 ×15 ×5 glass plates. These were coated to 0.5 mm thickness with silica gel GF_{254} (purchased from Merck Co.) and kept for activation at 100°C over night Sample stock of crude cannabinoids was prepared by dissolving 10 mg of the extract in 50 μl of chloroform. Crude cannabinoids extract was applied on TLC plate as spots of 5 μl volume containing 1 mg cannabinoids. Developing chamber was saturated with Hexane: diethyl ether in the ratio of 4:1 for minimum of 15 mins. The TLC plates were developed till the

solvent front reached the distance of 17 cm. TLC chromatogram was observed under short UV and sprayed with 0.5 % Fast Blue B salt (*O- dianisidine tetrazotized zinc chloride*) (Sigma) followed by 0.1 N NaOH spray to visualize different cannabinoids. Specific colors relative to different cannabinoids were developed. Retention factor (Rf) was calculated for every band of each spot. Different cannabinoids were scrapped off from the TLC chromatogram and the fractions were collected separately based upon Rf values and FBB colors (Verpoorte, 2005). TLC for run approximately 50 times to collect different Cannabinoids according to their Colors and Rf

RF value was calculated as the ratio of Distance move by solute to solvent front:

Rf = Distance moved by solute/ Distance moved by solvent front

4.2.2 Column Chromatography

The extract contained different cannabinoids and these were separated by column chromatography. For this silica gel for column chromatography purchased from Merck Co. was used (Mechoulam and Gaoni, 1970). 6 g of crude, oily cannabinoid material was charged onto a 50 mm column of Merck silica gel. Column length was kept to 35 cm and was wet packed in pure hexane (Merck). Fractions of 150 ml were collected. The column was packed in hexane and eluted with hexane: diethyl ether in ratio of 4: 1. Total 15 fractions were collected. All fractions were examined with TLC and UV spectroscopy. Fractions having similar compositions were combined. To avoid variations with temperature fluctuations the CC was carried out at 26$\pm$2°C. The fractions were rechromatographed to achieve better separation of different cannabinoids.

4.3 UV-VISIBLE SPECTROSCOPIC ANALYSIS

The column fractions were examined by UV-Visible Spectroscopic Analysis. The samples were prepared by dissolving 0.1 mg per 1 ml of ethanol. The THC scrapped from the TLC plates was also analysed in the same way. The parameters were setup having start and stop wavelength as 800 nm and 190 nm respectively. The base line was established using pure ethanol and wavelength scan of all the samples was performed. λ max values of standard cannabinoids are shown in the table 2 (Verpoorte, 2005).

4.4 ISOLATION OF PATHOGENIC FUNGI FROM *Cannabis*

This involved collection and surface sterilization of *Cannabis* leaves, preparation of Potato Dextrose Agar (PDA) plates, inoculation of surface sterilized leaves on PDA plates, sub culturing and preparing pure isolates.

4.4.1 Preparation of Potato Dextrose Agar (PDA) Plates

Weighed 39.0 g of PDA (Himedia) was dispensed in litre lukewarm single distilled water (SDW) and stirred thoroughly. This was then dispensed in 250 ml Erlenmeyer Flasks and autoclaved at 121°C, 15 *psi* for 15 minutes. Glass Petri plates were made aseptic by sterilization at 121°C, 15 psi for 20 minutes. Then under sterile conditions 25 ml of the autoclaved PDA was dispensed in sterile 90mm Petri plates and allowed to solidify at room temperature. The plates were stored in cold store temperature 0.7°C till further use.

4.4.2 Surface Sterilization of Cannabis Leaves and culturing

The diseased plants were collected from the campus in sterile disposable bags. The separated leaves were washed under running tap water for 15 minutes to remove dirt and attached particulate matter. Then the leaves were 70 % ethanol

for 5 minutes. Then the leaves were transferred to sterile water for 5 minutes. After rinsing with sterile water the leaves were immersed in 1 % sodium hypochlorite solution for 5 minutes. The leaves were again washed with sterile water. The surface sterilized leave were now cut into 1 mm pieces with the help of sterile (autoclaved) blade which was further heated to red hot on flame before cutting the leaves. These 1m pieces were inoculated on PDA plates with the ventral side facing media surface. Maximum of 8 leaf pieces were inoculated in single plate. The plates were incubated at 26±2°C for maximum of 5 days or till fungal growth was seen. All procedures were carried out under aseptic conditions under laminar airflow hood.

4.4.3 Sub-culturing

The fungi grown obtained on PDA plates were further sub cultured on fresh PDA plates to get pure isolates.

4.4.4 Identification of Fungal Isolates

The isolated fungal cultures were studied for their morphological and spore characteristics. These were stained with lactophenol blue dye to stain the spores and hyphae and examined under microscope. The fungi were identified based upon their spore structure and other morphological characteristics microscopically.

4.4.5 Evaluation of Koch's Postulates

Infected *Cannabis* leaves were collected from the Thapar Institute's campus. These were surface sterilized as described above. Glass petri dishes were prepared by placing cotton at the bottom and wetting it by (SDW). The plates were autoclaved at 121°C, 15 *psi*, 20 minutes. The surface sterilized infected leaves were sprayed with different concentrations of fungal spores (2×10^6 cfu/ml

of spores, 1.5 × 10^6 cfu/ml of spores, 1× 10^6 cfu/ml of spores, 0.5× 10^6 cfu/ml of spores). The spore concentrations were made using haemocytometer. The plates were incubated at 26±2°C, 2500 lux for a week (Saxena and Pandey, 2002). The grown fungus was inoculated on fresh PDA plates to evaluate that the disease is caused by the same fungi that was isolated from infected *cannabis* leaves.

4.5 BIOTRANSFORMATION METHODS

The separated cannabinoids were biotransformed using growing cultures of fungi. For this the fungus was first grown and then cannabinoid was inoculated into the growing culture. Test flasks were contained 50ml of presterilized PDB. This was inoculated with the different fungal isolates by using a 5mm mycelial plug of 7-days old culture grown on PDA and incubated at 26±2°C, 120 rpm for 48 hrs. When the culture started growing culture it was inoculated with test cannabinoid in the concentration of 25 mg/ml of ethanol and further incubated at 26±2°C, 120 rpm for 120-240 hrs. Three control flasks were prepared: in first, the cannabinoids were added after killing the fungus by autoclaving it at 121°C, 15 psi, for 15 min. (two sterilizing cycles were operated). In second control flask no cannabinoids were added to the growing fungal culture in PDB. Finally in third control experiment cannabinoids were added directly in PDB without inoculating any fungus. All the control flasks were incubated at same conditions as that of test flask. After the completion of the incubation duration the transformed products were extracted in 1:1 ratio using ethyl acetate. The solvent was evaporated and the extract was examined by TLC. The solvent system consisted of Hexane: diethyl ether in the ratio of 4:1. The TLC chromatograph was observed under short UV and sprayed with FBB to observe cannabinoids and transformed products. These were scrapped by running multiple TLC's.

RESULTS AND DISCUSSION

5.1 EXTRACTION OF CANNABINOIDS

12 grams of crude cannabinoid residue (hashish) from 600 grams of leaves was obtained using petroleum benzene (2% yield). The extract was dark brown in color; oily and sticky in nature therefore it was recovered in minimal volume of chloroform and stored in glass vials in dark area at room temperature. Matsunaga *et al.* (1990) has used benzene and Mechoulam (1970) have used petroleum ether as an extractant of cannabinoids. Petroleum ether is more commonly used as it extracts both neutral as well as acidic forms of cannabinoids.

5.2 ISOLATION OF CANNABINOIDS BY TLC AND COLUMN CHROMATOGRAPHY

After extraction of cannabinoids, they were examined by two different chromatographic methods, namely, Thin Layer Chromatography (TLC) and column chromatography (CC).

5.2.1 Thin Layer Chromatographic Analysis (TLC)

Fast Blue B is specific visualizing agent for cannabinoids that undergoes coupling reaction with cannabinoids and provides them specific colors. Each cannabinoid is observed as a specifically colored spot after reacting with FBB has been previously confirmed (Verpoorte, 2005). Six major cannabinoids were identified on the basis of FBB staining. The R_f of the cannabinoids identified was in the range of 0.18 to 0.96. Different mobile phases have been used to separate cannabinoids and identify them using FBB. The major component of interest was

THC which was having Rf value of 0.91 and appeared purple on the TLC plate (Table 2 and Fig.3).

Fig 3. TLC Chromatogram of Crude Cannabinoids.

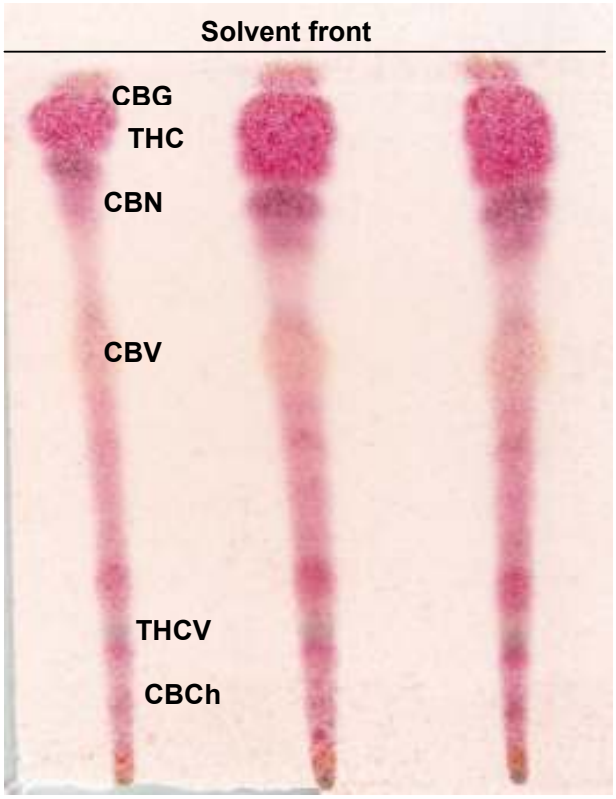


Table 2: Retention factors and FBB colors obtained by TLC using 4 Hexane: 1 diethyl ether

CANNABINOIDS	Rf	FBB COLORS
CBG	0.96	Red
THC	0.91	Purple
CBN	0.84	Red
CBV	0.52	Orange
THCV	0.28	Red
CBCh	0.18	Red

5.2.2 Column Chromatographic (CC) Analysis

15 fractions of 150 ml each were collected by column chromatography and analysed for their yields, spectrum and FBB colour by TLC for identification of the cannabinoids. Fraction color and yields are given in Table 3. Fractions with similar compositions were combined. TLC analysis of column fractions exhibited CBG

Table 3: Colour, yield and λ max of Column Fractions

CC FRACTION	COLOR	YIELD (mg)
Fraction 1	Dark black brown	27 8.9
Fraction 2	Dark Brown	75.0
Fraction 3	Light Orange	52.0
Fraction 4	Orange	115.0
Fraction 5	Orange	355.3
Fraction 6	Orange	355.3
Fraction 7	Orange	332.0
Fraction 8	Light Orange	203.0
Fraction 9	Light Orange	27
Fraction 10	Dark Brown	460.5
Fraction 11	Dark Green	314.8
Fraction 12	Dark Green	160.8
Fraction 13	Dark Green	352.6
Fraction 14	Dark Brown	141.3
Fraction 15	Dark Brown	137.1

content in fractions 3 and 4 and a higher content of THC in fractions 7, 8, 9 and 10. Fractions 7, 8, 9 and 10 were clubbed for further isolation of TLC by scrapping it and using it as pure component to study biotransformation. The Rf values of THC is 0.91 and CBN is 0.84

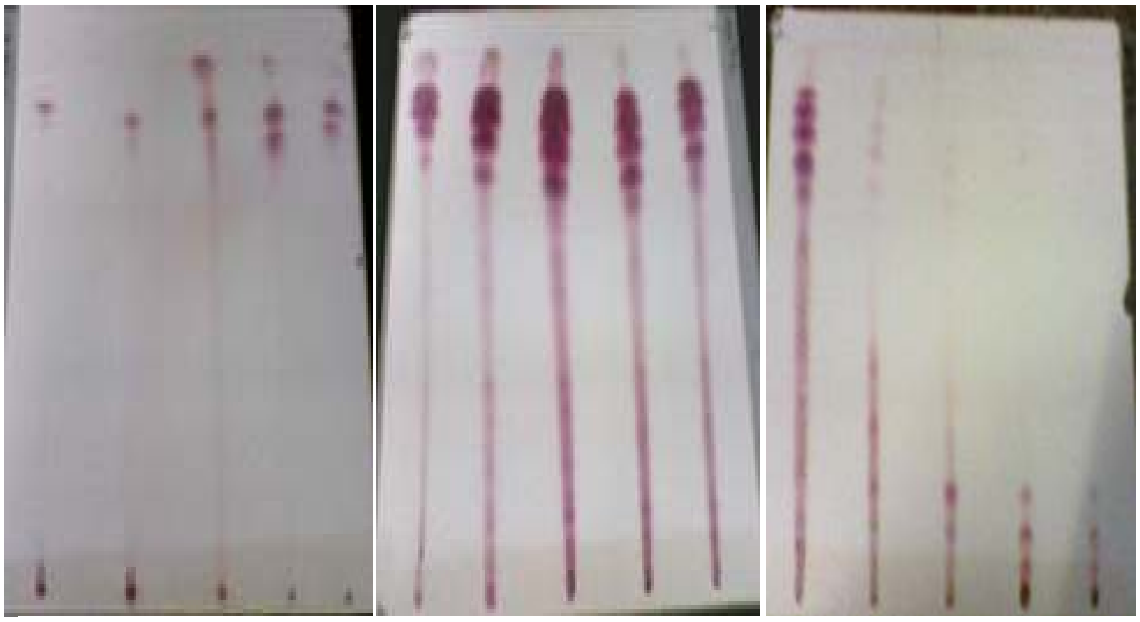


Fig. 4: TLC Chromatogram of Column Fractions

5.3 UV-VISIBLE SPECTROSCOPIC ANALYSIS

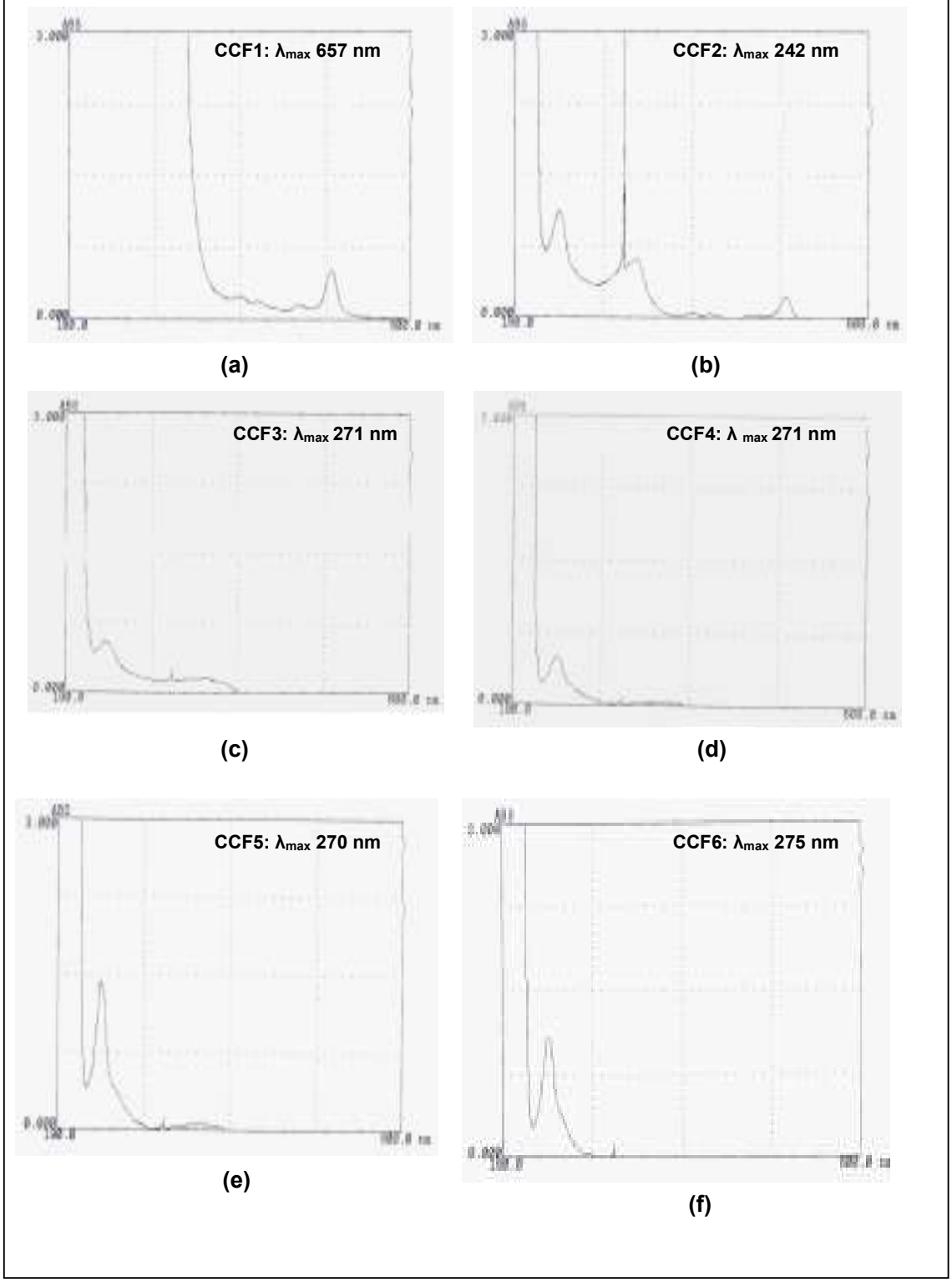
Peak data of the different column fractions was obtained by UV spectroscopic analysis (Table 4) The UV Spectral analysis shows that Fraction 7, 8, 9 and 10 clearly exhibit a good separation of THC and were

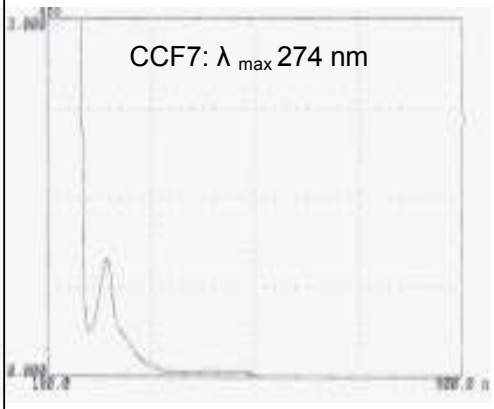
Table 4: λmax of Column Fractions

CCF	λmax
1	657
2	242
3	271
4	271
5	270
6	275
7	274
8	275
9	277
10	276
11	275
12	275
13	275
14	277
15	277

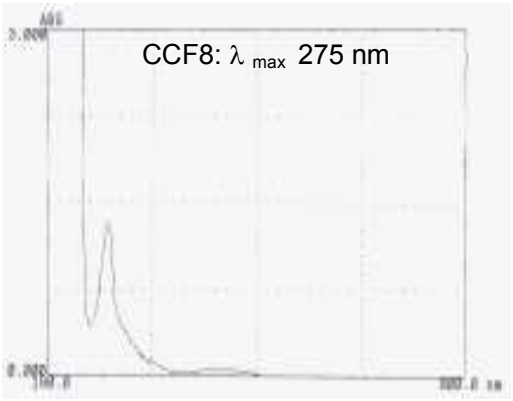
therefore remixed and rechromatographed by TLC to obtain purest THC biotransformation (Fig. 5)

Fig. 5 UV-Visible Spectrophotometric Analysis of Column Fractions

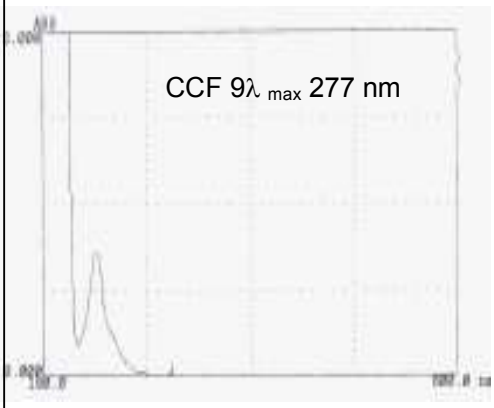



CCF7: λ max 274 nm

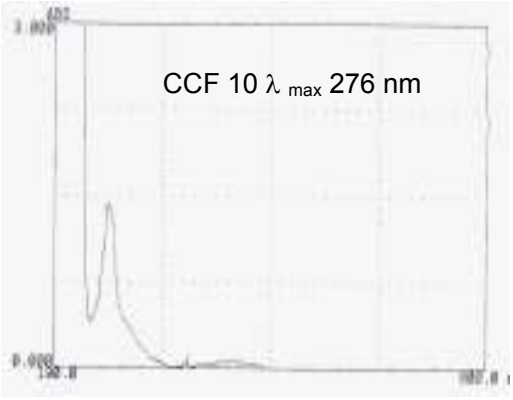
(g)


CCF8: λ max 275 nm

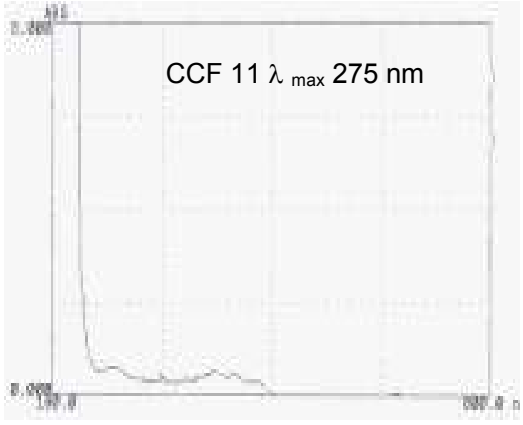
(h)


CCF 9λ max 277 nm

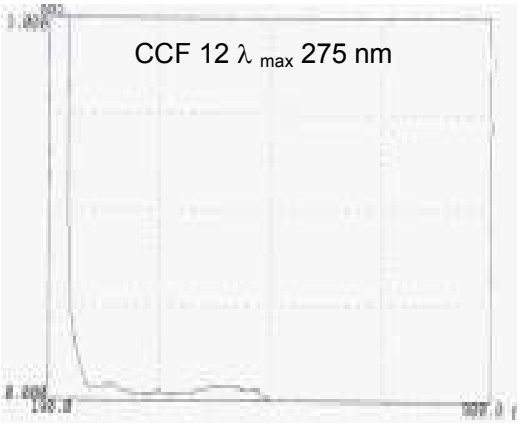
(i)


CCF 10 λ max 276 nm

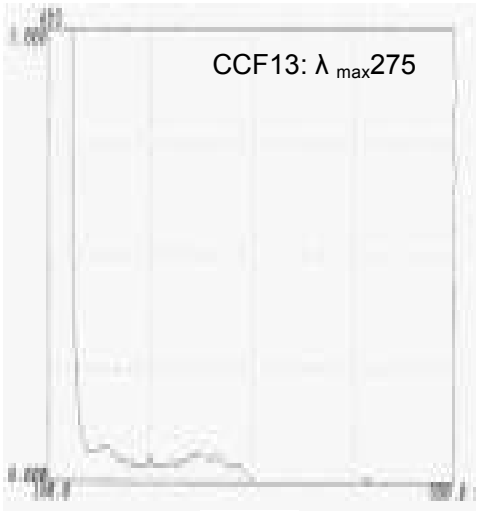
(j)


CCF 11 λ max 275 nm

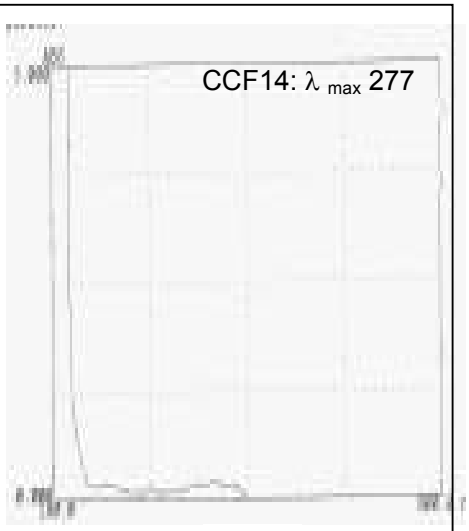
(k)


CCF 12 λ max 275 nm

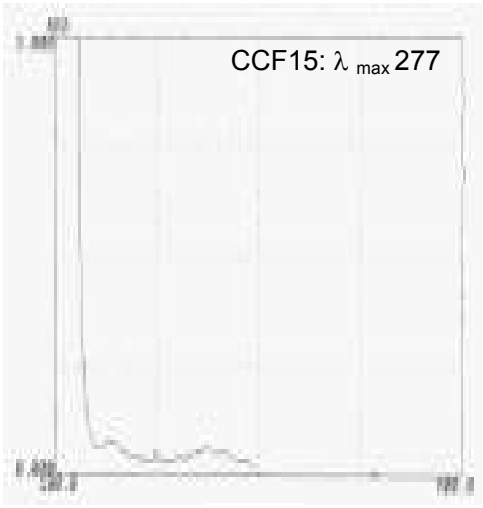
(l)



(m)



(n)



(o)

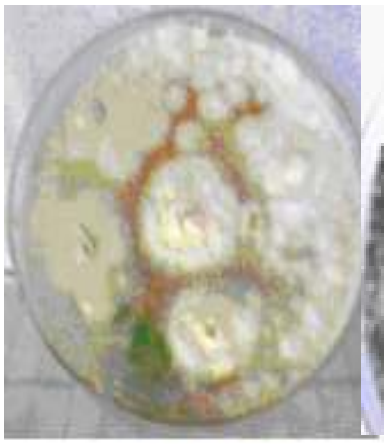
5.4 ISOLATION AND IDENTIFICATION OF PATHOGENIC FUNGI FROM *CANNABIS* LEAVES

6 different fungi were isolated from the infected/diseased *Cannabis* leaves. All the different fungi were identified as follows: *Alternaria alternata, Aspergillus niger, Mucor sp*., Unidentified Sanbio, CSM 7and CSM g. Photographs of fungal isolates are shown below (Photo 2).

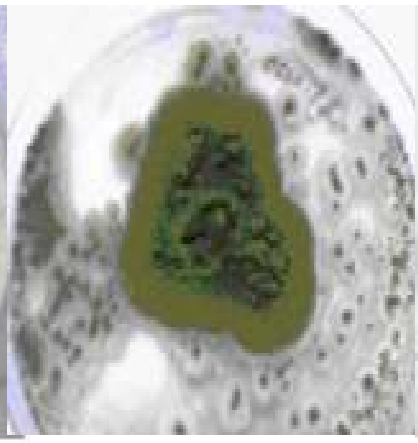
Aspergillus niger

Alternaria alternata

Mucor spp.



Unidentified fungus-SANBIO1



Unidentified fungus-CSM 7

Unidentified fungus-CSM g

Photo 2. Fungal Isolates from *Cannabis*

5.5 EVALUATION OF KOCH'S POSTULATES

The fungi isolated from the *cannabis* leaves were examined for their pathogenecity by evaluating them against Koch's postulates. The young and healthy surface sterilized leaves were sprayed with spore concentrations of 2×10^6 cfu/ml of spores, 1.5×10^6 cfu/ml of spores, 1×10^6 cfu/ml of spores and 0.5×10^6 cfu/ml of spores. Spores of three different fungi namely, Aspergillus *niger and Alternaria alternata and unidentified sabio1* were sprayed. Plates were prepared in triplicates having three leaves in each plate. Plates were incubated at $26\pm2^{\circ}C$ and checked for disease development. Leaves were observed to develop damage due to infection.

For all the three fungi no damage was seen for first 48 hrs. For *Aspergillus niger* and *Alternaria alternata* ~20% damage was observed after 72 hr. and ~40% after 120 hrs. The leaf area began to develop yellow spots after 48 hrs and with the passage of time complete chlorosis of the entire leaf was observed. The entire leaf area was covered with fungal mycelia and developed cottony appearance. The fungus continued to grow and feed on the leftover leaf material, which was finally blackened and killed. After 144 hr the leaves were totally damaged indicating maximum value of 5 on "five point damage rating scale".

For Sanbio1, only ~10% damage was observed in first 72 hrs and ~20 % damage in 120 hrs. Chlorosis and brown spots were observed in all cases after 3 days (72 hrs). In case of *Aspergillus niger* and *Alternaria alternata* the complete death occurred after 144. About 50% damage occurred on third day in Sanbio1 after 168 hr.

Fig 6. Damage Rating Table (Five Point Scale)

Table 5. Damage Rating (Five Point Scale) of *Aspergillus niger* on *Cannabis sativa*								
Concentration of Spores (cfu/ml)								
	2X 10^6		1.5 X 10^6		1 X 10^6		0.5 X 10^6	
HPT	M	SD	M	SD	M	SD	M	SD
0 hr	0	0	0	0	0	0	0	0
24 hr	0	0	0	0	0	0	0	0
48 hr	0	0	0	0	2	0	0	0
72 hr	2	0.5	2	0.3	2	0	0	0
96 hr	2.5	0.76	2.5	0.3	4	1	1.5	0
120 hr	4	0.5	4.5	0.3	5	1	3	0.29
144 hr	5	0	5	0	5	0	4.5	0.29
168 hr	5	0	5	0	5	0	5	0
192 hr	5	0	5	0	5	0	5	0
218 hr	5	0	5	0	0	0	5	0

Table 6. Damage Rating (Five Point Scale) of *Alternaria alternata* on *Cannabis sativa*								
Concentration of Spores (cfu/ml)								
	2X 10^6		1.5X 10^6		1X 10^6		0.5X 10^6	
HPT	M	SD	M	SD	M	SD	M	SD
0 hr	0	0	0	0	0	0	0	0
24 hr	0	0	0	0	0	0	0	0
48 hr	0	0	0	0	0	0	0	0
72 hr	2.5	0.29	2	0.3	3	0	1	0.29
96 hr	3	0.29	3	0.3	4	0	2	0.29
120 hr	4	0.5	4	0.3	4	0	3.5	0.29
144 hr	5	0	5	0	5	0	4	0
168 hr	5	0	5	0	5	0	4.5	0.29
192 hr	5	0	5	0	5	0	5	0
218 hr	5	0	5	0	5	0	5	0

Table 7. Damage Rating (Five Point Scale) of unidentified fungus –sanbio1 on *Cannabis sativa*								
Concentration of Spores (cfu/ml)								
	2X 10^6		1.5X 10^6		1.5X10^6		0.5 X 10^6	
HPT	M	SD	M	SD	M	SD	M	SD
0 hr	0	0	0	0	0	0	0	0
24 hr	0	0	0	0	0	0	0	0
48 hr	0	0	0	0	0	0	0	0
72 hr	1.5	0.29	1	0.3	2	0	1	0.29
96 hr	2	0	2	0.3	2	1	2	0.29
120 hr	1.5	0.5	2	0.3	3.5	0	2.5	0.58
144 hr	2.5	0.29	2.5	0.3	4	1	3.5	0
168 hr	3	1	3.5	0.3	4.5	0	4	0.29
192 hr	5	0	5	0	5	0	5	0
218 hr	5	0	5	0	5	0	5	0

HPT- Hours per Treatment; M-Mean; SD-Standard Deviation

Thus, *Aspergillus niger* and *Alternaria alternata* were put on 5 points on the “five point damage scale” only after 144 hr infection, while sanbio1 still showed only 40% damage at this point of time. Mean values and standard deviations (of triplicate plates) of damage rating data of *Alternaria alternata, Aspergillus niger* and sanbio1 are shown in the fig 6.

5.6 BIOTRANSFORMATION OF CANNABINOIDS

Spots of 1mg/ml concentration from ethyl acetate extracts of test 1, test 2, test 3 and test 4 were applied on TLC plates (silica gel GF_{254}, Merck, Germany). Occurrence of new spot and change in Rf indicated the original test compound has been biotransformed. Rf of the new spot came out to be 0.16. The biotransformed product is further analyzed with UV spectrophotometery by dissolving in ethanol as a solvent. The stock concentration was kept as 1 mg/ml. The wavelength scan was done from 800 nm – 190 nm. The λ max of the biotransformed compound comes out to be 339 with absorbance of 0.344 at 1 mg/ml concentration.

Lane 1 of TLC chromatogram showed new spot indicating that test THC has been biotransformed with Rf of 0.16. While the Lane 2 exhibiting the test 2 i.e. having extract of spent broth of autoclaved *Aspergillus niger* showed no change in the Rf or spot characteristics, indicating that the new band appearing in lane 1 is actually a biotransformed product formed after the growing fungus (test 1) modified it. Lane 3 shows standard or the

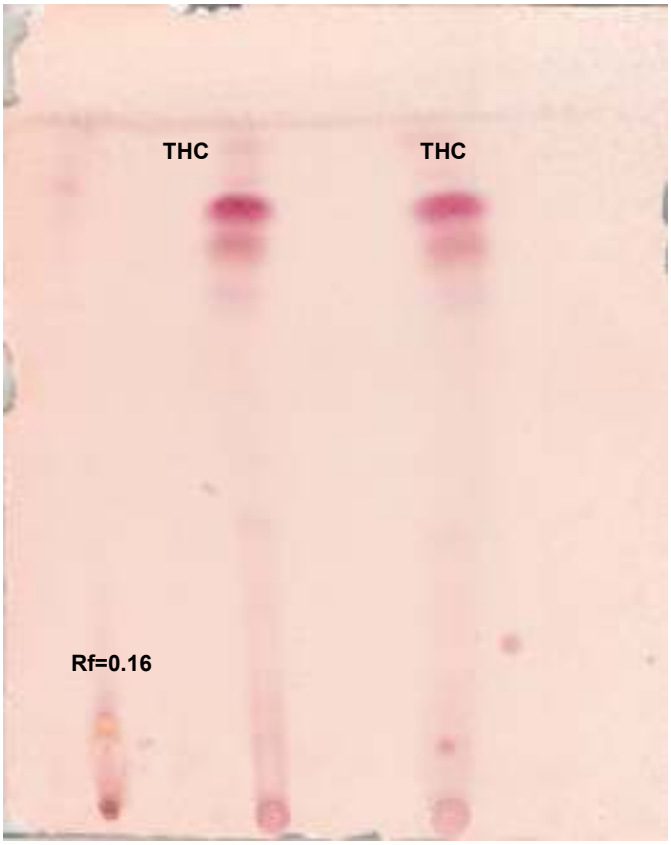
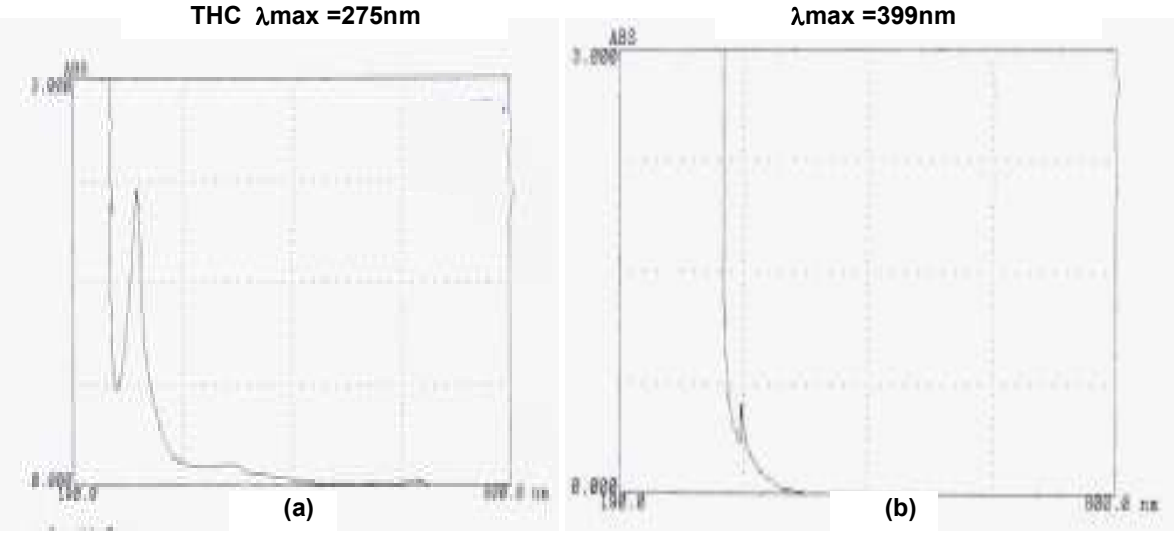


Fig 7. TLC Chromatogram of THC biotransformed with *Aspergillus niger*

THC fraction isolated and purified by TLC and used as test compound for biotransformation. Lane 4 of the TLC chromatogram shows no bands or spots indicating that the fungus did not produced any newer compounds that could be interfering in the interpretation of the results. TLC Chromatogram of biotransformed cannabinoid (THC) as shown in the Fig 7 and UV wavelength scan graphs of biotransformed cannabinoids are also shown below. UV spectroscopic studies Lane 1 and Lane 2 compounds indicate that the λmax was 399nm and 275/277 nm indicating the biotransformation occurring in the former and later exhibiting the characteristic absorption of THC (fog 8).



Fig, 8: Spectral Comparison of pure THC and the biotransformed product by *Aspergillus niger*

λ max of pure THC is 275 nm while after biotransformation it changes to 399 nm. This means some change in its structure has occurred which caused the change in UV absorption. The present study indicates that *Aspergillus niger* has the capacity to transform THC. Further studies are warranted after complete characterization of the biotransformed compound.

CONCLUSION

Cannabinoids are the active drug ingredients of the plant *Cannabis sativa.* These are secreted by glandular trichomes that are present on leaf surfaces and flower bracts. The plant has been used for self-medication since ages and this age-long use has been explored by ethnopharmacoloists. The cannabinoids, especially, THC has entered the field of medicine and drugs and is currently being used to cure many side effects associated with the major life threatening diseases like, wasting syndrome of AIDS, cancer chemotherapy, nervous disorders, multiple sclerosis and tumors.

THC pills are being approved by USA-FDA under the name of dronabinol (marinol). Sativex is the newer cannabinoid drug going to the market very soon. Thus, THC has many potent roles to play in the fields of pharmacology and medicine. But THC has many side effects associated with its intake like, drowsiness, sleepiness, headache, intoxication, clumsiness, dizziness, dry mouth, lowered blood pressure or increased heart rate.

Thus ways are thought to reduce all such unwanted side effects of THC while retaining its pharmacological potential. One such method is via microbial biotransformations. The microbes carry out different transformation reactions thus metabolizing the foreign compound given to them as substrate. Many microbes including wide species of bacteria, fungi and molds etc., have been successfully experimented to biotransform the different cannabinoids. In many cases, the transformed products are being used as intermediates to design newer synthetic cannabinoids.

In this dissertation work, we here tried to assess biotransform the THC extracted from the *Cannabis sativa* leaves with a pathogenic fungus *Aspergillus niger.* It transforms THC into some other different compound as is evident by the Rf of 0.16 and λ max of 344 nm. Which is different from the THC. Further studies are warranted after complete characterization of the biotransformed compound.

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