

A Review On Anti Microbial And Anti Fungal Properties Of Hemp Seed Oil

¹Harsha Indoriya, ²Sakshi Sapkal, ³Bhagyashree Ambapure, ⁴Anuradha Bombale

Department of Pharmaceutics, P.R.Pote Patil College of Pharmacy, Amravati-444602

Corresponding Author

Harsha Indoriya

Department of pharmaceutics, P.R.Pote Patil College of pharmmac, Amravati-444602

ABSTRACT : *Cannabis sativa L.* has many interesting features. Although, presently it is mainly view as a narcotic, only few known that *cannabis sativa L.* is great source of fiber and medicinal activity. The metabolic profile of the *cannabis* is extremely rich. More than 480 active compounds of *cannabis* has already isolated among which 180 are belong to cannabinoids family. Most studied secondary metabolites are the delta-9-tetrahydrocannabinol (Δ^9 -THC) which is known to have psychoactive properties, and the cannabidiol (CBD) known for its medicinal potential. Antimicrobial properties are attributed mostly to some of these active compounds. Several studies investigated these properties.

Keywords: Hemp seed oil, cannabis, metabolites, THC, CBD, B sitosterol, essential fatty acid.

INTRODUCTION :

In the era of 19th century in Western Europe, cannabis procure attention in the medical science. Later, in 1860, the first clinical conference about cannabis took place in the United States and afterwards many scientific papers have been published (Zuardi 2006). The situation changed when in 1942 cannabis was removed from United States Pharmacopoeia and lost its medical statute due to its potential to lead to "insanity" (Fankhauser 2002). Following the US, most of European countries adapted in 1971 the Convention on Psychotropic Substances instituted by United Nations by which cannabis became illegal (Amar 2006).

Although cannabis is mostly know for its medicinal and psychoactive properties it is widely used as a source of fibers in textile productions. Furthermore, hemp cannabis grown for industrial purposes gain recently some importance as a biocomposite material used for construction and insulation National Non-Food Crops Centre. Whole seeds and, seed oil are consumed by human, seeds and leaves are frequently used as a feed to animals. More over seed oil and stalks can be burned as fuel Clark 2002.[1]

Synonyms : Indian hemp, Indian cannabis, hashish, bhang, ganja, charas, *Cannabis indica*, marihuana.[2]



Fig.1.1 CANNABIS SATIVA PLANT

Biological Source :

Cannabis consists of dried flowering tops of the pistillate plants of *Cannabis sativa* Linn., belonging to family Cannabinaceae.[2]

Geographical Source :

Cannabis occurs in India, Bangladesh, Pakistan, Iran, Central America, United States, East Africa, South Africa, and Asia Minor.[2]

Cultivation and Collection :

Cannabis is an annual dioecious herb, which is cultivated by seed sowing method. The seeds are sown on seedbeds in the month of August and after a month the seedlings are transplanted into the open field. The male plants, which have attained the maturity, are taken and shaken over the female plants so as to facilitate pollination. The flowering tops of female plants are collected in February or March. They are made into bundles and treated under the foot to form flat masses. The flat masses are dried under the shade to obtain 'ganja'. In India the tops are treated to form rounded masses called as 'ganja'. [2]

Cannabis Products :

The following products are prepared from Cannabis. Ganja: It contains up to 10% of its fruits, large foliage leaves and stems over 3 cm. It is known as Flat or Bombay ganja when 30 cm long pieces of the herb are made into bundles and pressed. Round or Bengal ganja is prepared by rolling the wilted tops between the hands. Ganja is legally produced only by a few licensed growers in Bengal and southern India. The seeds are sown in rows about 1.3 m apart and male plants are discarded. The resinous tops of the unfertilized plants are cut about 5 months after sowing and pressed into cakes. The yield is nearly 120 kg per acre. [2]

Bhang or Hashish :

It consists of the larger leaves and twigs of both male and female plants. It is smoked with or without tobacco. It is unfit for medicinal use owing to deficiency of resin. It is also taken in the form of an electuary made by digestion with melted butter.

Charas:

It is the crude resin obtained by rubbing the tops between the hands and beating them on a piece of cloth. This is an inferior product. It may be collected by beating the flowering tops in coarse cotton cloths spread on the ground. A greenish-brown soft mass adheres, and may be purified by pressing it through the cloths. The resin is scraped off. It is mixed with many smoking mixtures.[2]

Morphology :

Cannabis occurs in flattened, rough, dull dusky green masses. The dried resin is hard, brittle, and does not stick. The flat-ganja is flattened mass of a dull green colour. The odour is very marked in the fresh drug and becomes faint afterwards; taste is slightly bitter.

The flat- or Bombay ganja occurs in agglutinated flattened masses of a dull green or greenish-brown colour. The resin is not sticky but hard and brittle; the odour, which is very marked in the fresh drug, is faint. The drug has a slightly bitter taste. The lower digitate leaves of the plant are not found in the drug. The thin, longitudinally furrowed stems bear simple or lobed; stipulate bracts which subtend the bracteoles, enclosing the pistil late flowers. The bracts are stipulate and the lamina may be simple or three-lobed. The bracteole enclosing each flower is simple.[2]

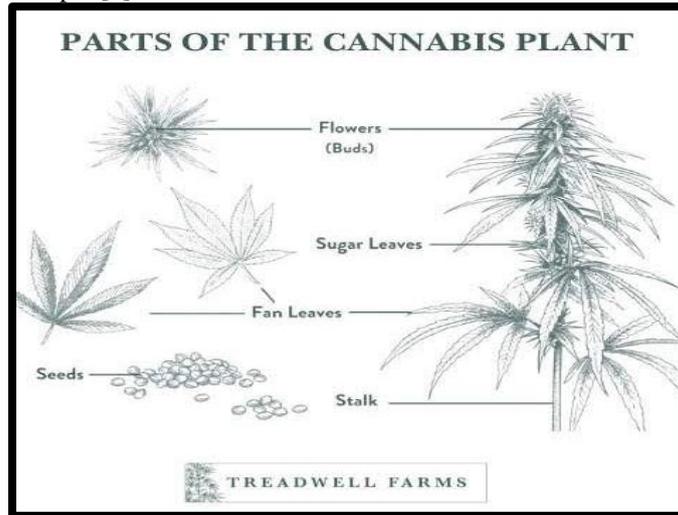


Fig.1.2PARTS OF THE CANNABIS PLANTS

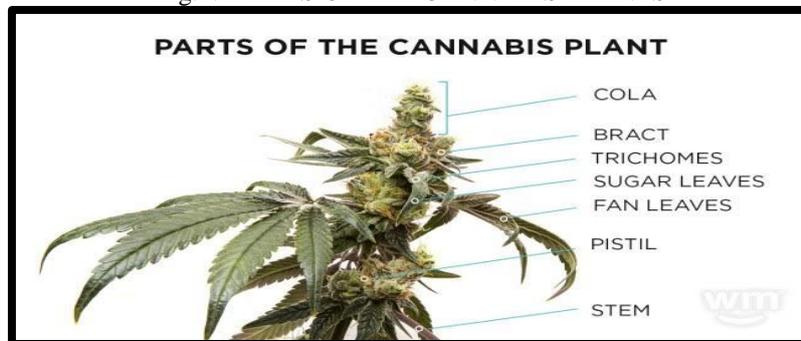


Fig.1.3 PARTS OF THE CANNABIS PLANTS

Microscopy :

The resin is secreted by numerous glandular hairs. The head is usually eight-celled and the pedicel multiseriate or unicellular. Corrigan and Lynch, a reagent consisting of vanillin in ethanolic sulphuric acid, stains the cannabis glands a deep reddish-purple. Abundant conical, curved, unicellular hairs are also found, many having cystoliths of calcium carbonate in their enlarged bases. These cystolith hairs are not confined solely to the genus Cannabis. Cluster crystals of calcium oxalate are abundant, particularly in the bracteoles.[2]

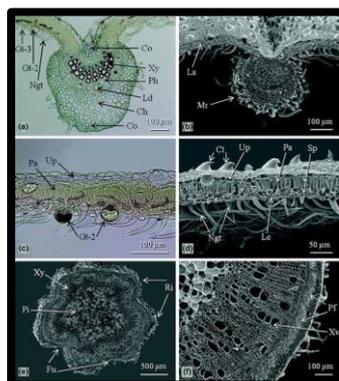


Fig 1.4.T.S OF CANNABIS SATIVA

Chemical Constituents :

Cannabis consist of 15 to 20% resin, the resins are amorphous, semisolid, brown coloured, soluble in ether, alcohol, and carbon disulphide. The most important active constituents present in cannabis are: cannabidiol, cannabidiolic acid, cannabinol, cannabichromene, and trans-tetrahydrocannabinol. Cannabis also contains Cannabidiolic acid, cannabidiol A 9, tetrahydrocannabinol, cannabinol A9, Tetrahydrocannabinol (THC), volatile oil, trigonelline, and cholene.[2]

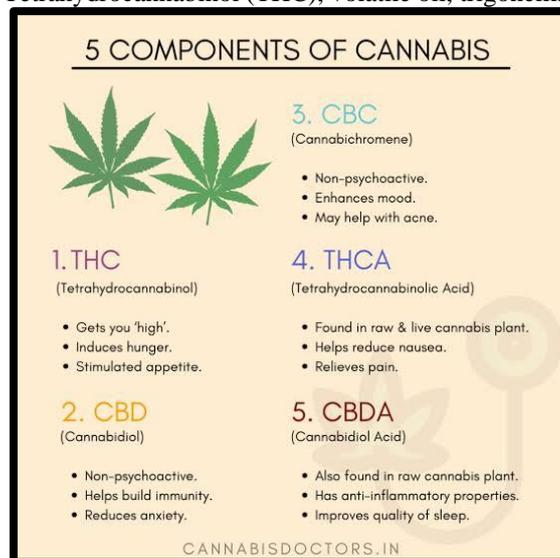


Fig.1.5 COMPONENTS OF CANNABIS

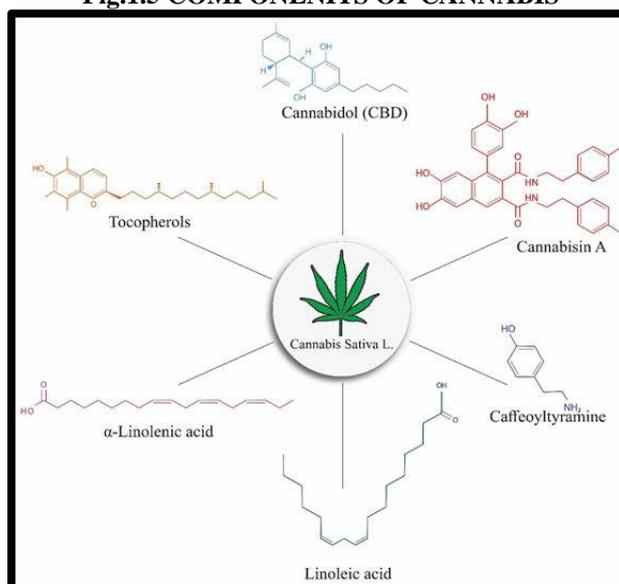


Fig.1.6 STRUCTURES

What are the effects of cannabis?

The effects experienced by the cannabis (marijuana) user are variable and will depend upon the dose, method of administration, prior experience, any concurrent drug use, personal expectations, mood state and the social environment in which the drug is used.[3]

Effects of cannabis (marijuana) include:

An altered state of consciousness. The user may feel "high", very happy, euphoric, relaxed, sociable and uninhibited.

Distorted perceptions of time and space. The user may feel more sensitive to things around them, and may also experience a more vivid sense of taste, sight, smell and hearing.[3]

Increased pulse and heart rate, bloodshot eyes, dilated pupils, and often increased appetite ("the munchies").

Impaired coordination and concentration, making activities such as driving a car or operating machinery difficult and dangerous.

Negative experiences, such as anxiousness, panic, self-consciousness and paranoid thoughts.

People who use large quantities of cannabis may become sedated or disoriented and may experience toxic psychosis -- not knowing who they are, where they are, or what time it is. High doses may also cause fluctuating emotions, fragmentary thoughts, paranoia, panic attacks, hallucinations and feelings of unreality.

Various concentrations of THC, cannabidiol (CBD), or hybrid products exist in products found cannabis dispensaries in states that have legalized recreational marijuana use.[3]

How long does it take cannabis to work?

The effects of inhaled cannabis are felt within minutes, reach their peak in 10 to 30 minutes, and may linger for two or three hours. However, edible cannabis, which may contain higher amount of THC, can take a longer period of time to reach full effect as it must be absorbed from the gastrointestinal tract. The effect of edible cannabis can last up to 12 hours.

THC is highly lipid soluble and can be stored in fat cells possibly for several months. Stored THC is released very slowly, and unevenly, back into the bloodstream.[3]

Epidiolex: use in childhood seizures :

In June 2018, the FDA approved Epidiolex (cannabidiol or CBD) from GW Pharmaceuticals. Epidiolex is an oral solution for patients two years and older to treat Lennox-Gastaut syndrome and Dravet syndrome -- two severe forms of seizures that begin in childhood.

Epidiolex is the first FDA-approved treatment in the U.S. that contains a purified drug substance derived from marijuana and the first treatment for Dravet syndrome. In April 2020 the DEA notified the manufacturer GW Pharma that it no longer considers Epidiolex a controlled substance (was previously a C-V controlled substance) under the Federal Controlled Substance Act.

In robust Phase 3 studies with 516 patients with either seizure type, Epidiolex, taken along with other medications, was shown to be effective in reducing the seizure frequency when compared with placebo.

Common side effects with Epidiolex can include:

Tiredness

Elevated liver enzymes

Diarrhea

Lowered appetite.[3]

Uses :

Cannabis resin is tonic, sedative, analgesic, intoxicant, stomachic, antispasmodic, antianxiety, anticonvulsant, antitussive, and narcotic. Cannabis causes only psychic dependence and act upon the nervous system. While cannabis (marijuana) remains a federal DEA Schedule 1 controlled substance, research has resulted in development and marketing of medications which are synthetic prescription cannabinoid products.

Marinol (dronabinol) is used for the control of nausea and vomiting caused by chemotherapeutic agents used in the treatment of cancer and to stimulate appetite in AIDS patients.

Cesamet (nabilone) is used for the control of nausea and vomiting caused by chemotherapeutic agents used in the treatment of cancer.[3]

ANTI BACTERIAL PROPERTIES :

Cannabis and its antibacterial activity For a long time cannabis has been regarded as possessing an antibacterial activity against a wide range of pathogenic bacteria as well as against some fungi. The antibacterial character is contributed mainly from Δ^9 -THC and CBD. However number of studies demonstrated that plant extracts or essential oils also present this activity. Wasim and coworkers (1995) tested ethanol and petroleum cannabis extracts prepared out of leaves against different microorganisms[1]. The results showed that the extracts have strong inhibitory effects on both Gram positive (*Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Micrococcus flavus*) and Gram negative bacteria (*Proteus vulgaris*, *Bordetella bronchioseptica*). The antibacterial activity of extracts was compare with the effect of the common antibiotic Cephalexin. The results were comparable, however the concentration of leaf extract was 250 times higher than the concentration of the antibiotic. It can be explain in two ways. First of all the plants that were used for this study were wild varieties with unknown content of cannabinoids, therefore the concentration of active compounds could be very low. Second of all the extracts were prepared out of leaves. Although cannabis leaves can contain some cannabinoids, it is well known that the highest concentration of these substances are usually found in the inflorescences. the in-vitro antimicrobial activity of the essential oils extracted from the inflorescence of three hemp varieties of low-THC content. The antimicrobial activity was tested against Gram (+), opportunistic and moderate pathogenic bacteria such as *Clostridium* spp. and *Enterococcus* spp., and against Gram (-), phytopathogens bacteria including *Pseudomonas* spp. and *Pectobacterium* spp. Results showed that oil made of the Futura variety was the only oil that was able to inhibit all Gram (+) and Gram (-) bacteria, as well as yeasts. Characterization of essential oils revealed that this variety had a significantly higher concentration of terpinolene compare to the three others. Therefore, it was assumed that the antimicrobial activity was attributed to this compound. The results suggest that although Δ^9 -THC and CBD are the most studied compounds, there are still many compounds out of 480 already discovered in the cannabis plant that have not yet been tested for antimicrobial properties. It is possible that some of these substances are even more efficient in antibacterial agents. Furthermore, the interactions between compounds of essential oils are still not clear. It is highly probable that the synergic and antagonistic effects of oil compounds exist and are the cause of different activities of the oils. Indeed, the synergistic activity of some monoterpenes, such as terpinolene and pinenes, have been already reported. studied the effect of *Cannabis sativa* L. seed oil as well as petroleum ether and methanol extracts of the whole plant on two Gram (+) organisms (*Bacillus subtilis*, *Staphylococcus aureus*), and two Gram (-) organisms (*Escherichia coli*, *Pseudomonas aeruginosa*). The *Cannabis sativa* seed oil demonstrated a strong antibacterial activity (21 - 28 mm) against *Bacillus subtilis* and *Staphylococcus aureus*, and moderate activity (15 mm) against *Escherichia coli* and *Pseudomonas aeruginosa* (16 mm). although the extracts were prepared from different plant materials. Cannabis seeds are known for their nutritional values and they are being considered as a great source of fatty acids, however the concentration of secondary metabolites is rather low. Whole plant extracts based on methanol and petroleum ether showed a slightly higher antimicrobial activity, particularly in the case of *Bacillus subtilis* where the inhibition zone was 29 and 28 mm respectively.[1]

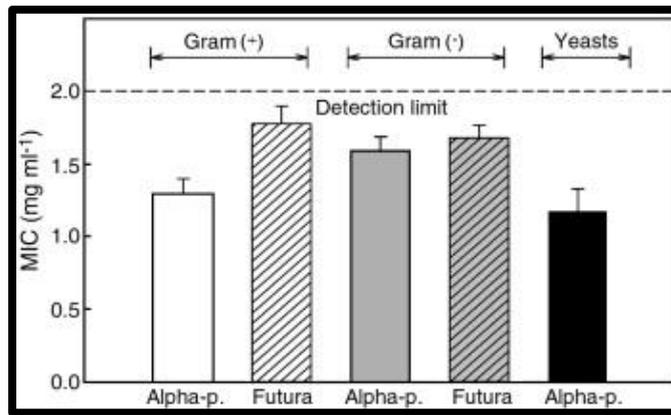


Fig.1.7 GRAPH OF GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

ANTI FUNGAL PROPERTIES :

Antifungal properties of cannabis Few researchers investigated antifungal properties of the cannabis and its secondary metabolites. Although this effect is not as extensively studied and as strongly pronounced as in the case of antibacterial activities, some papers report that plant extracts can be successfully used in the control of pathogenic fungi. demonstrated that the ethanol and petroleum extract of cannabis leaves are effectively inhibiting the growth and development of the common human pathogenic fungi *Candida albicans* and *Aspergillus niger*, responsible for the black mould in fruits and vegetables. The zone of inhibition in both cases was significantly higher compare 80 , however the concentration of the leave extract was 10 times higher compare to antibiotic. Similarly, studied the effect of *Cannabis sativa L.* seed oil as well as petroleum ether and methanol extracts of the whole plant on two fungi *Aspergillus niger* and *Candida albicans*. The seed extract as well as the whole plant methanol extract turned out to be inactive against the two fungi tested, but the whole plant petroleum ether extract showed a modest activity against *Candida albicans*. Pal and coworkers (2013) tested the extracts of eleven weed plants for their antifungal activity against seed-borne phytopathogenic fungi *Alternaria SPP.* All plants demonstrated antifungal properties, some performed significantly better than others. Although *Cannabis sativa L.* was not the most efficient among studied plants, it did show quit height percentage of mycelial growth inhibition. Among 5 different types of extracts, the acetone based extract turned out to be the most powerful antifungal agent.[1]

The Composition of Hemp Seed Oil and Its Potential as an Important Source of Nutrition :

Cannabidiol (CBD) has been found to be present in hemp seed oil as well. Although not explicitly produced within the seed, traces of cannabinoid contamination have been reported to result from the pressing of the oil Reports of cannabinoid contamination have been focused primarily on delta-9-tetrahydrocan-Leizer et al. 37nabinol (THC) with THC levels in oil reported at up to 50 ppm .The production and storage ofboth CBD and THC occur in the glandular structures of the plant andthe concentrations of CBD are typically much higher than THC inmost fiber and oil varieties of hemp.

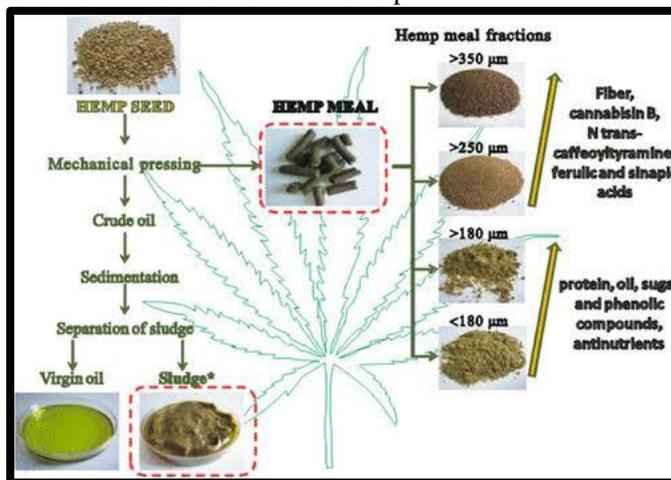


Fig1..8 EXTRACTION OF HEMP OIL

Therefore, it can be assumed that the concentration of CBD as a contaminant in the oil would be greaterthan the concentration of THC which has been reported in the literature. The presence of CBD is significant because it has documented anticonvulsive, anti-epileptic, and antimicrobial properties . Although the levels of CBD within the oil are typically small, many health benefits may still be gained from its presence.[1]

Fatty Acids in Hemp Oil :

The hemp seed oil used in this study was pressed from Canadiangrown seed of the French variety Fedora-19 and was provided by CGPCanada, Ltd. The results of fatty acid analysis .These results further strengthen previous reports that the relative ratiosand composition of hemp oil fatty acids are ideal for human nutrition.Benefits of Essential Fatty AcidsWhile there are many sources for omega-3 PUFA in the diet, hempseed oil is exceptionally rich in these compounds, which are usually present in the nutritionally optimal ratio of omega-6 to omega-3 PUFA(LA to LNA) of 3:1 , LAconcentrations ranged from 52-62% of total fatty acid compositionwhile LNA concentrations ranged from 12-23%. The range of concentrations of fatty acids results from the natural variation of individual samples of the Fedora hemp oil being tested. Several factors, including processing and storage methods, as

well as age of the samples being tested, could contribute to the variability of the fatty acid profile. As a result of the change in dietary habits within the past century, the intake of trans fatty acids has increased dramatically. Studies have shown conclusively that trans fatty acids increase total cholesterol levels and diminish the levels of “good” high density lipoprotein (HDL). By supplementing the diet with high levels of unsaturated cis fatty acids, some of these negative effects can be reversed (Erasmus, 1999). With respect to modern diets, the amount of LA consumed compared to the amount of LNA consumed has increased exceptionally in the past 100-150 years.

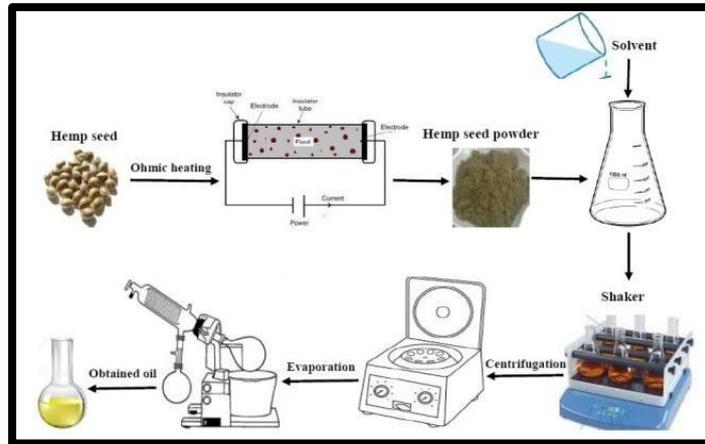


Fig.1.9 PROCESS OF EXTRACTION OF HEMP SEED OIL

Omega-3 PUFA have been reported to have an inhibitory effect on cancer and tumor growth. Increased consumption of omega-3 PUFA have not been shown to exhibit any negative side effects, but their beneficial qualities have been repeatedly confirmed. In addition to their anticancer properties, omega-3 PUFA have been shown to lower blood pressure and blood cholesterol levels, help normalize fat metabolism and decrease insulin dependence in diabetics, increase overall metabolic rate and membrane fluidity, and exhibit anti-inflammatory properties, specifically with regard to relieving arthritis. The benefits of omega-3 PUFA are not only present when taken in large quantities but the regular intake of recommended levels (2-2.5% of caloric intake/day) can be sufficient to provide many of its nutritional qualities. [4]

Natural Products in Hemp Oil :

Cannabidiol :

Pharmacological Properties of Cannabidiol. Cannabidiol (CBD) has been shown to possess several desirable pharmacological properties. Journal of Nutraceuticals, Functional & Medical Foods 44 ties which are exhibited in absence of the psychoactive properties of THC, which are usually associated with the cannabinoids. Although the levels of CBD detected in the oil were low at 10 mg/kg, its presence could still provide some benefit. CBD has been reported to reduce tremors in dystonic movement disorders with minimal side effects (Consroe et al., 1986). Patients receiving doses of CBD ranging from 100-600 mg/day had tremor reductions of 20-50%. The anti convulsant and anti-epileptic activity of CBD has also been well documented. CBD has been found to be relatively selective with respect to the central nervous system (CNS), in contrast to THC. Its anticonvulsant activity is on the same order of magnitude of THC, but unlike THC, it lacks psychoactivity. CBD's added efficacy as an anti-epileptic, without the associated side effects of psychoactivity, give it great pharmacological potential. [4]

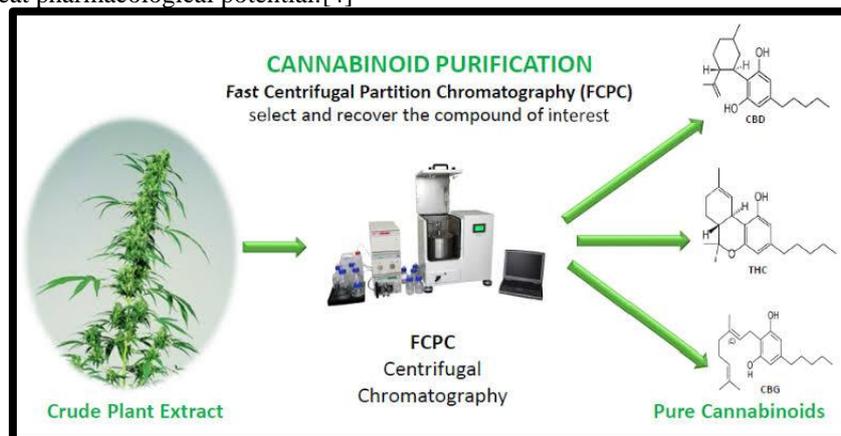


fig1.10 PURIFICATION OF CANNABINOID

β - Sitosterol :

Another component of hemp seed oil with several reported activities is β-sitosterol. Although studies have primarily demonstrated the efficacy of β-sitosterol in reducing hypercholesterolemia, additional antiviral, antifungal, and anti-inflammatory properties have been studied and observed. Plant sterols have been known to affect plasma cholesterol levels by blocking cholesterol absorption through crystallization and coprecipitation. Within the intestinal lumen, phytosterols reduce cholesterol solubility by excluding it from micelles, thereby preventing its absorption. In addition, competition exists between the sterols and cholesterol for uptake into the intestinal mucosa. A quantitative representation of this can be seen in human studies. Patients given 500 mg of cholesterol daily in their diets in addition to 1 g of β-sitosterol showed decreased cholesterol absorption. Leizer et al. 47. Mean reduction levels were

42%, demonstrating the efficacy of β -sitosterol even at low concentrations, sterol concentrations based on β -sitosterol were measured in sufficient quantities at 100-148 g/L.

Although β -sitosterol was the predominant sterol, other minor sterols may have contributed to this measurement. At these levels, many of β -sitosterol's beneficial qualities will be obtainable.[4]

Terpenes :

The presence of several terpenes were confirmed in the seed oil, the most abundant of which were β -caryophyllene and myrcene which were found at 740 mg/L and 160 mg/L, respectively. The terpene compounds, in general, are primarily found in the essential oil of Cannabis rather than in the seed oil (Hendriks et al., 1978) as a result of their production in the glandular structures on the aerial portions of the plant. These compounds are a component of the characteristic aroma of Cannabis and may impart some of these properties to the seed oil. Additional benefits may be provided to the oil as well. Some previously noted pharmacological properties of β -caryophyllene would include anti-inflammatory and cytoprotective activities which may too be active in the seed oil. In addition, it has been reported that myrcene exhibits antioxidant properties (Duke, 1999). The presence of β -caryophyllene and myrcene, even if only present as contamination components, add beneficial value to an already nutritionally important food product.[4]

Methyl Salicylate (Oil of Wintergreen) :

The medical benefits of plant salicylates have been enjoyed by people for centuries. Today aspirin or acetylsalicylic acid, a closer relative of methyl salicylate, is one of the most widely used drugs in the world because of its antipyretic, anti-inflammatory and analgesic properties. Once injected, methyl salicylate can be hydrolyzed to salicylic acid, a common active ingredient of aspirin and most other salicylates. Thus, pharmacological effects of methyl salicylate are similar to those of aspirin. Also, millions of people regularly take low doses of salicylates (aspirin) to reduce the risk of heart attacks, strokes and cancer. Methyl salicylate deserves particular attention as a beneficial component of hemp oil, even if present in trace quantities.[4]

CONCLUSION :

Cannabis sativa L. is a very powerful plant which presents many interesting properties due to its rich metabolic profile. Although most people associate it with drugs, many scientific data showed that its medicinal features should not be neglected. Number of studies showed its potential as an antimicrobial agent. An important amount of them focus on antibacterial properties, however there is less studies analyzing antifungal properties. The physiology of cannabis is already well understood. It is known that the highest concentration of active compounds is concentrated in inflorescent, therefore it is surprising that in most studies assessing antimicrobial properties of cannabis, leaves and seeds are usually used to prepare extracts. Furthermore, many of the studies look at the response of the same pathogenic fungi such as Aspergillus and Candida, or bacteria such as Bacillus or Staphylococcus. Due to environmental issues associated with pesticides use, many studies nowadays focus on finding alternatives to synthetic disease control chemicals. Natural plant extracts might be one of them. Therefore, future studies should search for cannabis use against plant pathogenic bacteria and fungi instead of focusing on the same model organisms[1].

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