Potential Health Benefits of Cannabis Extracts: A Review

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Citation

Abstract
A central tenet underlying the use of plant preparations is that herbs contain many bioactive compounds. Cannabis contains tetrahydrocannabinols (THC) a primary metabolite with reported psychotropic effects. Therefore, the presence of THC makes controversial the use of Cannabis to treat diseases by which their uses and applications were limited. The question then is: is it possible to use the extracts from Cannabis to treat the diseases related with it use in folk medicine? More recently, the synergistic contributions of bioactive constituents have been scientifically demonstrated. We reviewed the literature concerning medical cannabis and its secondary metabolites, including fraction and total extracts. Scientific evidence shows that secondary metabolites in cannabis may enhance the positive effects of THC a primary metabolite. Other chemical components (cannabinoid and non-cannabinoid) in cannabis or its extracts may reduce THC-induced anxiety, cholinergic deficits, and immunosuppression; which could increase its therapeutic potential. Particular attention will be placed on non-cannabinoid compounds interactions that could produce synergy with respect to treatment of pain, inflammation, epilepsy, fungal and bacterial infections. The evidence accessible herein pointed out for the possible synergism that might occur involving the main phytocompounds with each other or with other minor components.

1. Introduction

Plant extracts were regularly employed by people in prehistoric time until at the present. In ancient civilizations plant extracts were used for biomedically curative and psychotherapeutic option. Although some of the therapeutic properties attributed to natural products have proven to be erroneous, medicinal plant therapy is often based on speculation and superstition. On the other, approximately 80% of the world’s population (mainly in non-developed countries), still relies upon natural preparations for medication in primary health care. Almost 50,000 species of plants have been used for medicinal purposes [1]. Of all the species plants with biomedical reports, Cannabis sativa is one of the most remarkable because it is considered to be useful for almost any use [2].

The use of the C. sativa on medicinal issues varies, and the literature reports that it is used to treat enteric infections, inflammatory conditions, disorders of motility, emesis and abdominal pain etc [3]. Interestingly, other biomedical benefits where the potential use of cannabis has been suggested include epilepsy, glaucoma and asthma.

The chemical composition of Cannabis sativa has been studied previously [4]. It was found to contain an enormous variety of chemicals compounds [5]. At least of the 483 chemical compounds identified are exclusive to Cannabis, the more than 60 of which are phytocannabinoids (group of C21 terpenophenolic). They are divided into 10 subtype: 1) Cannabigerol, 2) Cannabichromene, 3) Cannabidiol, 4) ∆9 Tetrahydrocannabinol, 5) ∆8
THC, 6) Cannabicyclol, 7) Cannabielsoin, 8) Cannabinol and Cannabinodiol, 9) Cannabittrol, and 10) Miscellaneous.

The presence of noncannabinoid-type constituents was also reported, such as terpenoids, hydrocarbons, phenols fatty acids, flavonoids, alkaloids, phytosterols and carbohydrates. In addition, simple Alcohols, Aldehydes, Ketones, Acids, Esters, and Lactones, have been identified. These phytochemicals altogether contribute to the unique biological properties of cannabis plant [6].

Since, $\Delta^9$-THC is the toxicologically most important and main studied chemical compounds of the Cannabis plant, responsible for most of the behavioral and physiological effects of current Cannabis preparations. The chemical structure was described in 1964 and its synthesis was published in 1965 [4-6]. In the 1980, the demonstration of the existence of cannabinoids receptors and the discovery of their endogenous ligands (named endocannabinoids) was an important point in establishing how Cannabis exerted its biological effects [7, 8].

Moreover, it was observed that $\Delta^9$-THC, is very lipophilic get distributed in adipose tissue, liver, spleen and lung. This chemical compound is metabolized in the liver by microsomal hydroxylation and oxidation catalyzed by enzymes of cytochrome P450 complex. The hydroxylation of $\Delta^9$-THC produces the psychoactive compound 11-hydroxy $\Delta^9$-Tetrahydrocannabinol, and the posterior oxidation generates the inactive chemical entities 11-nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol [9].

On the other hand, it is known that Cannabidiol, a non-psychotropic component of Cannabis is capable of affecting the function of cytochrome P450 3A11, which biotransforms THC to the four time more psychoactive 11-hydroxy-THC. Cannabidiol also reduces anxiety and the other disagreeable psychological side effects of THC. The dual effects reported are important, considering the folk use of this plant to activate the Nervous System [7-10].

Cannabis plant also contains cannabinoid carboxylic acids which lack psychoactive properties and whose connotation is based on the fact that they transform into an active form of THC after they are heated, especially during cooking or when smoking. On the other hand, it has been demonstrated that, $\Delta^8$ - THC may oxidize into non-active chemical compounds when cannabis resin is stored. These finding contributed to characterize the biological activity of Cannabis and to understand the ability of its extracts to enhance behavioural and pathophysiological responses [11-14].

2. Noncannabinoid-Type Constituents and Its Potential

The presence of terpenoids was also reported. These metabolites were chosen as chemo type markers as they are considered to be one of the main bioactive constituents in Cannabis plant [15, 16]. Various studies showed a variation of relative content of terpenes between species and suggest that, terpene variation can be used as a tool for characterization of cannabis bio types. In these studies different terpenes profile was found; therefore the authors concluded that the different biological effects observed may be attributed to variations in the chemical profile of these metabolites [15-17].

These volatile compounds are lipophilic and permeate lipid membranes. That property probably facilitates its passage across the blood-brain barrier (BBB) after inhalation [17, 18]. For instance, terpenoids of Cannabis have been related to the modulation of THC activity through the action on receptors level by sequestering THC, by altering lipid around the receptor, or by increasing the fluidity of neuronal membranes [19-21]. Terpenoids have been related to the modulation of various receptors and neurotransmitters. For example, some terpenes can affect serotonin reuptake, increase norepinephrine and dopamine activity as well as GABA [19-23].

Although in folk medicine, Cannabis terpenoids is widely used as anti-inflammatory, there are some studies documenting this activity. De Oliveira et al. (2006) determined the anti-inflammatory effect of Cannabis but using only a myrcene (monoterpene) or caryophyllene (sesquiterpene) extracts. In these studies a decrease of prostaglandin (PG$_{2}$) levels were observed, a similar activity was observed in terpenes present in marijuana smoke such as carvacrol, eugenol, p-vinylphenol [24, 25].

With the aim to identify the active compounds of the Cannabis extract, the authors made a fractionation of it. Subfractions with Cannabis essential oil were obtained and analyzed in experimental model. They observed that, subfraction essential oil exhibits significant anti-inflammatory activity than its individual compounds. These data suggest an additive and synergic effect of the total oil extract. Others authors observed that, essential oil affect serotonergic receptors 5-HT$_{1A}$ and 5-HT$_{2A}$. These results could explain the analgesic effect (pain) and the mood alteration caused by Cannabis [26].

The studies performed with Cannabis extracts in the field of infections revealed that plants are potential sources of antimicrobial agents. However, essential oil on agar plate was inactive against staphylococcus aureus and Streptococcus faecalis, at 0.5 mg/mL; and produced weak activity against Pseudomonas fluorescens and Escherichia coli, minimum inhibitory concentration 10 mg/mL and 5 mg/mL, respectively [27-33]. On the other hand, myrcene, the most abundant monoterpen in Cannabis displays remarkable activity against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli obtaining an equivalence of $15.2 - 1.0 \mu$g/mL with Cephazolin used as reference antibiotic. Studies demonstrated the antimalarial activity of essential oil rich in caryophyllene and terpineol at 100 mg/mL against two strains of Plasmodium falciparum [27-34].

A gastric cytoprotective property of individual caryophyllene was also showed in rodents against challenge...
with absolute ethanol and hydrochloric acid, and this effect does not influence the production of gastric juice and pepsin. Besides, these results support the popular use of Cannabis in gastric disturbances such as gastric ulcers and gastric disorders associated with microorganism [34-36].

Chemoprevention by medicinal plants has received growing attention in recent years as a promising approach in controlling the incidence of cancer and other related disease [37]. For instance, cytochrome P450 (CYP) dependent monooxygenases are a superfamily of enzymes involved in the metabolism of endogenous and xenobiotic compounds, including pharmaceuticals. Terpenoids compounds, in general have been associated to multiple CYP enzymes. This observation is supported on previously results, that shows that, β-myrcene, a noncyclic monoterpene, induces expression of CYP 2B enzymes in rodents, and others monoterpenoids inhibit CYP 2B1 function in rat liver microsomes [36, 37].

Aflatoxin B1 is a promutagen agent produced by Aspergillus flavus and Aspergillus parasiticus, these are fungal contaminants present in moldy marijuana. After aflatoxin B1 is metabolized by P450 2B1, it becomes a carcinogenic compound. A survey of the relevant literature revealed that, myrcene inhibit this biotransformation as do other terpenoids constituents of cannabis, such as limonene, α pinene, terpinene and citronellal [36, 38]. Some reports have indicated that monoterpenes, such as those present in Cannabis resin have been related to the inhibition of cholesterol synthesis, induction of apoptosis in cells with damaged DNA, and inhibition of protein isoprenylation implicated in malignant deterioration, among others activities [20, 36].

Cannabis contains about twenty-three flavonoids compounds, which apparently retain their bioactivity in Cannabis smoke [39]. These chemical compounds existing mainly as C=0- and O-glycosides of the flavon and flavonol type aglycones, such as apigenin, luteolin, quercetin, kaempferol, vitexin, isovitexin and orientin. These chemical compounds may be significant for the overall pharmacological effects of THC and the other phytocannabinoids by either synergistically enhancing them, or by decreasing their side effects. Flavonoids are able to inhibit the cytochrome P450 monooxygenase enzymes, thereby altering the pharmacokinetics of THC, which is transformed into 11-hydroxy-THC by same enzymes. Flavonoids may therefore act as chemoprotective agents by blocking the conversion of procarcinogens such as benzopyrene and aflatoxin B1 two harmful compounds potentially found in cannabis smoke, as previously mentioned [19, 38].

Among the flavonoids, Cannflavin A was also found [40]. This compound is one of the two of prenylated flavonoids apparently unique to cannabis plant. Evans et al. 1987 determined the anti-inflammatory effect of cannflavin but using only an alcohol extract. They observed that, cannflavin A inhibits cyclooxygenase enzymes and lipooxygenase enzymes more potently than THC. Others authors showed that cannflavin at 31 ng/mL inhibit prostaglandin E2 in human rheumatoid synovial cells; and this effect was about 30 times more potent than aspirin [41].

Apigenin is the primary anxiolytic agent found in several plants. It selectively binds to central benzodiazepine receptors, which are located in α- and β-subunits of GABA A receptors [7, 39]. Apigenin like other flavonoids interact with estrogen receptors (in particular β-estrogen receptors), and appear to be the main estrogenic agents in cannabis smoke. Apigenin extract inhibits estradiol induced proliferation of breast cancer cells; however, the mechanism involved in the inhibition of proliferation by apigenin remains unclear and requires further investigation. Cannabis also contains β-Sitosterol, a phytosterol with reported anti-inflammatory effects [42]. This chemical compound was found in the red oil extract of Cannabis plant. With regards to anti-inflammatory properties, some reports have revealed an antiedematogenic effect of β-Sitosterol in some animal models of acute and chronic inflammation.

In one study, ajulemic acid (a nonpsychoactive cannabinoid acid) was administered to healthy human adults and patients with chronic neuropathic pain [43, 44]. The authors observed that the extract does not produce psychotropic effects, and demonstrated that ajulemic acid (AJA) was more effective than placebo in reducing this chronic pain as measured by the visual analog scale. Besides, signs of dependency were not observed at the end of the treatment period. AJA also produces analgesia in the mouse hot plate, the p-phenylquinone writhing, the formalin and the tail flick assays. These data are in agreement with the findings in the paw edema, subcutaneous air pouch and rat adjuvant-induced arthritis models of inflammation (0.2 mg/kg p. o).

Other studies related to anti-arthritis effect were done with an ajulemic acid of Cannabis [44, 45]. The results indicated that oral administration of AJA reduced joint tissue damage in rodents with adjuvant arthritis. Peripheral blood monocytes and synovial fluid monocytes were isolated from healthy subjects and patients with inflammatory arthritis. The examples obtained were treated with AJA in vitro and then stimulated with lipopolysacaride. The experimental results show that ajulemic acid attenuates the production of LPS-induced pre-inflammatory cytokines interleukin-1. However, extract did not influence tumor necrosis factor-α (TNFα) gene expression in or secretion from PBM. These results support that the anti-inflammatory effect exerted by Cannabis could be due to a decrease of pro-inflammatory cytokines [45].

3. Bioactivity of Crude Extracts

On the other hand, several studies evaluated the biological effect of total extract of Cannabis. A clinical study evaluated the effects of the Cannabis extract on amyotrophic lateral sclerosis. One hundred thirty-one patients were interviewed, 13 of whom reported using Cannabis in the last 12 months. Those thirteen patients were examined and the results revealed that Cannabis could be effective in reducing the
symptoms of loss of appetite, depression, pain spasticity and drooling [46].

It was shown that, water extract of the dried entire plant administered intravenously to Rhesus monkeys and rabbits at a dose of 0.01 µg/animal, display remarkable antiglaucomic activity [47]. Green et al. (1999), showed that extract at dose of 25 µg/animal, administered intravenously to rabbits also inhibits the intraocular pressure. Kausar et al. (1995), observed that water extract of the dried leaf and stem, applied ophthalmically to rabbits, was effective in prevention of intraocular pressure [48].

In one work, the effects of tincture from Cannabis resin (12.5 mg/kg) were studied in several behavioral animal models. In these animals a significant decrease in mounts and attempted mounts were observed. However, no differences were detected in other behavioral trials [49]. Other studies demonstrated that crude ethanolic extract of aerial parts of Cannabis exhibited a pronounced anti-androgenic effect in mice at a dose of 2 mg/animal, while the dried leaf smoked by males adult for 21 days had no significant effect [50].

About anti-fertility studies performed with the Cannabis extract there are some evidences. Some authors determined the anti-fertility effect of Cannabis but using aqueous, alcoholic and chloroform crude extracts of the leaves. They observed that, extracts administered by gastric incubation to female mice at dose of 100 mg/bw, 200 mg/bw and 400 mg/bw, was effective in this experimental assay [51]. However, administration of alcoholic extract (400 mg/bw) showed maximum abortifacient activity as compared to aqueous and chloroform extracts. The extract also exhibited estrogenic activity and prolonged the estrous cycle in rats. In addition, these extract caused a significant decrease in the ovarian and uterine weight. In contrast, the petroleum ether extract at dose of 3 mg/kg induced weak effect [52–54].

It has been demonstrated the antagonizing effect of cannabinoids in the male reproductive system, some investigators, by using experimental models in male rats showed depression of spermatogenesis and decrease in circulating testosterone levels. Ethanol extract of the dried aerial parts, administered intraperitoneally to mice at a dose of 2 mg/animal daily for 45 days resulted in a total arrest of spermatogenesis, but this effect was reversible [55]. Others investigators found that administration of Cannabis reduces levels of fructose and citric acid, and decreases glucuronidase, glycosidase, and acid phosphatase levels in accessory reproductive organs of male rats [56].

Considering, that several of these organs are regulated by circulating levels of testosterone, this observation suggests a possible anti-androgenic effect of Cannabis. In addition it has been observed in chronic marijuana smokers a reduction in the total sperm count, after 4 weeks of high-dose smoking (approximately 8–20 cigarettes days). Analogous results were obtained by other works, where daily inhalation of dry aerial part of the plant, decreased the quantity and quality of spermatozoa [57].

In folk medicine, Cannabis is widely used as anti-inflammatory, there are several works documenting this activity. Authors determined the anti-cyclooxygenase effect of Cannabis using ethanol extract and essential oil of the aerial parts. They observed that, the ethanol extract and essential oil of Cannabis showed cyclooxygenase activities (IC50 67 mg/L – 7.5 mg/L). Other authors studied in mice the anti-nociceptive potential of extracts of Cannabis plant in vivo. They found that, the total extract administered intraperitoneally to animals at a dose of 250 mg/kg, was active versus tail pressure method [58]. In addition, unfractonated Cannabis essential oil extract inhibits prostaglandins more effectively than its individual constituents, suggesting synergy [59].

Two non-psychotropic phytocannabinoids, cannabidiol and cannabinichromene, are known to modulate in experimental conditions, the activity of several targets involved in nociceptive response these include, transient receptor potential, channels of vanilloid type-1, and of ankyrin type-1, the equilibrative nucleoside transporter and proteins related in the inactivation of endocannabinoid. Investigators determined the antinociceptive effect of Cannabis but using only cannabidiol or cannabinichromene extract purified. In these animals an increase of endocannabinoid levels in the ventrolateral periaqueductual grey were observed. Moreover, the author shows that phytocannabinoids isolated stimulated descending pathways of antinociception, in vivo and caused analgesia by interacting with several target proteins related in the control of pain [60].

The anti-inflammatory and anti-nociceptive activities of standardized plant extracts derived from the Cannabis sativa L., clone 202 (rich in CBD) were studied in mice. This research group showed that, in contrast to the purified CBD, the extract obtained from clone 202, when administered either orally or intraperitoneally, presented anti-inflammatory and antinociceptive effects in a dose-dependent manner. The clone 202 extracts reduced zymosan-induced paw swelling and pain in mice, and prevented TNF produced in vivo. These results suggest an additive and synergistic effect between the CBD and other constituents of the extract [58-60].

In other study, petroleum ether (containing the cannabinoids) and ethanol (cannabidiol free) extracts of the dried aerial parts were administered intra gastrically to mice. The extracts were active vs phenylbenzoquinona induced writhing, at 0.013 mg/kg and 0.045 mg/kg respectively. Besides, the same extracts applied superficially on rodents at a dose of 100 µg/ear, was effective vs tissue plasminogen activator-induced erythema of the ear. However, the inflorescence extract administered orally to rodents produced little effects, vs paw pressure assay effective dose 35, 5 mg/kg and hot plate test 53 mg/kg. These finding contributed to characterize the biological activity of Cannabis and to understand the ability of its extracts to enhance nociceptive responses [61].

Previous studies also suggest that total extract and phytocannabinoids fraction, in particular cannabidiol and cannabidivarin, have significant anticonvulsant effects which are mediated by the endocannabinoid system. In addition,
human studies suggest that cannabidiol has anticonvulsant effects in adult and infantile epilepsy and is well tolerated after prolonged treatment [62]. The mechanism by which phytocannabinoids exerts its antiepileptic effects is not well established, and possibly comprises several mechanisms. Among these can be mentioned modulation of equilibrative nucleoside transporter, the orphan G-protein-coupled protein receptor, and the transient receptor potential of melastatin type 8 channel [62].

Moreover CBD is an agonist at the 5-HT1a and the glycine receptors (a3 and a1) and the transient receptor potential of ankyrin type 1. These phytocompounds modulates the intracellular Ca2+ concentration and inhibits T-type calcium channels. Other studies also showed the antiapoptotic, neuroprotective and anti-inflammatory action of CBD [64-66].

Anticonvulsant potential was carried out using the tincture of the resin, petroleum ether and ethanolic, extracts of Cannabis by employing the electroshock seizure test and the pentylenetetrazole seizure test, in rodents. The results showed that ethanolic extract and tincture of the resin (2-4mL/kg), were effective against electroshock-induced seizures (25 mg/kg) [67, 68]. Petroleum ether extract obtained from Cannabis were assessed in vivo as well. The authors observed that the crude extract, when administered intraperitoneally in male rats, inhibits gastric secretion and also showed anticonvulsant activity [68-70].

The effect of ethanolic extract of the dried seeds was assayed on hair loss in mice, as a possible therapeutic agent destined to induce hair growth in animals. The experimental results showed that Cannabis at dose of 0.33 g for 14 days attenuates the hair loss [71]. Antifungal activity was carried out using the water and ethanolic extracts of Cannabis. The water extract inhibits the in vitro growth of the following fungi: Fusarium oxysporum, Ustilago maydis and Ustilago nuda, but was inactive on Helminthosporium Turcicum. Ethanolic extract showed 65.99% of mycelial inhibition against Rhizoctonia soloni [72-74].

Others investigators demonstrated that dried leaf of Cannabis administered intraperitoneally to rats of both sexes at a dose of 7 mg/kg/week, exhibited a pronounced carcinogenic effect. The animals were irradiated between 40 and 50 days of age and observed for 78 weeks. They found that the Cannabis extract and radiation are able to induce tumors in animals given extract and radiation than either marihuana or radiation alone [75]. About toxicity studies performed with the total extract of Cannabis there are some evidences. These studies evaluated the effects of the ethanolic and water total extracts of Cannabis in in vitro assays on cell culture. The different concentrations of the extracts did not cause a statistically significant alteration in culture of CA-9Kb and Ca mammary-microalveolar [76]. The results obtained showed that the mixed compounds present in the extracts have no toxicological effects on some cell types in vitro.

In another study, the healthy female albino rats, starved for 3-4 hrs were subjected to acute toxicity studies. The animals were observed continuously for 2 hrs for behavioral, neurological and autonomic profiles and intermittently after 24 and 72 hrs for any lethality or death. Toxicity signal such as respiratory distress, salivation, weight loss and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment. Besides, the extract did not modify the behavioural, neurological and autonomic profile in treated groups at dose of 4000 mg/kg body weight, in fact no toxicity signals were observed [77, 78].

On the others hand, investigators demonstrated that resin of Cannabis administered orally to pregnant rabbits at a dose of 1 mL/kg, induces teratogenic effects. In addition, the alcoholic and water extracts (at dose of 125mg/kg or 125-800 mg/kg respectively) of the dried leaves, administered intragastrically to pregnant rats from days 7 to 16 of gestation, produced various types of malformations in the fetuses, such as visceral anomalies, and skeletal malformations. However, Petroleum ether extract of the aerial parts, administered orally to rats and rabbits, did not alter fetal development [79, 80].

4. Conclusions

Chemical compounds from traditional medicinal plants have been introduced in the development of new drugs. The objective of this review has been to show the advances in the exploration of extracts and individual phytocompounds of Cannabis as potential therapeutic agents. With the current information, it is evident that the chemical compounds from Cannabis have pharmacological activities including antimicrobial, antinociceptive, antiglaucomic, anticonvulsant, and antiinflammatory effects etc.

We suggest that the effects mentioned above may result from a synergy between the principal components of Cannabis, and others not investigated. However, more studies are needed to determine the efficacy of the total extract and contributions of different constituents to use it in phytomedicine. For these reasons, extensive pharmacological studies, referring to bioavailability, selectivity distribution pattern, pharmacodynamic and pharmacokinetic should be performed in animals and humans. Also, a phytochemical study may be a focus for future studies.

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References

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