International Journal of Chemical and Biomedical Science
2016; 2(1): 1-8
Published online March 1, 2016 (http://www.aascit.org/journal/ijcbs)

American Association for Science and Technology

Keywords
Non-cannabinoids Compounds,
Cannabis Extracts,
Bioactivity

Received: January 24, 2016
Revised: February 10, 2016
Accepted: February 12, 2016

Potential Health Benefits of Cannabis Extracts: A Review

Maria Rosana Ramirez

CITER-The National Scientific and Technical Research Council (CONICET), Argentine Government, Agency, Argentina

Email address
mr.ramirez@conicet.gov.ar

Citation
Maria Rosana Ramirez. Potential Health Benefits of Cannabis Extracts: A Review. *International Journal of Chemical and Biomedical Science*. Vol. 2, No. 1, 2016, pp. 1-8.

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 biomedicinal 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 C_{21} terpenophenolic). They are divided into 10 subtype: 1) Cannabigerol, 2) Cannabichromene, 3) Cannabidiol, 4) Δ^9 Tetrahydrocannabinol, 5) Δ^8

THC, 6) Cannabicyclol, 7) Cannabielsoin, 8) Cannabinol and Cannabinodiol, 9) Cannabitriol, 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 phytocompounds altogether contribute to the unique biological properties of cannabis plant [6].

Since, Δ^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 Δ^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 Δ^9-THC produces the psychoactive compound 11-hydroxy Δ^9 Tetra hydrocannabinol, and the posterior oxidation generates the inactive chemical entities 11-nor-9-carboxy-Δ^9-tetrahydrocannbinol [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, Δ^9 - 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 reveled 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 monoterpene in *Cannabis* displays remarkable activity against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* obtaining an equivalence of 15.2 - 1.0 µg/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-/O- 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 monoxigenase 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 benzopyreno 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 flavones 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 lipoxygenase enzymes more potently than THC. Others authors showed that cannaflavin at 31 ng/mL inhibit prostaglandin E_2 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 phytoesterol 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-arthritic 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 healty 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 exerts 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 opthalmically 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-cyclooxigenase 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 cycloxigenase 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, unfractionated *Cannabis* essential oil extract inhibits prostaglandins more effectively than its individual constituents, suggesting synergy [59].

Two non-psychotropic phytocannabinoids, cannabidiol and cannabichromene, 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 cannabichromene extract purified. In these animals an increase of endocannabinoid levels in the ventrolateral periaqueductal 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 TNFα production *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 (cannabinoid 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 ug/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 hs 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.

Acknowledgements

Financial support for this work was provided by The National Scientific and Technical Research Council, (Argentine). Dr. Maria Rosana Ramirez, is member of the CONICET, Argentina.

References

[1] Núñez-Sellés AJ, Delgado-Hernández R, Garrido-Garrido G, García-Rivera D, Guevara-García M, Pardo-Andreu GL. The paradox of natural products as pharmaceuticals: Experimental evidences of a mango stem bark extract. *Pharmacol Res* 2007, 55: 351-358.

[2] Dreher M. Crack heads and roots daughters: the therapeutic use of cannabis in Jamaica. *J Cannabis Therap* 2002, 2: 121-133.

[3] Russo EB. History of cannabis and its preparations in saga, science, and sobriquet. *Chem Biodivers* 2007, 4: 1614-1648.

[4] Gaoni Y & Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of hashish. *J Am Chem Soc* 1964, 86: 1646-1647.

[5] Turner CE, Elsohly MA, Boeren EG. Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. *J Nat Prod* 1980, 43: 169-304.

[6] Sparacino CM, Hyldburg PA, Hughes TJ. Chemical and biological analysis of marijuana smoke condensate. *NIDA Res Monogr* 1990, 99: 121-140.

[7] Devane WA, Dysarz FA III, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* 1988, 34: 605-613.

[8] Barros DM, Carlis V, Maidana M, Silva ES, Baisch AL, Ramirez MR, Izquierdo I. Interactions between anandamide-induced anterograde amnesia and post-training memory modulatory systems. *Brain Res* 2004, 30, 1016 (1): 66-71.

[9] Musshoff F & Madea B. Review of biologic matrices (urine, blood, hair) as indicators of recent or ongoing cannabis use. *Ther Drug Monit* 2006, 28: 155-163.

[10] Happyana N, Agnolet S, Muntendam R, Van Dam A, Schneider B, et al. Analysis of cannabinoids in laser-microdissected trichomes of medicinal Cannabis sativa using LCMS and cryogenic NMR. *Phytochemistry* 2013, 87: 51-59.

[11] Gill EW & Paton WDM, Pertwee RG. Preliminary experiments on the chemistry and pharmacology of *Cannabis*. *Nature* 1970, 228: 134-6.

[12] Mechoulam R, Ben-Zvi Z, Shani A, Zemler H, Levy S. 1972. Cannabinoids and Cannabis activity In: *Cannabis and its derivatives*. Paton WDM, Crown J, eds. London: Oxford University Press, pp. 1-13.

[13] McPartland JM & Pruitt PP. Side effects of pharmaceuticals not elicited by comparable herbal medicines: the case of tetrahydrocannabinol and marijuana. *Altern Therap* 1999, 5: 57-62.

[14] Ethan B Russo. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *Br J Pharmacol* 2011, 163, 1344-1364.

[15] Ahmad UK, Muniandy Y, Hassan MS. Physical Analysis and Chemical Profiling of Illicit Herbal Cannabis using Multivariate Analysis. *MJOFS* 2014, 5: 26-34.

[16] Elzinga S, Fischedick J, Podkolinski R and Raber JC. Cannabinoids and Terpenes as Chemotaxonomic Markers in Cannabis. *Nat Prod Chem Res* 2015, 3: 4.

[17] Thompson GR, Rosenkrantz H, Schaeppi UH, Braude MC. Comparison of acute oral toxicity of cannabinoids in rats, dogs and monkeys. *Toxicol Appl Pharmacol* 1973, 25: 363-73.

[18] Pate D. 1994. Chemical ecology of cannabis. *J Internat Hemp Assoc* 1994, 2: 32-37.

[19] Bornheim LM, Kim KY, Li J, Perotti BY, Benet LZ. Effect of cannabidiol pretreatment on the kinetics of tetrahydrocannabinol metabolites in mouse brain. *Drug Metab Dispos* 1995, 23: 825-31.

[20] ElSohly M. Chemical constituents of Cannabis, in *Cannabis and cannabinoids-Pharmacology, Toxicology and Therapeutic Potential* (Grotenhermen, F. and Russo, E., eds.), Haworth Press, New York, 2002, pp. 27–36.

[21] Agrawal AK, Kumar P, Gulati A, Seth PK. Cannabis-induced neurotoxicity in mice: effects on cholinergic (muscarinic) receptors and blood brain barrier permeability. *Res Commun Subst Abuse* 1989, 10: 155-68.

[22] Russo E. Hemp for headache: an in-depth historical and scientific review of cannabis in migraine treatment. *J Cann Ther* 2001, 1, 21–92.

[23] De Oliveira AC, Ribeiro-Pinto LF, Paumgartten JR. *In vitro* inhibition of CYP 2B1 monooxygenase by beta-myrcene and other monoterpenoid compounds. *Toxicol Lett* 1997, 92: 39-46.

[24] Evans AT, Formukong EA, Evans FJ. Actions of cannabis constituents on enzymes of arachidonate metabolism: anti-inflammatory potential. *Bioch Pharmacol* 1987, 36: 2035-7.

[25] Burstein S, Varanelli C, Slade LT. Prostaglandins and *Cannabis*–III. Inhibition of biosynthesis by essential oil components of marihuana. *Biochem Pharmacol* 1975, 24: 1053-4.

[26] Burstein S, Taylor P, El-Feraly FS, Turner C. Prostaglandins and *Cannabis*– V. Identification of p-vinylphenol as a potent inhibitor of prostaglandin synthesis. *Biochem Pharmacol* 1976, 25: 2003-2004.

[27] Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, Stavri M *et al*. Antibacterial cannabinoids from Cannabis sativa: a structure-activity study. *J Nat Prod* 2008, 71: 1427-1430.

[28] Arruda DC, D'Alexandri FL, Katzin AM, Uliana SR. Antileishmanial activity of the terpene nerolidol. *Antimicrob Agents Chemother* 2005, 49: 1679-1687.

[29] Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ *et al*. Beta-caryophyllene is a dietary cannabinoid. *Proc Natl Acad Sci USA* 2008, 105: 9099-9104.

[30] Nigam MC, Handa KL, Nigam IC, Levi L. Essential oils and their constituents. XXIX. The essential oil of marihuana: composition of the genuine Indian *Cannabis sativa* L. *Canad J Che* 1965, 43: 3372-3376.

[31] Novak J, Zitterl-Eglseer K, Deans SG, Franz CM. Essential oils of different cultivars of *Cannabis sativa* L. and their antimicrobial activity. *Flavour Fragr J* 2001, 16: 259-262.

[32] Onawunmi GO, Yisak WA, Ogunlana EO. Antibacterial constituents in the essential oil of *Cymbopogon citratus* (DC.) Stapf. *J Ethnopharmacol* 1984, 12: 279-86.

[33] Ross SA & ElSohly MA. The volatile oil composition of fresh and air-dried buds of *Cannabis sativa*. *J Nat Prod* 1996, 59: 49-51.

[34] Santos FA & Rao VS. Anti-inflammatory and antinociceptive effects of 1, 8-cineole a terpenoid oxide present in many plant essential oils. *Phytother Res* 2000, 14: 240-4.

[35] Tambe Y, Tsujiuchi H, Honda G, Ikeshiro Y, Tanaka S. Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, beta-caryophyllene. *Planta Med* 1996, 62: 469-70.

[36] Rao VSN, Menezes AMS, Viana GSB. Effect of myrcene on nociception in mice. *J Pharm Pharmacol* 1990, 42: 877-8.

[37] Jones CLA. Monoterpenes: Essence of a cancer cure. *Nutr Sci News* 1999, 4 (4): 190.

[38] Greene-McDowelle DM, Ingber B, Wright MS, Zeringue HJ, Bhatnagar D, Cleveland TE. The effects of selected cotton-leaf volatiles on growth, development and aflatoxin production of *Aspergillus parasiticus*. *Toxicon* 1999, 37: 883-93.

[39] Paris RR, Henri E, Paris M. Sur les c-flavonoïdes du *Cannabis sativa* L. *Plantes Médicinales et Phytothérapie* 1976, 10: 144-54.

[40] Barrett ML, Scutt AM, Evans FJ. Cannflavin A and B, prenylated flavones from *Cannabis sativa* L. *Experientia* 1986, 42: 452-463.

[41] Barrett ML, Gordon D, Evans FJ. Isolation from Cannabis sativa L. of cannflavin—a novel inhibitor of prostaglandin production. *Biochem Pharmacol* 1985, 34, 2019-2024.

[42] Fenselau C & Hermann G. Identification of phytosterols in red oil extract of cannabis. *J Forens Sci* 1972, 17: 309-312.

[43] Burstein SH, Karst M, Schneider U, Zurier RB. Ajulemic acid: A novel cannabinoid produces analgesia without a "high." *Life Sci* 2004, 75: 1513-1522.

[44] Vann RE, Cook CD, Martin BR, Jenny L, Wiley JL. Cannabimimetic properties of ajulemic acid. *J Pharmacol Exp Ther* 2007, 320: 678-686.

[45] Zurier, R. B., R. G. Rossetti, S. H. Burstein, and B. Bidinger. Suppression of human monocyte interleukin-1beta production by ajulemic acid, a nonpsychoactive cannabinoid. *Biochem Pharmacol* 2003, 65 (4): 649-655.

[46] Amtmann D, Weydt P, Johnson KL, Jensen MP, Carter GT. Survey of cannabis use in patients with amyotrophic lateral sclerosis. *Am J Hosp Palliat Care* 2004, 21: 95-104.

[47] Kausar W, Muhammad A, Ul-Haq I. Effect of cannabis on intraocular pressure. *Pak J Pharmacol* 1995, 12: 43-48.

[48] Green K, Symonds CM, Elijah DR, Zalkow LH, Deutsch HM, Bowman KA, Morgan TR. Water soluble marihuana-derived material: Pharmacological actions in rabbit and primate. *Curr Eye Res* 1981, 1: 599-608.

[49] Cutler MG, Mackintosh JH, Chance MRA. Cannabis resin and sexual behaviour in the laboratory mouse. *Psychopharmacologia* 1975, 45: 129-131.

[50] Mendelson JH, Ellingboe J, Kuehnle JC, Dixit VP, Lohiya NK. Effects of cannabis extract on the response of accessory sex organs of adult male mice to testosterone. *Indian J Physiol Pharmacol* 1975, 19: 98-100.

[51] Mello NK. Effects of chronic marihuana use on integrated plasma testosterone and luteinizing hormone levels. *J Pharmacol Exp Ther* 1978, 207: 611-617.

[52] Varsha Zade, Manisha Wikhe, Dinesh Dabhadkar, Sagar Dawada, Uddhao Patil. Antifertility efficacy of *Cannabis sativa* leaves on female albino rats. IJSIT, 2013, 2 (2), 107-117

[53] Kostellow AB, Ziegler D, Kunar J, Fujimoto GI, Morrill GA. Effect of cannabinoids on estrous cycle, ovulation, and reproduction capacity of female A/J mice. *Pharmacology* 1980, 21: 68-75.

[54] Frischknecht HR, Sieber B, Waser PG. Effects of multiple, chronic and early hashish exposure on mating behavior, nest-building, and gestation in mice. *Comp Biochem Physiol* 1982, 77: 363-368.

[55] Hembree III, WC, Nahas GG, Zeidenberg P, Huang HFS. Changes in human spermatozoa associated with high dose marihuana smoking. *Adv Biosci* 1997, 1979: 429-439.

[56] Dixit VP. Effects of Cannabis sativa extract on testicular function of Presbytis entellus. *Planta Med* 1981, 41: 288-294.

[57] Dixit VP, Sharma VN, Lohiya NK. The effect of chronically administered cannabis extract on the testicular function of mice. *Eur J Pharmacol* 1974, 26: 111-114.

[58] Formukong EA, Evans AT, Evans FJ. Analgesic and antiinflammatory activity of constituents of Cannabis sativa L. *Inflammation* (NY) 1988, 12: 361-371.

[59] Dhar ML, Dhar MM, Mehrotra BN, Ray C. Screening of Indian plants for biological activity. Part I. *Indian J Exp Biol* 1968, 6: 232-247.

[60] Rousinov KS & Athanasova Shopova S. Experimental screening of the anticonvulsive activity of certain plants used in popular medicine in Bulgaria. C R *Acad Bulg Sci* 1966, 19: 333-336.

[61] Franke C, Trovato AE, Giagnoni G. Oral anti-inflammatory activity of cannabidiol, a non-psychoactive constituent of cannabis, in acute carrageenan-induced inflammation in the rat paw. *Naunyn Schmiedebergs Arch Pharmacol* 2004, 369: 294-299.

[62] Sofia RD, Vassar HB, Knobloch LC. Comparative analgesic activity of various naturally occurring cannabinoids in mice and rats. *Psychopharmacologia* 1975, 40: 285-295.

[63] Segelman AB, Segelman FP, Sofia RD, Star AE. Some pharmacological effects of certain cannabinoid free marijuana extracts. *Lloydia* 1974, 37: 645A.

[64] Dwivedi C & Harbison RD. Anticonvulsant activities of delta-8- and delta-9-tetrahydrocannabinol and uridine. *Toxicol Appl Pharmacol* 1975, 31: 452-458.

[65] Feeney DM. Marihuana and epilepsy. *Science* 1977, 197: 1301.

[66] Hill AJ, Williams CM, Whalley BJ, Stephens GJ. Phytocannabinoids as novel therapeutic agents in CNS disorders *Pharmacol Ther* 2012, 133: 79-97.

[67] Cilio MR, Thiele EA, Devinsky O. The case for assessing cannabidiol in epilepsy. *Epilepsia* 2014, 55: 87-90.

[68] Robson PJ. Therapeutic potential of cannabinoid medicines. *Drug Test Anal* 2014; 6: 24-30.

[69] Maa E & Figi P. The case for medical marijuana in epilepsy. *Epilepsia* 2014, 55: 783-786.

[70] dos Santos RG, Hallak JEC, Leite JP, Zuardi AW, Crippa JAS. Phytocannabinoids and epilepsy. *J Clin Pharm Ther* 2015, 40: 135-143.

[71] Kubo M, Matsuda H, Fukui M, Nakai Y. Development studies of cuticle drugs from natural resources. I. Effects of crude drug extracts on hair growth in mice. *Yakugaku Zasshi* 1988, 108: 971-978.

[72] Singh KV & Pathak RK. Effect of leaves extracts of some higher plants on spore germination of Ustilago maydes and U. nuda. *Fitoterapia* 1984, 55: 318-320.

[73] Renu. Fungitoxicity of leaf extracts of some higher plants against Rhizoctonia solani Kuehn. *Nat Acad Sci Lett* 1983, 6: 245-246.

[74] Nene YL, Thapliyal PN, Kumar K. Screening of some plant extracts for antifungal properties. *Lab Dev J Sci Tech B* 1968, 6: 226-228.

[75] Sato A. Studies on anti-tumor activity of crude drugs. The effects of aqueous extracts of some crude drugs in short-term screening test. *Yakugaku Zasshi* 1989, 109: 407-423.

[76] Montour JL, Dutz W, Harris LS. Modification of radiation carcinogenesis by marihuana. *Cancer* 1981, 47: 1279-1285.

[77] Wright PL, Smith SH, Keplinger ML, Calandra JC, Braude MC. Reproductive and teratologic studies with delta 9-tetrahydrocannabinol and crude marijuana extract. *Toxicol Appl Pharmacol* 1976, 38: 223-235.

[78] Sethi N, Nath D, Singh RK, Srivastava RK. Antifertility and teratogenic activity of *Cannabis sativa* in rats. *Fitoterapia* 1991, 62 (1): 69-71.

[79] Cozens DD, Clark R, Palmer AK, Hardy N, Nahas GG, Harvey DJ. The effect of a crude marihuana extract on embryonic and fetal development of the rabbit. *Adv Biosci Marihuana Biological Effects* 1978, 22: 469-477.

[80] Agnihotri PK, Singh RK, Sethi SN. Fototoxic effects of crude alcoholic *Cannabis sativa* extract in rats. *Fitoterapia* 1992, 63: 489-492.