11. Cannabis sativa L. and its antimicrobial properties – A review

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Abstract

Cannabis sativa L. is a very potent plant that exhibit many interesting features. Although, presently it is mainly perceived as a narcotic, not many knows that it is also a great source of fiber and a powerful medicinal plant. The metabolic profile of the *cannabis* is extremely rich. More than 480 active compounds have already been isolated, among which 180 belong to the cannabinoids family. The 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. Therefore, the goal of this paper is to review available scientific data about the antibacterial and antifungal properties of the *cannabis* extracts.

1. Introduction

Cannabis sativa L. is an annual herbaceous plant that belongs to the *Cannabis* genus, a species of the *Cannabaceae* family. Originating from Central Asia cannabis is one of the oldest psychoactive plant known to man But also it has been used all over the world either as a medicinal plant or as a source of food and fibers (Jiang 2006). One of the first use of this plant was reported in China: archeological findings pointed out that cannabis was cultivated for fibers and further used in textiles, ropes and paper production, since 4000 B.C. (Zuardi 2006). In Europe, cannabis was introduced by Muslims in paper manufacture techniques, first in 1150 in Spain and later in Italy (Aldrich 1997). Although cannabis is presently perceived mostly as a recreational drug, the plant has been appreciated and applied in ancient medicine since centuries. The first written information about the medicinal use of cannabis comes from ancient China, in the oldest pharmacopoeia dated from the first century of this Era. At the time cannabis was used in the treatment of rheumatic pain, intestinal constipation, disorders of the female reproductive system, malaria, and other health problems (Touwn 1981). Some sources reports that cannabis was used in the twentieth century B.C in Egypt to treat sore eyes. In India, before the tenth century B.C., bhang, (edible preparation of cannabis, traditionally used in food and drink for centuries) was used as an anesthetic and anti- phlegmatic (Sachindra and Pradhan 1977). It is still used among Hindu and Muslims as spasmolytic, analgesic in mental conditions and to increase resistance to severe physical stress (Mechoulam and Lander 1980). It is not uncommon that cannabis is recommended to patients suffering from rabies, cholera, rheumatism, and tetanus. In the middle of 19th century in Western Europe, cannabis gained some 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). It is the main reason why cannabis gained a bad reputation, interest in this plant drastically decreased and the access became limited to the black-market. Since a few years however, an increasing number of scientific evidences demonstrate the efficiency of cannabinoids in the treatment of epilepsy, Parkinson disease, analgesia, antiemetic effect, appetite disorders, multiple sclerosis, Tourette's syndrome and other neurological diseases, carefully reviewed by Amar (2006) in "Cannabinoids in medicine: A review of their therapeutic potential". Nowadays, many countries cultivate cannabis for the fiber and seeds production.

Furthermore, an increasing number of countries have legalized the medicinal use and cultivation of plants with an increased THC and CBD content. New varieties with desired characteristics and suitable for certain climate conditions are constantly being developed. Therefore, cannabis becomes a very important crop of great potential and economical value. Most of the available literature concerning cannabis rather describes its medical properties and its use in clinical research. Similarly, an important number of studies focus on psychological effects for humans. Besides that there is quite a lot known about its antimicrobial activity against human pathogens but only little is known about its antimicrobial properties against crop pathogens. Therefore the main goal of this review is to gather scientific data about the inhibitory effect of cannabis and some of its secondary metabolites on the microorganisms that cause some of the crop diseases. Although cannabis is mostly know for it 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 bio-composite 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. Moreover, seed oil and stalks can be burned as fuel (Clark 2002).

2. Cannabis active compounds

Cannabis sativa L. is known to have numerous active compounds representing different chemical classes. Some of them belong to primary metabolites, for example, amino acids, fatty acids and steroids, while cannabinoids, stilbenoids, flavonoids, lignans, terpenoids, and alkaloids belongs to secondary metabolites. Generally, the metabolic profile of this plant is extremely rich, more than 480 compounds have been discovered, from which 180 belong to cannabinoids family (Fischedick et al. 2010). Cannabinoids are usually a group of compounds build of 21 carbons (C21) and they are in the form of carboxylic acids. The cannabinoids are the most known among secondary metabolites of *cannabis* (Amer 2006). The classical cannabinoids are usually concentrated in the viscous resin produced in structures known as glandular trichomes (Figure 2).

Tetrahydrocannabinol (Δ^9-THC) (Figure 1a) is the most studied among them and it attracts lot of attention due to its psychoactive properties. On the other hand cannabidiol (CBD) (Figure 1b) is a non-psychoactive cannabinoid that gained lot of interest in recent years due to the increasing number of evidences demonstrating its efficiency in the treatment of several neurological diseases. The concentration of active compounds depends on many factors such as variety, age, tissue type, growing conditions, harvest time as well as storage conditions. Hemphill et al. (1980) found that the quality as well as quantity of cannabinoids varies importantly between organs of geographically distinct hemp plants. It the analysis of the leaves of different ages it was demonstrated that the youngest leaves from the upper part of the flowering plant contained the highest concentrations of cannabinoid. Some study report that the production of cannabinoids increases in plants under stress conditions (Pate 1999). Also, it was reported that hemp grown in the northern latitudes has usually higher concentration of Δ^9-THC and CBD (Leizer et al. 2000). Cannabinoids are found in all parts of hemp organs, however the highest concentrations are found in the resinous exudate of flowering tops (Figure 2).

Theoretically all genotypes of industrial hemp contain Δ^9-THC. Nonetheless, unlike marijuana (*cannabis* with the high content of THC, used as a recreational drug), industrial cultivars contain minimal amount of THC, usually not exceeding 0.2% (w/v), which is about 50 times lower than that of marijuana (Nissen et al. 2010). Most of available studies assessing antimicrobial properties of plant extracts, based on the high-THC varieties, which are known to have powerful antimicrobial characteristics (Appendino et al. 2008). Nevertheless, remarkably little studies focus on the antimicrobial features of compounds isolated from low-THC varieties, which are much more common and can be cultivated without any legal restrictions.

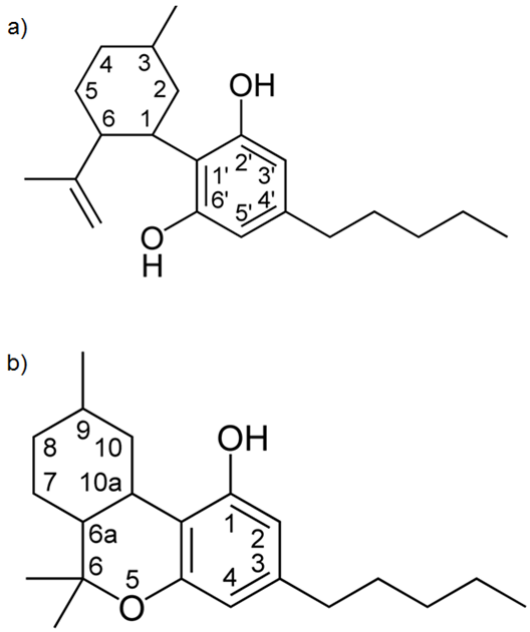

Figure 1. Two most important compound from cannabinoid family
a) cannabidiol (CBD) and b) tetrahydrocannabinol (Δ^9-THC)

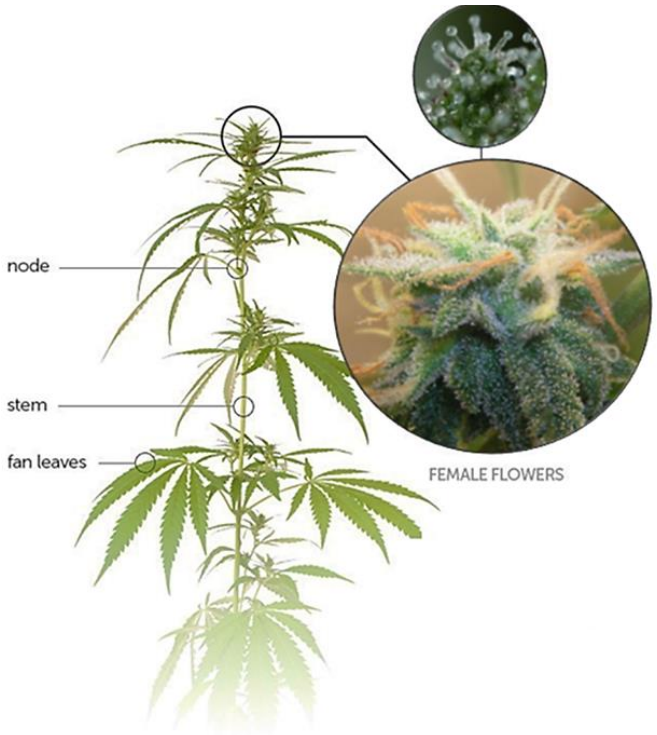

Figure 2. Phenology of Cannabis plant and its inflorescence

3. *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. The results showed that the extracts have strong inhibitory effects on both Gram positive (*Baccilus subtilus, Baccilus pumilus*, *Staphlococcus aureus*, *Micrococcus flavus*) and Gram negative bacteria (*Proteus vlugaris*, *Bordetella bronchioseptica*). The antibacterial activity of extracts was compare with the effect of the common antibiotic Celphalexin. 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 wiled 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. Nissen et al. (2010) assessed 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 Garam (+) 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 monoterpens, such as terpinolene and pinenes, have been already reported (Gallucci et al. 2009). Ali et al. (2011) 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). These results are similar to those reported by Wasim et al. (1995), 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.

4. 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. Wasim and coworkers (1995) 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

to antifungal antibiotic (Nystatin), however the concentration of the leave extract was 10 times higher compare to antibiotic. Similarly, Ali and coworkers (2011) 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.

5. Conclusion

Cannabis sativa L. is a very powerful plant which present 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 know 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 *Baccilus* or *Staphlococcus.* 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.

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