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ANTIMICROBIAL EFFECT OF *CANNABIS SATIVA* L.

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In the last 25 years, the therapeutical potential of Cannabis (*Cannabis sativa* L.) came back to our attention, many states in the world legalizing the medical use of this plant. At the same time, the antimicrobial resistance became a major healthcare problem all over the world, requiring new alternative therapies. This review proposes to synthesize research connected to the antimicrobial effect of phytocannabinoids, as basis for future studies in the field.

INTRODUCTION

Antimicrobial resistance became a major healthcare problem all over the world, requiring new alternative therapies. The therapeutical products derived from plants are the most targeted for some time, especially due to the fact that they have less adverse effects in comparison to the allopate products.[1,2] In 2014, over 700 000 people died of diseases related with some form of microbial resistance, worldwide. In the scenario that the phenomenon of antimicrobial resistance continues to increase, an estimated 10 million deaths will be due to this cause, by 2050 (Figure 1).[3]

World Health Organization (WHO) published a prioritized list of germs for which alternative therapies are imperative to be found, according to the prevalence of antimicrobial resistance, clinical relevance, contribution to global morbidity, mortality and economic impact.[4,5]

In the last 25 years, the therapeutic potential of cannabis (*Cannabis sativa* L.) has emerged, most states in the world legalizing the medical use of this plant. *Cannabis sativa* L. is an annual herbaceous plant that belongs to the *Cannabis* genus, a species of the *Cannabaceae* family, originating from Central Asia. Although cannabis is presently perceived mostly as a recreational drug, the plant has been used in medicine since centuries. Among the multiple non-psychotropic effects of medicinal cannabis, one can include: analgesic,[6] anti-vomiting, anti-nausea, antiepileptic (Dravet and Lennox-Gastaut syndromes), anti-psychotic, antidiabetic, neuroprotective, anti-inflammatory[7] and immunomodulatory properties[8], muscle relaxant (Parkinson's disease, multiple sclerosis, Huntington's chorea),[9] anti-arthritic, anti-anxiety, antiglaucoma action, as well as anti-tumoral properties,[10,11,12,13] but also the antimicrobial activity, on which more and more studies are being performed.

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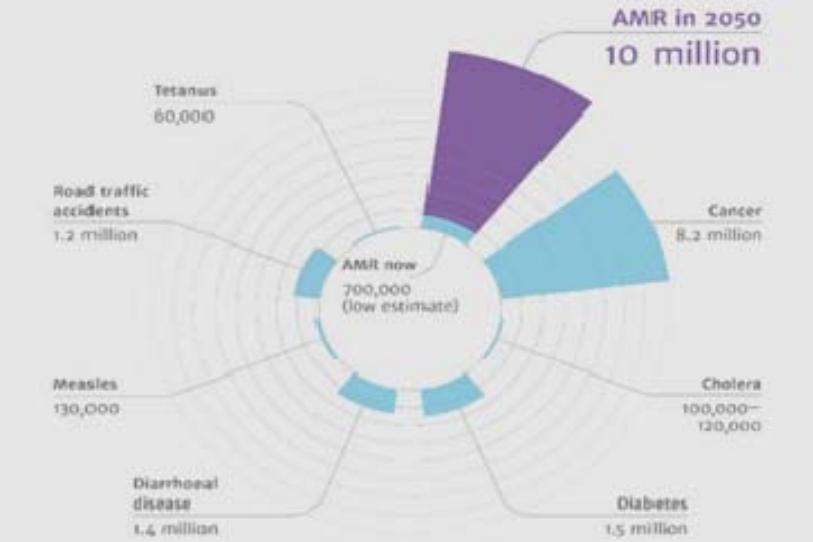

Fig. 1 – Annual deaths due to anti-microbial resistance (2014 and estimation for 2050)[3].

Antimicrobial effect of Cannabis Sativa phytocannabinoids

Although the antimicrobial effect of cannabis is recorded in old medicine ever since the end of the 19th Century, the first studies on the antibacterial action of *Cannabis sativa* L. emerged in the 1950s. These were independent studies published by Drobotko *et al.* in 1951, Krejci in 1958 (publish of PhD Thesis results in 1950)[14] and Ferenczy *et al.* in 1956. [15] The first two studies mentioned above state the inhibiting effect of *Cannabis sativa* L. extract, on the growth of some Gram-positive bacteria. The third study, mentioned above stated the antibacterial effect of the extract obtained from cannabis seeds. Subsequently, numerous other studies confirmed the antibacterial effect (on Gram-positive or on Gram-negative bacteria), or anti-fungal effect of cannabis mixtures.[16,22]

Cannabis sativa L. is known to have numerous active compounds representing different chemical classes. Generally, the metabolite profile of this plant is extremely rich, with more than 480 compounds discovered, of which 180 belong to the cannabinoids family. [17] The antibacterial character is considered to have been given mainly by Δ9-THC and CBD (Figure 2). In 1958, Schultz and Haffne isolated CBD and CBD-A and compared their antimicrobial effect on *Staphylococcus aureus, Bacillus subtilis and Escherichia coli*. Both cannabinoids had an antibacterial activity on Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*), but not on Gram-negative ones (*Escherichia coli*). In 1965, Mechoulam *et al.* Published a study on the antibacterial action of CBG and of CBG-A (Figure 2) on Gram-positive bacterias.

In 1976, Klingeren and Ham showed the antibacterial effect of Δ9-THC and CBD on the following Gram-positive bacteria: *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus milleri and Enterococcus faecalis*. In 1982, Turner and ElSohly mentioned the antibacterial and antimycotic effect of the cannabinoid derivates, like CBC and CBG, namely on: *Staphyloccocus aureus, Bacillus subtilis*, *Mycobacterium smegmatis, Candida albicans, Saccharomyces cerevisiae, Trichophyton mentagrophytes*.[18]

In 2009, Radwan *et al*. identified an antibacterial action (*Staphylococcus aureus* multi-resistant on antibiotics, *Escherichia coli*, *Mycobacterium intracellulare*), anti-mycotic (*Candida albicans, Candida krusei*), anti-leishmania (*Leishmania donovani*) and antiprotozoal (*Plasmodium falciparum*).[19] Moreover, starting with the study of Appendino *et al.* in 2008, numerous studies showed the existence of the antibacterial action of all five major cannabinoids CBD, Δ9-THC, CBG, CBC and Cannabinol (CBN) (Figure 2), on numerous types of *Staphylococcus aureus* multi-resistant to antibiotics, including *Staphylococcus aureus* methicillin – resistant (MRSA),[20] a germ included by WHO in the category of high priority according to the need of a therapeutical solution.[4,21]

In Table 1, recent studies regarding the antibacterial activity of cannabinoids are included.[22]

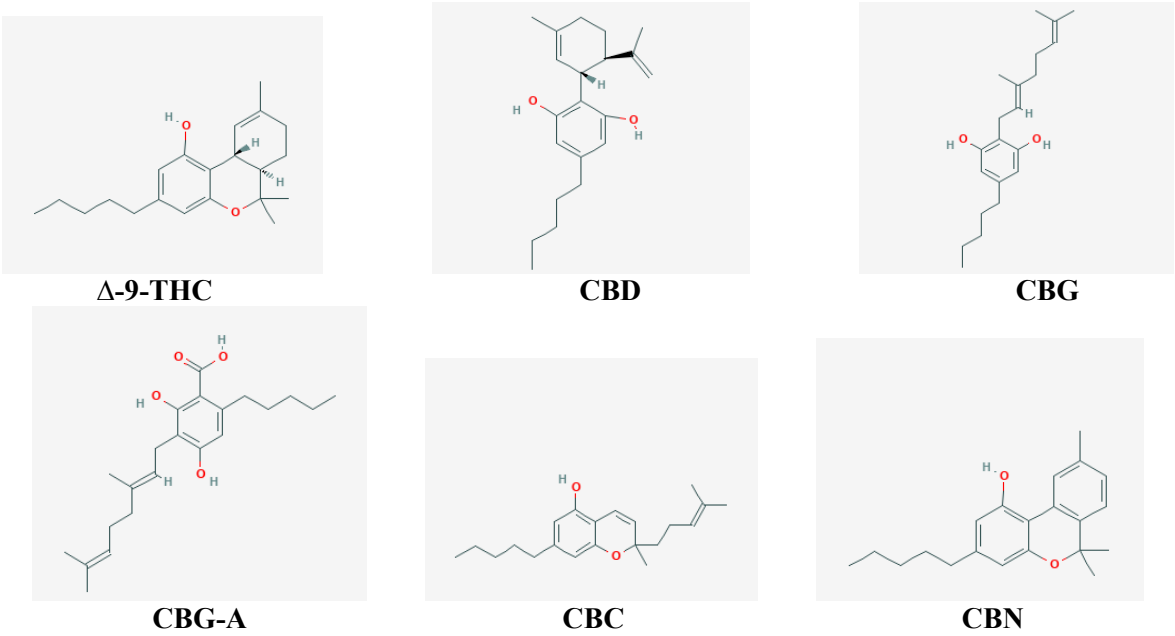

Fig. 2 – Molecular formulas of the first and most studied phytocannabinoids, for their antibiotic effect.
Source: https://pubchem.ncbi.nlm.nih.gov.

Table 1

Antimicrobian activity of *Cannabis sativa* extracts, adapted from[22]

Cannabis plant part, extracted	**Solvent used for extraction**	**Aactivity of the extracts against tested microorganisms in various studies**
Leaf	Aqueous, ethanolic and petroleum ether	*Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Micrococcus flavus, Proteus vulgaris, Bordetella bronchiseptica, Candida albicans and Aspergillus niger*[23]
	——	Bacterial strains representative of skin, mouth and ear microflora and beta strain of *Escherichia coli* [24]
	Aqueous and acetone	*Pseudomonas aeruginosa, Vibrio cholerae, Cryptococcus neoformans, Candida albicans and Aspergillus niger*[25]
	Aqueous and ethanol	*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans*[26]
	Ethanol and aqueous	*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Salmonella typhi, Klebsiella pneumoniae* [27]
	Ethanol, methanol, acetone and aqueous	*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Saccharomyces cerevisiae*[28]
	Methanol, ethanol, acetone and aqueous	*Escherichia coli, Staphylococcus aureus, Streptococcus pneumonia, Salmonella typhi* [29]
	Acetone and methanol	*Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger* [30]
	Methanol and n-hexane	*Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* [31]
Stem and leaf	Aqueous ethanolic	*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* [32]
Seed's oil	Hexane and methanol	*Aspergillus niger, Escherichia coli, Staphylococcus aureus, Saccharomyces cerevisiae, Pseudomonas aeruginosa* [33]
Whole bud	Hexane, dichloromethane, ethyl acetate, ethanol, aqueous ethanol and aqueous	*Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Aspergillus fumigatus* ATCC 90906, methicillin-resistant *Staphylococcus aureus* ATCC 33591, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Mycobacterium intracellulare* ATCC 23068 [34]
Whole plant	Acetone	Methicillin-resistant *Staphylococcus aureus* [23]
	Petroleum ether and methanol	*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* [35]
	Hydro-alcoholic	*Escherichia coli* 25922, *Escherichia coli* ESBL+, *Staphylococcus aureus* 25923, Methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* ESBL+, *Pseudomonas*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* [36]

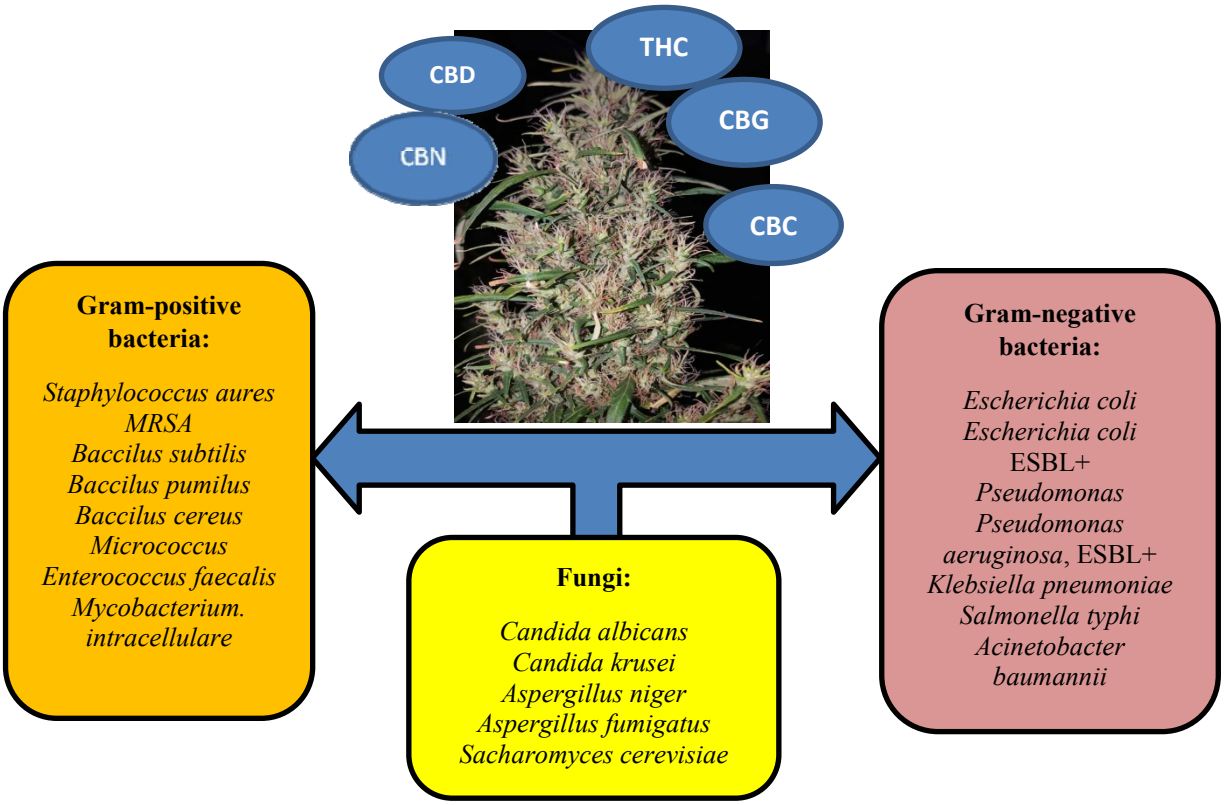

Fig. 3 – Five major cannabinoids are effective against bacteria and fungi.

Besides the direct antimicrobial effects, the study published by Brown and co-workers in 2014 mentioned the effect of CBG to increase the activity of polymyxin B on Gram-negative bacteria (*Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*)[37] (Figure 3).

Recent research also remind us about the synergistic action and/ or entourage effects of terpenes from the cannabis plant (namely they sum up with, and potentiate the cannabinoids effects when they are used in natural cannabis full-spectrum and broad-spectrum products, respectively,[38] including for the antimicrobial action.[39,40] These results suggest that the antibacterial potential of *Cannabis sativa* L. is far from being completely known.

In 2011 EB Russo published such notorious synergistic actions,[41] of which we bring the following to the reader's attention:

- Limonene is active against acne bacteria, CBD being a synergistic action cannabinoid;[42]
- Limonene is active against *Dermatophytes*, CBG being a synergistic action cannabinoid;[43,44]
- Caryophyllene oxide is antifungal in onychomycosis, comparable to ciclopiroxolamine and sulconazole,[45] CBC and CBG being synergistic action cannabinoids;
- CBD and CBN are effective versus MRSA,[46] pinene being a synergistic action terpene;
- CBC has antifungal activity, Caryophyllene oxide being a synergistic action terpene (Figure 4).

Finally, but very hopefully, the Bo Wang *et al.*, 2020 research[47] showed *Cannabis sativa* L. extracts, high in CBD might become a preventive treatment of SARS CoV2 infection in humans, by decreasing virus entry in gateway tissues, mostly oral tissue, following mouth washes with such extracts.

It is already known that oral, lung and intestinal tissues have an important ACE2 (angiotensin converting enzyme II) gene expression.[48,49,50] SARS CoV2 uses receptor-mediated entry into the human host via ACE2.[51]

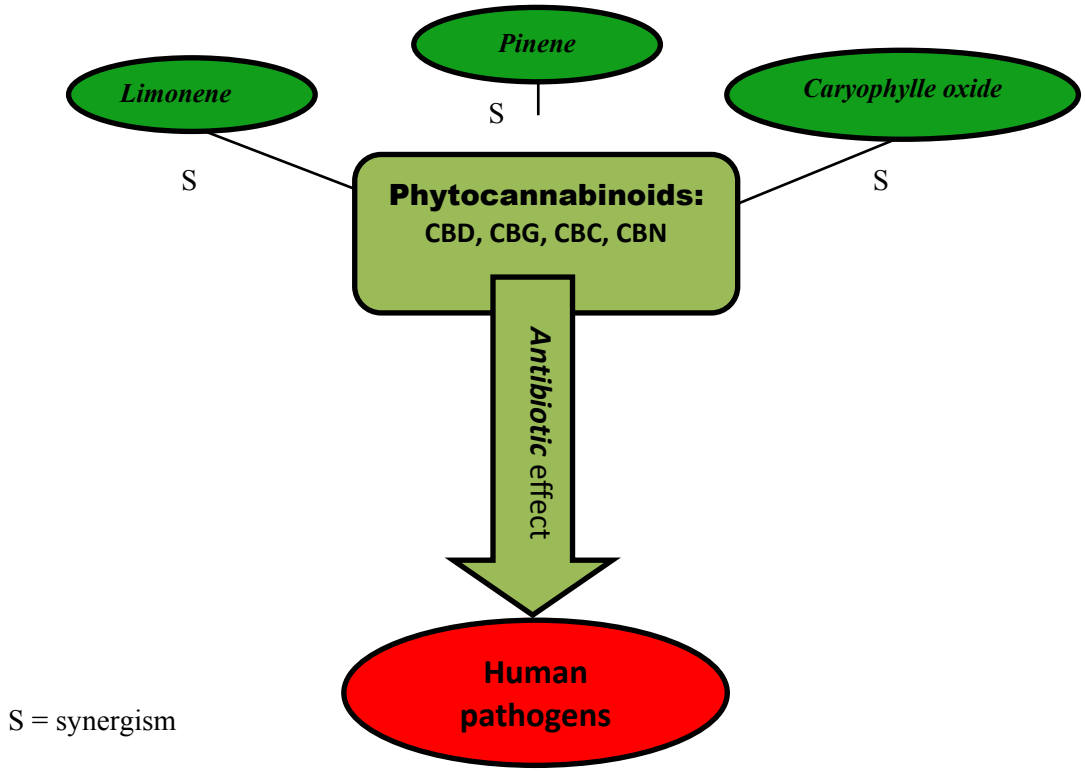

Fig. 4 – Synergistic actions of terpenes and phytocannabinoids, for the antibiotic effect.

CONCLUSIONS

Cannabis sativa L. is a plant with a high therapeutical potential, including an antimicrobial one, which is still not sufficiently investigated. Most of the studies performed by now were on the antimicrobial effect and very few on the antifungal, antileishmanial, antiviral or antiprotozoal effect. On the other hand, great part of the already discovered 480 compounds have not been tested yet, regarding their antibacterial properties, while the entourage effect, as well as the synergistic effect are still under study.

As such, the resolution of the European Parliament from February 13th 2019 regarding the use of cannabis for medical purposes shows the necessity that all member states of EU should stimulate, at their medical staff, the improvement of the knowledge level regarding medicinal cannabis, based on independent and extended research and provide them with the appropriate professional training[52]. Moreover, serious efforts are required for educating the population regarding the fundamental difference between the medicinal use of cannabis ant its recreational use.

List of abbreviations:
Δ9-THC = Δ9- tetrahydrocannabinol
CBD = Cannabidiol
CBD-A = Cannabidiolic acid
CBG = Cannabigerol
CBG-A = Cannabigerolic acid
CBC = Cannabichromene
CBN = Cannabinol
Enterobacteriaceae ESBL+ = Enterobacteriaceae that release Extended Spectrum Beta Lactamases, which are enzymes that inactivate penicillins, cephalosporins or monobactams, through hydrolyzing the oxyimino- group of their beta-lactam ring.

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