Antimicrobial Efficacy of Cannabis sativa L. (Bhang): A Comprehensive Review

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ABSTRACT
Therapeutic products derived from plants are gaining popularity since long time and this is primarily due to their ability to overcome the side effects of allopathic forms of medicine. Besides this, multi-drug resistance by pathogens to currently used antibiotics triggered the search for identifying natural antimicrobial products that are effective in combating infections. Considering this, we have focused on the various investigations related to antimicrobial properties of the well-known wildly growing plant i.e., Cannabis sativa. Cannabis sativa is an annual herbaceous plant belonging to the family Cannabaceae. It is known by various names worldwide as Marijana, Bhang, Dogga, Hashish, Grifa etc. The plant and its preparations have many medicinal properties including antimicrobial properties, which are thought to be beneficial for human health. From our review study, we have found that the leaves of a plant can act as most prominent source for isolating antimicrobial compounds. Also Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis and Candida albicans were found most susceptible towards the different plant extracts, indicating the greater effectiveness of the plant in diseases caused by these pathogens. Furthermore ethanol, methanol and distilled water can be considered as the best solvents for extracting different plant secondary metabolites. This review is intended to encourage utilization of Cannabis sativa in treating diseases related to microbial infections. Moreover, it will also help to provide us with an idea of selecting plant parts, pathogens and solvents for conducting further study.

Keywords: Cannabis sativa, antimicrobial properties, multi-drug resistance, secondary metabolites, microbial infections.

INTRODUCTION
Plants are the richest source of many therapeutic agents, which are used for powerful drug discovery in different countries.1, 2 The therapeutic value of plants is due to the presence of some chemical substances within the plant tissues which produce a definite physiological action on the human body; include alkaloids, flavonoids, glucosides, tannins, gums, resins, essential oils etc.3 It is estimated that there are 250,000 to 500,000 species of plants on the earth with biologically and chemically diverse groups.4 Though, plants have been used as traditional medicines for the treatment of various diseases throughout most of human history, but actually they have gained popularity in the late 1990s.5 However, plants are still an important source of medicines, especially in developing countries where the plant-based therapeutic products are still used to meet the health care needs.6 The World Health Organization (WHO) estimates that the traditional systems of medicines are accepted by almost 80% of the population throughout the world.7 Further, the National Health Interview Survey (NHIS) conducted by the Centers for Disease Control and Prevention (CDC) in 2007 indicate that around 40% of adults in the United States used some form of complementary and alternative medicine, indicating that such medical practices are prevalent even in the developed nations.8 In recent years, there are considerable challenges with the treatment of infections caused by bacterial strains of clinical importance that show multi-drug resistance (MDR) properties, such as methicillin-resistant Staphylococcus aureus (MRSA) and the recently emerged extremely drug-resistant Mycobacterium tuberculosis XDR-TB. Therefore, due to the potent antimicrobial activity of many plant secondary metabolites9 and the ability of some of them to modify the resistance associated with MDR strains10, researchers are increasingly turning their attention towards the development of new effective drugs from natural sources.

Cannabis sativa is an angiosperm belonging to the family Cannabaceae.11 It is known by various names worldwide as Marijana in America; Bhang, Ganja and Charas in India; Kif in North Africa; Dogga in South Africa; Krori in Tunisia, Habak in Turkey; Hashish in Middle East; Djomba or Liamba in Central Africa and Brazil; Sodom, Tampl, Gum, Gauge and Stuff in Kinshasa, Swala and Whiskt in Ghana; Grifa in Mexico and Macohna in some parts of South America.12 The plant grows well at low temperature, and well-adjusted to moderate climates. Today, the plant is commonly known as a powerful psychoactive substance, but for many years it was cultured primarily for its fibers, that were used in the production of rope, clothes and ship sails.13 Cannabis sativa extracts as medicine was described in China and India before the birth of Christ.14 The plant and its preparations have been used for its sedative, narcotic, antispasmodic, analgesic and many other properties including its use for photo phobia, asthma and piles.15, 16 Cannabis also induces an increase in heart rate, lowers blood pressure due to vasodilatation and stimulates appetite.17 Its extracts may represent an efficacious and
safe alternative for treating insomnia, sick headaches, neuralgia, migraine, mania, whooping cough, dysuria, hyperemesis gravidarum and in relieving pain in dysmenorrhea and menorrhagia. Cannabidiol (CBD) is used as a pain killer and for other cosmetic purposes. It can be administered to patients suffering from rabies, cholera, rheumatism, epilepsy and tetanus. Also observation is that Cannabis sativa have been used for the treatment of specific human ailments, such as allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies, smallpox and sexually transmitted diseases.

Gender identification of Cannabis is done on the basis of its flowers. The flowers (and to a lesser extent the leaves, stems, and seeds) of this plant contain psychoactive and physiologically active chemical compounds known as cannabinoids that are consumed for recreational, medicinal, and spiritual purposes. The term “cannabinoids” represents a group of C21 terpenophenolic compounds found uniquely in Cannabis sativa and includes their analogs and transformation products. The male variety of this plant is known to produce greater amount of cannabinoids. Cannabiodial yield is also higher in more tropical environment; this is mainly due to reactions, such as alklylation and condensation. The 86 known cannabinoids from Cannabis plant can be classified into 11 structural types (figure): Cannabigerol (CBG), Cannabichromene (CBC), Cannabidiol (CBD), Tetrahydrocannabinol (THC), Δ8-THC, Cannabicyclol (CBL), Cannabielsoin (CBE), Cannabinol (CBN), Cannabinodiol (CBND), Cannabidiol (CBD) and miscellaneous types. Of which, the most prevalent cannabinoids are Δ9-Tetrahydrocannabinol (THC), Cannabidiol (CBD), Cannabinol (CBN), Cannabigerol (CBG), Cannabichromene (CBC) and Cannabinodiol (CBND). Tetrahydrocannabinol (THC) is one of these cannabinoids which is considered the most active element of this plant. Other compounds from this plant have little or no psychoactive effects. Cannabinoids induce their effects by interacting with various neurotransmitters and neuromodulators, such as gamma-amino butyric acid (GABA), histamine, serotonin, dopamine, glutamate, norepinephrine, prostaglandins and opioid peptides. Basically, there are three sources of cannabinoids; phytocannabinoids occur uniquely in the Cannabis plant; endogenous cannabinoids or endocannabinoids are produced in the bodies of humans and animals and synthetic cannabinoids which are similar compounds produced in the laboratory. The main objective of this review is to encourage utilization of Cannabis sativa in developing potential therapeutic agents based on their antimicrobial performance.

Antimicrobial activity of Cannabis sativa

Antimicrobial studies on the Cannabis sativa grown in different parts of the world have been carried out by many researchers (Table 1). Wasim et al. (1995) studied the antimicrobial activity of aqueous, ethanolic and petroleum ether extracts of the leaves of Cannabis sativa against Baccillus subtilis, Baccillus pumilus, Staphlococcus aureus, Micrococcus flavus, Proteus vulgaris, Bordetella bronchiseptica, Candida albicans and Aspergillus niger using well diffusion method. Results showed that ethanolic and petroleum ether extract exhibited activity both against Gram-positive and Gram-negative bacteria and also against the fungi, whereas aqueous extract did not show any antimicrobial activity. The antimicrobial activity of seed’s oil of Cannabis sativa extracted with hexane and methanol solvent was evaluated by Leizter et al. (2000) against Aspergillus niger (mycelium-forming fungi), Escherichia coli, Staphylococcus aureus, Saccharomyces cerevisiae (yeast, single cell fungi) and Pseudomonas aeruginosa. Results showed that the oil extracted with methanol exhibited potent activity against Saccharomyces cerevisiae, however, it did not displayed antibacterial properties. Appendino et al. (2008) extracted all five major cannabinoids from Cannabis sativa: Cannabidiol (CBD), Δ9-Tetrahydrocannabinol (THC), Cannabigerol (CBG), Cannabichromene (CBC), and Cannabinol (CBN), and observed their antibacterial activity. They found that all of them showed potent activity against a variety of methicillin-resistant Staphylococcus aureus (MRSA) strains of current clinical relevance. Borchardt et al. (2008) studied the antimicrobial activity of aqueous ethanolic extract of Cannabis sativa (stem and leaf) against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans using disc diffusion method. The extract was found to have very good antibacterial activity only against Staphylococcus aureus.

Radwan et al. (2009) isolated and studied antimicrobial property of nine new cannabinoids [(±)-4-acetoxycannabinol, (±)-3-hydroxy-Δ(4",5")-cannabichromene, (±)-7-hydroxycannabinol, (±)-7R-cannabicornamacronic acid A, 5-acetyl-4-hydroxycannabinol, 4-acetoxy-2-geranyl-5-hydroxy-3-n-pentylphenol, 8-hydroxy cannabinol, 8-hydroxy cannabinol acid A, and 2-geranyl-5-hydroxy-3-n-pentyl-1,4-benzoquinone]. Cannabinoids were isolated from the hexane, dichloromethane, ethyl acetate, ethanol, aqueous ethanol and aqueous extracts of whole buds of a high-potency variety of Cannabis Sativa through 1D and 2D NMR spectroscopy, GC-MS and HRESIMS. Antimicrobial property of these cannabinoids were studied against Candida albicans ATCC 90028, Candida kruusei ATCC 6258, Aspergillus fumigatus ATCC 90906, methicillin-resistant Staphylococcus aureus ATCC 33591, Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 27853 and Mycobacterium intracellulare ATCC 23068. Compound 2, 5, 6, 7 and 8 displayed significant antimicrobial activities compare to other isolated compounds. The antibacterial activity of freshly extracted essential oils from industrial hemp varieties was evaluated by Nissen et al. (2010) against Gram positive bacteria, with regard to food-borne pathogens or gastrointestinal bacteria and...
Gram negative bacteria. They characterized essential oils through the gas chromatography and gas chromatography-mass spectrometry. Their results showed that essential oils of industrial hemp can significantly inhibit the microbial growth, depending on variety and sowing time. Antibacterial activity of crude alkaloid extracted from Cannabis sativa leaf was investigated by Das and Mishra in 2011 against bacterial strains representative of skin, mouth and ear microflora and also against β strain of E. coli. This study revealed the effectiveness of the plant against all the tested strains.

Figure 1: Structures of different cannabinoids found in Cannabis plant

Ali et al. (2012) investigated the antibacterial activity of the seed’s oil, petroleum ether and methanol extracts of the whole plant of Cannabis sativa against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa using the cup plate agar diffusion method. Their results showed that oil of the seeds of Cannabis sativa exerted pronounced antibacterial activity against Bacillus subtilis and Staphylococcus aureus, moderate activity against Escherichia coli and high activity against Pseudomonas aeruginosa. The petroleum ether extract of the whole plant exhibited pronounced antibacterial activity against both Bacillus subtilis and Staphylococcus aureus organisms, high activity against Escherichia coli and inactive against Pseudomonas aeruginosa. The methanol extract of the whole plant also showed pronounced antibacterial activity against Bacillus subtilis, low activity against Staphylococcus aureus and high activity against both Gram negative organisms. Lone and Lone in 2012 evaluated the antimicrobial activity of cannabinoids extracted by aqueous and acetone extraction from Cannabis sativa leaf against Pseudomonas aeruginosa,
**Table 1**: Antimicrobial activity of *Cannabis sativa* (summary of review)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Plant parts</th>
<th>Solvents</th>
<th>Test organisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaf</td>
<td>Aqueous, ethanolic and petroleum ether</td>
<td><em>Bacillus subtilis, Baccilus pumilus, Staphlococcus aureus, Micrococcus flavus</em>, <em>Proteus vulgaris, Bordetella bronchiseptica</em>, Candida albicans and <em>Aspergillus niger</em></td>
<td>Wasim et al., 1995</td>
</tr>
<tr>
<td>2</td>
<td>Seed’s oil</td>
<td>Hexane and methanol</td>
<td><em>Aspergillus niger</em> (mycelium-forming fungi), <em>Escherichia coli</em>, <em>Staphylococcus aureus</em>, <em>Saccharomyces cerevisiae</em> (yeast, single cell fungi) and <em>Pseudomonas aeruginosa</em>.</td>
<td>Leizer et al., 2000</td>
</tr>
<tr>
<td>3</td>
<td>Whole plant</td>
<td>Acetone</td>
<td>Methicillin-resistant <em>Staphylococcus aureus</em> (MRSA)</td>
<td>Appendino et al., 2008</td>
</tr>
<tr>
<td>4</td>
<td>Stem and leaf</td>
<td>Aqueous ethanolic</td>
<td><em>Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa</em> and <em>Candida albicans</em></td>
<td>Borchardt et al., 2008</td>
</tr>
<tr>
<td>6</td>
<td>Leaf</td>
<td>-</td>
<td>Bacterial strains representative of skin, mouth and ear microflora and β strain of <em>E. coli</em></td>
<td>Das and Mishra, 2011</td>
</tr>
<tr>
<td>7</td>
<td>Whole plant</td>
<td>Petroleum ether and methanol</td>
<td><em>Bacillus subtilis, Staphylococcus aureus, Escherichia coli</em> and <em>Pseudomonas aeruginosa</em></td>
<td>Ali et al., 2012</td>
</tr>
<tr>
<td>8</td>
<td>Leaf</td>
<td>Aqueous and acetone</td>
<td><em>Pseudomonas aeruginosa, Vibro cholera, Cryptococcus neoforms, Aspergillus niger</em> and <em>Candida albicans</em></td>
<td>Lone and Lone, 2012</td>
</tr>
<tr>
<td>9</td>
<td>Leaf</td>
<td>Aceton and methanol</td>
<td><em>Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans</em> and <em>Aspergillus niger</em></td>
<td>Mkpenie et al., 2012</td>
</tr>
<tr>
<td>10</td>
<td>Leaf</td>
<td>Methanol and n-hexane</td>
<td><em>Bacillus cereus, Bacillus subtilis</em>, <em>Escherichia coli, Pseudomonas aeruginosa</em> and <em>Salmonella typhi</em></td>
<td>Nasrullah et al., 2012</td>
</tr>
<tr>
<td>11</td>
<td>Leaf</td>
<td>Aqueous and ethanol</td>
<td><em>Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus</em> and <em>Candida albicans</em></td>
<td>Mathur et al., 2013</td>
</tr>
<tr>
<td>12</td>
<td>Leaf</td>
<td>Methanol, ethanol, acetone and aqueous</td>
<td><em>Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae</em> and <em>Salmonella typhi</em></td>
<td>Monika et al., 2014</td>
</tr>
<tr>
<td>13</td>
<td>Leaf</td>
<td>Ethanol and aqueous</td>
<td><em>Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis</em>, <em>Salmonella typhi</em> and <em>Klebsiella pneumonia</em></td>
<td>Naveed et al., 2014</td>
</tr>
<tr>
<td>14</td>
<td>Whole plant</td>
<td>Hydro-alcoholic</td>
<td><em>E. coli</em> 25922, <em>E. coli</em> ESBL+, <em>S. aureus</em> 25923, <em>MRSA, Pseudomonas aeruginosa</em> ESBL+, <em>Pseudomonas, Klebsiella pneumonia</em> and <em>Acinetobacter baumannii</em></td>
<td>Sarmaday et al., 2014</td>
</tr>
<tr>
<td>15</td>
<td>Leaf</td>
<td>Ethanol, methanol, acetone and aqueous</td>
<td><em>Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans</em> and <em>Saccharomyces cerevisiae</em></td>
<td>Kauret et al., 2015</td>
</tr>
</tbody>
</table>
Antibacterial and phytochemical investigations of methanol, ethanol, acetone and aqueous extracts of *Cannabis sativa* leaves was done by Monika et al. (2014) against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Salmonella typhi* using agar well diffusion method. Their results revealed that all the extracts exhibited antibacterial activity against all the tested strains. Phytochemical screening showed the presence of saponins, tanins, quinones, alkaloids in the plant extracts. The antibacterial activity of ethanolic and hot water extract of *Cannabis sativa* leaf was investigated by Naveed et al. (2014) against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Salmonella typhi* and *Klebsiella* following well diffusion method. Their results showed that the plant extracts exerted pronounced activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, while no activity against *Salmonella typhi* and *Klebsiella*. Sarmadyan et al. (2014) investigated the antibacterial effect of hydro-alcoholic extract of *Cannabis sativa* against *E. coli* 25922, *E. coli* ESBL+, *S. aureus* 25923, MRSA, *Pseudomonas aeruginosa* ESBL+, *Pseudomonas*, Klebsiella pneumonia and *Acinetobacter baumannii* following disc diffusion method. They also determined the minimum inhibitory concentrations (MIC) of the active extracts. Their results revealed that the extract was active against *S. aureus* 25923, MRSA, *E. coli* 25922, *E. coli* ESBL+ and *Klebsiella pneumonia*, while inactive against *Pseudomonas aeruginosa* ESBL+, *Pseudomonas* and *Acinetobacter baumannii*. In MIC determination assay, the lowest value was observed for *S. aureus*. Kaur et al. (2015) studied the antimicrobial potency of ethanol, methanol, acetone and aqueous leaf extracts of *Cannabis sativa* against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Saccharomyces cerevisiae* following agar well diffusion method. Results of their study showed that all the extracts were effective against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*, while ineffective against *Pseudomonas aeruginosa*, *Candida albicans* and *Saccharomyces cerevisiae*. Among all the extracts, methanolic extract was found most effective against the tested strains.

CONCLUSION

From the above review, we can conclude that *Cannabis sativa* can be exploited to prepare potent broad-spectrum antimicrobial drugs, because of its remarkable inhibitory activity against a wide range of organisms. More importantly, it can be included in the list of herbal medicines in appropriate concentrations due to its lesser side effects. This study also reveals that the leaves of a plant can be considered as a potential source for isolating antimicrobial compounds. In most of the studies, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* were found significantly inhibited by different plant extracts, suggesting the effectiveness of plant in diseases caused by these pathogens. Solvents used in the extraction methods have great influence on both; the amount of active compounds that can be extracted and the result of the antimicrobial performance of a plant. Considering this, our review also suggests that ethanol, methanol and distilled water can act as potent solvents for extracting different secondary metabolites. To further confirm the therapeutic applicability of *Cannabis sativa*, studies on mechanism(s) of action, in-vivo and toxicological effects of its compounds are required.

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Authors’ Contributions:

This review study was carried out in collaboration between both the authors. Each author has participated sufficiently in the work to take public responsibility for appropriate portions of the content. Both the authors have read and approved the final version of the manuscript.

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