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Computation of the Antimicrobial Efficacy and Phytochemical Properties of *Cannabis Sativa* Powder against Some Clinical Isolates

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Abstract

The phytochemical makeup and antibacterial qualities of *Cannabis sativa* powder are examined in this study, underscoring the plant's potential as a substitute therapy for bacterial infections, especially those caused by bacteria resistant to antibiotics. Clinical isolates of *Bacillus cereus, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa,* and *Serratia marcescens* were used to assess the antibacterial activity of *Cannabis sativa*. With differing degrees of inhibition against the microorganisms, the results showed strong antibacterial efficacy. The highest activity was observed against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, with zones of inhibition measuring up to 5 mm and 4 mm, respectively. The effectiveness of *Cannabis sativa* extracts in preventing bacterial growth, namely against *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, was further validated by the Minimum Inhibitory Concentration (MIC) experiments. Because of the expanding worldwide problem of antibiotic resistance, this study emphasizes the potential of *Cannabis sativa* as a source of antibacterial drugs. Future studies should identify the precise chemicals causing these effects and investigate their modes of action, which could lead to the development of new antimicrobial therapies. Overall, this study underscores the need to revisit traditional medicinal plants like *Cannabis sativa* in the search for innovative solutions to contemporary health challenges.

Keywords: *Cannabis sativa;* Phytochemical analysis; Antimicrobial properties; Clinical isolates.

1. Introduction

The plant *Cannabis sativa*, commonly known as hemp, is native to central and southwest Asia and belongs to the Cannabaceae family. For ages, people have utilized the herbaceous annual plant Cannabis sativa for religious, culinary, medicinal, recreational, and textile uses [1]. For thousands of years, this herb was widely employed in Ayurvedic medicine, traditional Chinese medicine, European medicine, and Arab culture. Chinese civilization utilized hemp seeds 5,000 years ago to treat inflammatory illnesses, malaria, and exhaustion. Traditional medicine is seen as the best treatment option for these new issues with antibiotic resistance [2]. Because of their therapeutic qualities, over 80 % of people still use plants for therapy today, even in the face of sophisticated synthetic pharmaceuticals [3]. Approximately 258,650 species of higher plants have been identified by global plant research. Over ten percent of them have been used as a treatment for residents who are ill. Pakistan is home to over 6,000 species of higher plants, of which up to 700 species are known to be used as herbal remedies [4]. Pakistan is fortunate to have a variety of medicinal plants due to its distinct climate [5]. Two major groups of medicinal plants have been identified. Physicians use plants in the first group to treat pain, while plants in the second group are used in pharmaceutical companies to make synthetic medications because of their bioactive chemicals [6]. In addition to volatile compounds like humulene-limonene, a-terpinolene, b-selinene, and others, the plant is known to contain nonvolatile substances like flavonoids, cannabinoids, phenolic acids, fatty acids, alkaloids, and steroids [7-9]. Additionally, the research commented on this plant's nutritional value and safe use [10]. An overview of *C. sativa's* history was recently published by Rull, *et al.* [11], and Schanknecht, *et al.* [12] provided scientific proof that cannabis can be used to cure melanoma. The effects of cannabinoids on the central nervous system, the therapy of COVID-19, and the methods for identifying and measuring cannabis phytochemicals have all been covered in other review publications [13-15].

A comprehensive review was conducted to better guide future research studies for potential use to enhance health conditions. The current study aims to highlight the current directions in C. sativa research by summarizing its antimicrobial action. Additionally, it provided information about the legalization and decriminalization of cannabis use, as well as an exploration and summary of the various bioactive chemicals found in hemp plants. Worldwide, bacterial resistance to antimicrobial treatment is a serious issue. According to Martens and Demain [16], bacterial

illnesses claim the lives of 17 million people annually, making infectious diseases the second greatest cause of death. It is unprecedented how many organisms are resistant to one or more antibiotic classes. No antibiotic class has escaped a resistance mechanism, and there are over 15 classes that target various bacterial cell structures. The absence of novel antibiotics created and approved in recent decades is the basis for the lack of alternatives to reduce antimicrobial resistance [17]. To reduce resistant bacterial strains in this situation, alternate tactics need to be investigated [18]. Many plant extracts and phytochemicals have demonstrated antibacterial qualities against pathogens, including clinical-resistant bacterial strains, and plants have long been utilized as sources of natural products for human health [19]. In certain instances, animal models of infection confirmed the antibacterial properties of plant extracts. Yunana, *et al.* [20] and Choi, *et al.* [21]. Additionally, combining plant extracts or their bioactive components with antibiotics may have synergistic effects that counteract the mechanisms of bacterial resistance [22].

2. Material and Method

2.1. Collection of Samples

Indian hemp samples had been collected in the Southwest Nigerian city of Ibadan. They were randomly collected in sterile conical flasks from local producers and retailers in motor parks and markets. The samples were transported in ice packs to the laboratory and stored under refrigeration at 4 ^{o}C until required for bacterial isolation and phytochemical activities.

2.2. Plant Extract Preparation

After collecting, the Indian hemp was thoroughly cleaned to get rid of any dirt. The plants were crushed to powder and allowed to dry at room temperature in the dark. For later use, the powder was kept at room temperature in a glass jar. 10 grams of each plant powder were dissolved in one hundred milliliters of ethyl acetate in a 250-milliliter flask and gently stirred. After a day at room temperature, the mixture was filtered using Whatman no. 1 filter paper to extract the plant extract.

2.3. Isolation of Microorganisms

Each sample of *hemp* powder was diluted repeatedly in sterile distilled water, and the corresponding dilutions were plated onto sterile plates of both nutrient agar and selective agars. (Mueller-Hinton agar, Mannitol salt agar)

2.4. Preparation of Media

Following the manufacturer's instructions, all the media used for microbiological examination were prepared and autoclave sterilized for 15 minutes at 121 ^{o}C. As needed, the sterile media were poured or dispensed into test tubes or Petri dishes that had been sterilized. Blindly chosen plates were incubated at 37 ^{o}C for the entire night to verify the prepared media's sterility.

2.5. Determination of Antibacterial Potential of Plant Extract

A common technique for figuring out whether a plant has antibacterial action or not is the agar well diffusion method. For this assay, *Bacillus* (KC 881030), *S. aureus* ATCC 35556 (80 %), and *Pseudomonas* (KC 881031) strains were employed as test strains, following the methodology of Irshad, *et al.* [23]. Ethyl acetate was employed as a negative control and ampicillin (10 µg/ml) as a standard. Plant extracts' zones of inhibition against strains of *Bacillus*, *Staphylococcus*, and *Pseudomonas* were assessed in millimeters (mm).

2.6. Minimum Inhibitory Concentration

A quantitative test called minimum inhibitory concentration (MIC) assessment is used to find the lowest extract concentration that stops bacterial growth. Bacterial growth was measured using tetrazolium salt. The 24-hour broth culture was supplemented with tetrazolium salt. Following incubation, a hue shift was noticed. A reaction with tetrazolium salt caused the wells whose extract concentration was unable to stop the growth of bacterial cells to change color. The extract's antimicrobial action prevented any color change, indicating that there were no vegetative cells in the wells [24].

2.7. Antibiotic Sensitivity Pattern of Bacteria Isolates

Mueller Hinton agar was used for antibiotic sensitivity tests. The 0.5 McFarland standards were used to prepare the inoculums. The test organism was selected using a sterile inoculating loop and suspended in two milliliters of sterile normal saline. The spectrophotometer was used to measure the absorbance of this suspension at a wavelength of 625 nm. When it was determined that the suspension was too light, more organisms were added, and when it was too turbid, sterile normal saline was added to dilute it. A sterile swab stick was left to soak after being dipped into the inoculum's suspension.The swab stick will next be used to uniformly streak the entire dried agar surface to inoculate it. To ensure adequate diffusion of the antibiotics on the agar, a Gram-negative antibiotic multidisc containing eight medications was aseptically placed on the plate and forcefully pressed onto the agar surface using sterile forceps. Ceftazidime (30µg), cefuroxime (30µg), ciprofloxacin (5µg), gentamicin (10µg), cefixime (5µg), ofloxacin (5µg), augmentin (30µg), and nitrofurantoin (300µg) were the antibiotics on the disk. All test organisms

underwent this procedure once more, and the plates were incubated for twenty-four hours at 37 ^{o}C. Following incubation, the inhibition zones were measured in millimeters using a transparent ruler [25].

2.8. Phytochemical Analysis of Selected Medicinal Plant Extracts

Several phytochemical components, including alkaloids, carbohydrates, cardiac glycosides, flavonoids, phlorotannins, saponins, tannins, terpenoids, steroids, oxalate, and proteins, were examined in Indian hemp plants for the current study [26-29]..

2.8.1. Test for Tannin/Polyphenol

When three to four drops of 10% FeCl3 were added to the diluted extract, gallic tannins turned blue and catechol tannins caused the solution to turn green [30].

2.8.2. Test for Reducing Sugar

One milliliter of water, five to eight drops of Fehling's solution, and 0.5 milliliters of plant extract were heated. Brick-red precipitation appeared, indicating the presence of decreased sugar

2.8.3. Test for Glycosides

Molisch's Reagent Test: Concentrated H2SO4 and 5 mL of Molisch's reagent were added to the extract. Glycosides were suggested by a violet hue.

2.8.4. Test for Flavonoids

Shinoda test: a tiny piece of magnesium was heated along with 4 milliliters of extract solution and 1.5 milliliters of 50 % methanol solution. When five to six drops of concentrated HCl were added, flavonoids showed a crimson hue.

2.8.5. Test for Terpenoids

Each sample's zero point two (0.2 g) was combined with 3 mL of concentrated H2SO4 and 2 mL of chloroform. Terpenoids were detected by reddish-brown coloring.

2.8.6. Test for alkaloids

Meyer's examination one milliliter of Meyer's reagent was added to two milliliters of extract. Alkaloids were detected by the appearance of a pale yellow precipitate.

2.8.7. Test for Saponins

Twenty milliliters of distilled water were used to boil two grams of the powdered material. Five milliliters of distilled water and ten milliliters of filtrate were shaken vigorously. Saponins were present because of the appearance of foaming.

2.8.8. Test for Steroids

A drop of conc and a few drops of acetic acid were used to dissolve one gram of plant extract. They added H2SO4. Steroids were present when a green tint appeared.

3. Results

Table 1 summarizes the phytochemical properties of *hemp* (*Cannabis sativa*) that were examined. The findings showed that the hemp plants under study contained chemicals with potential medical uses. Proteins, carbohydrates, phenols and tannins, flavonoids, alkaloids, glycosides, and steroids are all listed in Table 1. *Hemp* plants contained both terpenoids and saponins, with the largest concentration of these compounds being carbohydrates (31.05), followed by protein (19.69) and flavonoids (0.024 mg/gm).

Table-1. Phytochemicals properties of Aqueous extract of*Cannabis sativa*

S/N	Parameters	Units mg/mg
1	Alkaloids	0.267
2	Flavonoids	0.024
3	Saponin	0.326
4	Tannin	0.082
5	Glycoside	0.197
6	Phenol	0.285
7	Steroids	0.056
8	Terponoids	0.035
9	Carbohydrate	31.05
10	Protein	19.67

Table 2 provides information about the antibacterial properties of the aqueous extract of *Cannabis sativa*. It shows the zone of inhibition (in mm) for different bacterial isolates at two concentrations (500 mg and 250 mg).

Table-2. Antimicrobial Susceptibility of Aqueous extract of *Cannabis sativa* Zone of Inhibition Millimeter (mm)

Test Isolates	**Aqueous Extract Conc. (500mg)**	**Aqueous Extract Conc. (250mg)**
Bacillus cereus	3.00	0.00
Staphylococcus aureus	2.00	0.00
Klebsiella pneumonia	5.00	1.00
Escherichia coli	0.00	0.00
Pseudomonaa saeruginosa	4.00	1.00
Serratia marcescens	0.00	0.00

Table 3 shows the qualitative analysis results of the MIC for various bacterial isolates at two concentrations (500 and 250 µg/ml).

Table-3. Minimum Inhibitory Concentration (MIC)

Qualitative analysis Result		
Test Isolates Codes	Aqueous Extracts Concentration (500g/ml)	Aqueous Extracts Concentration (250g/ml)
Bacillus cereus	+	-
Staphylococcus aureus	+	-
Klebsiella pneumonia	+	+
Escherichia coli	-	-
Pseudomonas aeruginosa	+	+
Serratia marcescens	-	-

Keys: + Positive, - Negative

4. Discussion

Cannabis sativa L. a member of the family *Cannabaceae*, is grown globally as hemp and marijuana [31]. Plant extract analysis revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, carbohydrates, proteins, and alkaloids. Phenolic compounds also contribute to the plant's antimicrobial activity, as they can interfere with microbial cell wall synthesis [32].

Tannins are a type of polyphenolic chemical present in many plants, particularly their bark, leaves, and fruits. They are known for their ability to bind and precipitate proteins, which contributes to their astringent taste (Haslam, 1996). In cannabis, tannins may play a role in inhibiting the growth of harmful microorganisms, contributing to the antimicrobial properties observed in the study.

Saponins are a varied collection of naturally occurring chemicals present in many plant species. They are glycosides, meaning they consist of a sugar part (glycone) and a non-sugar part (aglycone). The aglycone can be a steroid or a triterpene, which contributes to their classification [33]. Saponins have been widely researched for their therapeutic potential, including their ability to reduce cholesterol and enhance immune function [32].

Several flavonoids found in cannabis, such as cannflavins, have shown promise as anti-inflammatory agents [34].

Alkaloids are naturally occurring organic molecules composed mostly of basic nitrogen atoms. They are typically generated from plants and are known to have pharmacological effects on people and animals [35].While alkaloids are well-known in plants like opium poppy and coca, their presence in cannabis may contribute to some of the plant's psychoactive effects, particularly in compounds like THC (tetrahydrocannabinol). Research has shown that alkaloids in cannabis extracts may have neuroprotective properties and can influence neurotransmitter systems [36]

Glycosides consist of a sugar molecule bound to a non-sugar molecule (aglycone). They can exert a variety of biological effects, including anti-inflammatory, antimicrobial, and anticancer properties. Glycosides in cannabis may have therapeutic benefits due to their ability to modulate cell signaling pathways and immune responses.

Terpenoids are a large and diverse group of organic compounds found in cannabis. They are primarily responsible for the plant's aroma and flavor but also contribute to its therapeutic properties. Terpenoids have been shown to possess antimicrobial, anti-inflammatory, and analgesic effects. Some terpenes found in cannabis, such as limonene and pinene, are known to have antimicrobial activity, making them important in the overall antimicrobial profile of cannabis [37].

Carbohydrates are essential for energy metabolism and can have additional health benefits through their fiber content, which supports digestive health ElSohly and Slade [36]. Proteins are vital for cellular function, and their presence in cannabis suggests that the plant may also have nutritional benefits. Proteins in cannabis may also play a role in modulating immune responses and inflammation [36]. According to the study, Cannabis sativa has a lot of phytochemicals, which gives it special qualities [38].

The antibacterial qualities of the *Cannabis sativa aqueous* extract are examined in this study, along with the zone of inhibition (measured in millimeters) for various bacterial isolates at two dosages (500 mg and 250 mg). The

extract exhibited a 3.00 mm zone of inhibition in Bacillus cereus at 500 mg, indicating modest antibacterial activity, which vanished at 250 mg. This strain, which is a common foodborne pathogen, showed some susceptibility to the extract. *Staphylococcus aureus* the extract showed weak activity (2.00 mm) at 500 mg but no activity at 250 mg. *Staphylococcus aureus*, a notorious pathogen responsible for skin infections and more serious conditions like MRSA, was only weakly inhibited by the extract. *Klebsiella pneumonia:* The extract showed a strong inhibitory effect (5.00 mm) at 500 mg, suggesting that this pathogen is highly susceptible to the antimicrobial compounds in cannabis. At 250 mg, the inhibition was reduced to 1.00 mm, indicating dose-dependent activity. *Escherichia coli*: No inhibition was observed at either concentration. This could be due to the specific resistance mechanisms of *E. coli* or the lack of effective antimicrobial compounds in the extract for this strain. *Pseudomonas aeruginosa:* The extract showed moderate inhibition at both concentrations (4.00 mm at 500 mg and 1.00 mm at 250 mg), suggesting that *Pseudomonas aeruginosa* is somewhat susceptible to the extract. *Serratia marcescens:* No inhibition was observed at either concentration, indicating that this strain is resistant to the aqueous extract of *Cannabis sativa*. The findings of Chakraborty, *et al.* [39], who investigated the antibacterial activity of Cannabis sativa, are consistent with the results of this study. Additionally, the results of this investigation are consistent with those of Anumudu1, *et al.* [38], who demonstrated that the presence of cannabinoids *Cannabis sativa* extracts leads in comparable antibacterial action.

The Minimum Inhibitory Concentration (MIC) is a critical measure of the smallest concentration of a substance that inhibits bacterial growth. It provides qualitative analysis results of the MIC for various bacterial isolates at two concentrations (500 and 250 µg/ml). *Bacillus cereus*, the extract was effective at 500, but not at 250 µg/ml, indicating that a higher concentration is required to inhibit its growth. *Staphylococcus aureus*: Similar to *Bacillus cereus*, the extract inhibited growth at 500 but not at 250 µg/ml. *Klebsiella pneumoniae:* The extract inhibited the growth of this bacterium at both concentrations, which suggests a higher sensitivity to the extract. *Escherichia coli* no inhibition was observed at either concentration, confirming its resistance to the extract. *Pseudomonas aeruginosa* the extract inhibited this bacterium at both concentrations, indicating its effectiveness even at lower doses *Serratia marcescens* no inhibition was observed at either concentration, reinforcing its resistance. In this investigation, four bacterial strains were tested positive, and two bacterial strains were tested negative for minimal inhibitory concentration, which is consistent with the findings of Martinengin, *et al.* [40].

5. Conclusion

The aqueous extract of *Cannabis sativa* contains a wide array of bioactive compounds with potential antimicrobial properties. The presence of alkaloids, flavonoids, saponins, phenols, and other phytochemicals likely contributes to the plant's observed antimicrobial effects. The extract exhibited antimicrobial activity against some bacterial strains, particularly *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, but was ineffective against others like *Escherichia coli* and *Serratia marcescens*. The findings imply that Cannabis sativa could be a promising source of antibacterial agents, but more research is needed to identify the exact chemicals responsible for these effects and to fully investigate the plant's therapeutic potential. Furthermore, future studies should examine the mechanism of action of these phytochemicals and assess the therapeutic efficacy of cannabis-derived antimicrobials.

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