Contents list available at Journal of e-Science Letters

Journal of e-Science Letters

journal homepage: http://scienceletters.researchfloor.org/

# Comprehensive Analysis of Antimicrobial Susceptibility Test of *Cannabis sativa* on Multidrug Resistant Bacterial Isolates

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ARTICLE INFO

*Article History:*
*Received 19 May 2022*
*Revised 26 June 2022*
*Accepted 29 July 2022*
*Available Online 05 September 2022*

*Keywords:*
*Antimicrobial susceptibility test,*
*Cannabis sativa,*
*multidrug-resistant bacterial isolates,*
*Nigeria*

ABSTRACT

*This study shows the effect of various parts of Cannabis sativa as a plant on some multidrug-resistant clinical isolates. Cannabis sativa plants and the ready-to-smoke popularly called skunk (SK) were obtained from the Nigeria Drug Law Enforcement Agency (NDLEA), Ado-Ekiti Unit, Ekiti State, Nigeria, and processed for laboratory analysis and standard microbiological assays. This study shows that Cannabis sativa's potential and antimicrobial activity are valuable in treating multi antibiotics clinically resistant microorganisms. It was observed that n-haxane extracts of Cannabis sativa seed -A1, Cannabis sativa stem -A3, Cannabis sativaSkurk -A5 as well as chloroform extracts of extract Cannabis sativa seed -D1 and Cannabis sativa leaves-D2 are inhibitive against test organisms. The most active was extract A1 which showed a bactericidal effect against some enteric organisms such as Escherichia coli, Salmonella typhi and Salmonella pullorum tested. Others are Staphylococcus aureus, Bacillus cereus as well as bacteriostatic effect against Proteus mirabilis and Pseudomonas aeruginosa. Extract A3 showed bactericidal effect against Bacillus cereus and bacteriostatic effect against Proteus mirabilis. Similarly, Extract A5 and Extract D1 showed a bactericidal effect against Bacillus cereus. In comparison, Extract D2 and Extract E3 showed bactericidal effects against Salmonella typhi and Proteus mirabilis, respectively. The observation in this study shows that Extract A1 is highly effective against test organisms such as Salmonella pullorum and Bacillus cereus, where its bactericidal effect is greater than that of the control (Ofloxacin) used. It further suggests that Extracts A3, A5, D1, and D2 can be further studied by increasing the concentration and changing the solvent used for extraction.*

## 1. Introduction

*Cannabis sativa* is a plant that is not yet been fully harnessed; it has numerous applications in the industrial and health sectors. It has a large and diversified medicinal value. Available commonly used antimicrobial agents have restricted value in overcoming the surge of multi-drug resistant microorganism and the toxicity related to extending treatment is a limiting factor for its usage; hence, the use of alternative antimicrobial agent from phytomedicine will be helpful scientifically (Ajayi *et al*., 2017; Atef *et al*., 2019). *Cannabis sativa* is among variety of cannabinoids that have been discovered with strong potentials to treat various infections. It also shows synergism with polymyxin B in previous stusdies. Considering the current surge of antimicrobial resistance in recent years, cannabinoids is found efficient for use as antibiotics. Fundamental reviews on *Cannabis* as antimicrobial of interest emerged from Kabelík *et al*., (1960) in which extracts from extraordinary components of *Cannabis sativa* var. *indica* had been screened for antibacterial activities towards some isolated micro-organisms

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DOI: http://dx.doi.org/10.46890/SL.2022.v03i03.001

and its bactericidal effect is confirmed mainly on Gram positive bacteria. Gram-negative bacteria in addition to fungi and yeast assays were unaffected (Kabelik*et al*., 1960).

Bioactivity of *Cannabis sativa* dates back to 1976 when Potter, (2009); Richins *et al*., (2018) located that the chain of 9- tetrahydrocannabinol as well as cannabidol are bacteriostatic in nature. In addition, some properties of *C. sativa* such as essential oils added to its antimicrobial value using different solvents (Khan *et al*., 2014). The use of various techniques for setting apart *C. sativa* extracts was reported. This includes the traditional strategies with better technology that enhance advanced merchandise for productivity (Wasim *et al*., 1995).

Lone (2012) examined the antimicrobial potential of *Cannabis sativa*. Their study shows the effectiveness of this plant source exemplified by the considerable zone of inhibition against *Cryptococcus neoformans*, *P. aeruginosa, C. albicans* and *Vibro cholera* at concentrations of five µg/ml and ten µg/ml (Lone and Lone, 2012). *C. sativa* leaf extracts has also been demonstrated to have good antimicrobial activity against some bacterial species (Kaur *et al*., 2015; Isahq *et al*., 2015 and Frassinetti *et al*., 2020).

The study of Chakraborty et *al*., (2018), on *C. sativa* with comparative analysis and other Indian medicinal plant sources shows a wide range of antimicrobial spectrum as well as synergies between cannabis and the other extracts. Van Klingeren and Ham (1976) generated the first record of the purified cannabinoids, which is a bioactive component from of *C. sativa* to quantify its antimicrobial activities. The study of Turner *et al*., (1981) also shows wide range biological activity of *C. sativa* positive assay results (Taura *et al*., 2007).

New forms of cannabinoids have been recently studied and shown to be active against selected bacterial species and some known etiologic agents (Appendino *et al*., 2008 and Appendino *et al*., 2011). Few purified sources among this were very powerful against dental plaque-associated bacterial strains in experimental studies (Stahl and Vasudevan., 2020; Farha *et al*., 2020). Complementary to this, bioactive components of *C. sativa* has the potential to inhibit biofilm formation, for cosmestics and in preservation of food (Feldman *et al*., 2020; Martinenghi *et al*., 2020; Galletta *et al*., 2020; Frassineti *et al*., 2020). The search for novel techniques and alternative antimicrobial agents was geared toward bacterial resistance to contemporary chemical antibiotics. *C. sativa* extracts amongst other antimicrobial agents were exploited for this reason and proven to possess several appealing and therapeutic components that necessitates investigation (Feldman *et al*., 2020). Further work by Galletta *et al*., (2020) in this regard on related microbiological perspectives shows the variations and differences in activity of *Cannabis* products on test organisms. Ethanol extracts of *C. sativa* leaves screened against test isolates had inhibition zone from 9 to 15 mm in diameter for the clinical isolates, this value was less than vancomycin which ranged 13 to 24 mm. However, synergies with plant sources in ratio 1:1 demonstrated a synergistic effect with zones of inhibition in diameter ranging from 25 to 30 mm in most cases.

The potential and bioactive components of *Cannabis sativa* had been shown to be valuable based on this investigations. This study, therefore, helps to determine the antimicrobial potential of different parts of *Cannabis sativa* plants using different extraction solvents for effective control of some aetiologic agents that are resistant to commonly used drugs.

## Materials and Methods

### Collection and Preparation of *Cannabis sativa*:

The *Cannabis sativa* plants along with the ready-to-smoke popularly called skunk (SK) were obtained from the Nigeria Drug Law Enforcement Agency (NDLEA with approved reference number NDLEA/ALS/156/VOL. IV), Ado-Ekiti Unit, Ekiti State, Nigeria. The plants were supplied in sacks and were severed into different plant parts (seeds, leaves, stem and root) on clean plastic sheets and allowed to air dry for 4 weeks in the laboratory. After complete drying, the individual parts and the seed plus leaf (SK) were milled and stored in a plastic jar.

**Extraction:** Extraction processes were done according to standard methods (AOAC, 2005). 5.00g of the powdered samples were measured into conical flasks and extracted with 5 different

solvents (petroleum ether, chloroform, butanol, ethanol, *N-hexane*, and water). Flasks used were shaken at an hour interval for the first six hours and shaken again after 24 hours (Azwanida, 2015). The flasks were allowed to sit for three days before filtration of the final extract. Rotary evaporator was used to dry the extracts. A total of 30 extracts were obtained and carefully kept in airtight sample bottles before analyses.

**Collection and identification of microorganisms**

Nine (9) bacterial isolates recovered from clinical specimens of patients were collected from the microbiology laboratory of the Adekunle Ajasin University Health Centre, Akungba-Akoko, Ondo State, Nigeria and the Centre for Infectious Disease Control and Drug Development (CIDCDD), using nutrient agar medium. A preliminary confirmatory test was earlier done to confirm the identity of the organisms using Microbact™ 24E (Oxford, UK) identification kit for this purpose. And the organisms were confirmed to multidrug resist for this clinical antimicrobial susceptibilities test purposes (Ajayi *et al.,* 2020).

**Antibiotic susceptibility test (AST)**

The isolates tested were subcultured using nutrient broth and incubated at temperature of 37°C overnight (24 hours). The broth cultures were diluted with normal saline to 0.5 McFarland turbidity standards. Appropriate inoculums were used accordingly. The test for antibiotic susceptibility was carried out on the bacterial isolates using a Kirby-Bauer's antibiotics disc diffusion method with Muller-Hinton agar (CM337 – Oxoid, UK) as culture medium incubated at 37 °C for 18 hours (Balouiri *et al*., 2016). The zones of inhibition were measured in diameter and interpreted standard guidelines (CLSI, 2016). Calibrated measuring rulers or devices are adopted for this purpose.

**Antimicrobial screening of *Cannabis* parts extracts used**

The antimicrobial screening of the *Cannabis sativa* (Leavess, seed, stem, root, SK- Skurk) extracts against some predetermined antibiotics resistant clinical isolates were carried out using the modified agar well diffusion method (Balouiri*et al*., 2016). A stock concentration of 100mg/mL was constituted by dissolving 1g of extract in a 10mL mixture of 30% Dimethyl sulfoxide (DMSO) and sterile distilled water (1:3). Two-fold serial dilution of the stock was done to obtain concentrations range of 50mg/mL, 25mg/mL and 12.5mg/mL of the extracts. 0.5 McFarland turbidity standard was set and 1 mL aliquot of this standardized inoculum was prepared by mixing 19mL of molten Mueller-Hinton agar and was left to solidify in a sterile Petri dish.

Wells were carefully made on the agar plate (at least 20 mm apart) using sterile cork borer (6mm in diameter). 50 µl concentration of each extract and the controls were introduced into each well and were left on the table to settle. The culture medium was incubated at 37 $^0$C for 20 hrs and the activity of extracts was determined by formation of zone of inhibition was measured across the diameter in milliliter. A potent antibiotic (Ofloxazin) and Dimethyl sulfoxide (DMSO) were used as controls (positive and negative respectively) for this study.
Cork borer size used: 6mm. While for Control: Water serves as, Negative Control and Ofloxacin as, Positive control.

**Table 1:** Solvents and Plant parts used for the antimicrobial analysis

| Solvents | Plant parts code |
|---|---|
| A – N-Hexane | Seed - 1 |
| B – Methanol | Leaves - 2 |
| C – Petroleum ether | Stems - 3 |
| D - Chloroform | Roots - 4 |
| E – Ethanol | SK- Skurk - 5 |
| W – Water | |

## Results

This study shows that *Cannabis sativa* has potential and antimicrobial activity that is valuable in the treatment of multi antibiotics clinically resistant microorganisms. It was observed that n-hexane extracts of *Cannabis sativa* (A1, A3, A5, D1, D2) are generally active throughout this study. They are inhibitive against test organisms, especially

n-hexane extracts of extract *Cannabis sativa* seed (A1) (Tables 2 to 13). n-hexane extracts of extract *Cannabis sativa* seed (A1) showed bactericidal effect against some enteric organisms such as *Escherichia coli, Salmonella typhi* and *Salmonella pullorum* tested. Others are *Staphylococcus aureus, Bacillus cereus* and bacteriostatic effect against *Proteus mirabilis* and *Pseudomonas aeruginosa*. n-hexane extracts of extract *Cannabis sativa* stem (A3) showed bactericidal effect against *Bacillus cereus* and bacteriostatic effect against *Proteus mirabilis*. Similarly, n-hexane extracts of extract *Cannabis sativa* Skurk (A5) and chloroform extracts of extract *Cannabis sativa* seed (D1) showed bactericidal effect against *Bacillus cereus.* Whilst, Chloroform extracts of *Cannabis sativa* leaves D2 and ethanol extracts of *Cannabis sativa* stem (E3) showed bactericidal effect against *Salmonella typhi* and *Proteus mirabilis* respectively. Complementary to this, chloroform extracts of *Cannabis sativa* leaves D2 had a bacteriostatic and bactericidal effect on *Pseudomonas aeruginosa* and *Acinetobacter baumannii*respectively. Water extracts of *Cannabis sativa* leaves W2 had a bactericidal effect on *Salmonella typhi* since there was a clear zone of inhibition. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* showed a clear resistance against the control used. Methanol extract of *Cannabis sativa* stem showed a bacteriostatic effect on *Bacillus cereus*. Other *Cannabis sativa* plant extracts tested gave relatively low antimicrobial responses. This might be partly due to the low concentration of extracts used as well as their general potentials which may not be active at low concentrations.

**Table 2:** *In-vitro* Antimicrobial Assay on Bacteria, Using Extract Labelled A1, N-Hexane/Seeds

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Staphylococcus aureus* | 30 | - | 13 | 15 | 20 |
| *Acinetobacter baumannii* | - | - | - | - | - |
| *Klebsiella pneumoniae* | - | - | - | - | - |
| *Bacillus cereus* | 26 | 22 | 22 | 24 | 26 |
| *Escherichia coli* | 16 | 13 | 16 | 16 | 16 |
| *Salmonella typhi* | 22 | - | - | - | 14 |
| *Proteus mirabilis* | 22 | 9 | 9.5 | 11 | 12 |
| *Pseudomonas aeruginosa* | 15 | - | - | 10 | 10 |
| *Salmonella pullorum* | 18 | - | - | 20 | 22 |

**Table 3:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled A2, N-Hexane Leaves

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 4:** *In-vitro* Antimicrobial Assay on Bacteria Using ExtractLabelled A3, N-Hexane/Stems

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Staphylococcus aureus* | 27 | - | - | - | - |
| *Acinetobacter baumannii* | 25 | - | - | - | - |
| *Klebsiella pneumoniae* | - | - | - | - | - |
| *Bacillus cereus* | 35 | - | - | - | 9 |
| *Escherichia coli* | 36 | - | - | - | - |
| *Salmonella typhi* | 12 (BS) | - | - | - | 6 |
| *Proteus mirabilis* | 14 | - | - | - | 14 (BS) |
| *Pseudomonas aeruginosa* | 19 (BS) | - | - | - | - |
| *Salmonella pullorum* | 25 | - | - | - | - |

**KEY:** (BS) – Bacteriostactic

**Table 5:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled A4, N-Hexane/Roots

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 6:** *In-vitro* Antimicrobial Assay on Bacteria Using ExtractLabelled A5, N-Hexane/Sk

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Acinetobacter baumannii* | 23 | - | - | - | - |
| *Bacillus cereus* | 36 | - | - | - | 9 |
| *Escherichia coli* | 37 | - | - | - | - |
| *Klebsiella pneumoniae* | 34 | - | - | - | - |
| *Proteus mirabilis* | 21 | - | - | - | - |
| *Pseudomonas aeruginosa* | 14 | - | - | - | - |
| *Salmonella pullorum* | 25 | - | - | - | - |
| *Salmonella typhi* | 21 | - | - | - | - |
| *Staphylococcus aureus* | 34 | - | - | - | - |

**Table 7:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled C1, Petroleum ether/Seed

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 8:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled C2, Petroleum ether/leaves

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 9:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled C3, Petroleum ether/Stems

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 10:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled C4, Petroleum ether/Roots

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 11:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled C5, Petroleum ether/SK

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Acinetobacter baumannii* | 21 | - | - | - | - |
| *Bacillus cereus* | 30 | - | - | - | 9 |
| *Escherichia coli* | 24 | - | - | - | - |
| *Klebsiella pneumoniae* | 28 | - | - | - | - |
| *Proteus mirabilis* | 31 | - | - | - | - |
| *Pseudomonas aeruginosa* | 35 | - | - | - | - |
| *Salmonella pullorum* | 33 | - | - | - | - |
| *Salmonella typhi* | 18 (BS) | - | - | - | - |
| *Staphylococcus aureus* | 15 | - | - | - | - |

**KEY:** (BS) - Bacteriostactic

**Table 12:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled D1, Chloroform/Seed

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Acinetobacter baumannii* | 23 | - | - | - | - |
| *Bacillus cereus* | 36 | - | - | - | 12 |
| *Escherichia coli* | 36 | - | - | - | - |
| *Klebsiella pneumoniae* | 34 | - | - | - | - |
| *Proteus mirabilis* | 17 | - | - | - | - |
| *Pseudomonas aeruginosa* | 12 | - | - | - | - |
| *Salmonella pullorum* | 25 | - | - | - | - |
| *Salmonella typhi* | 28 | - | - | - | - |
| *Staphylococcus aureus* | 36 | - | - | - | - |

**Table 13:** *In-vitro* Antimicrobial Assay on Bacteria Using ExtractLabelled D2, Chloroform/leaves

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Acinetobacter baumannii* | 23 | - | - | - | - |
| *Bacillus cereus* | 38 | - | - | - | - |
| *Escherichia coli* | 35 | - | - | - | - |
| *Klebsiella pneumoniae* | 36 | - | - | - | - |
| *Proteus mirabilis* | 24 | - | - | - | - |
| *Pseudomonas aeruginosa* | 10 | - | - | - | - |
| *Salmonella pullorum* | 20 | - | - | - | - |
| *Salmonella typhi* | 30 | - | - | - | 9 |
| *Staphylococcus aureus* | 33 | - | - | - | - |

**Table 14:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled D3, Chloroform/stem

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 15:** *In-vitro* Antimicrobial Assay on Bacteria Using ExtractLabelled D4, Chloroform/Roots

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 16:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled D5, Chloroform/SK

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Acinetobacter baumannii* | 10 | | | | 19 |
| *Bacillus cereus* | 36 | - | - | - | - |
| *Escherichia coli* | 30 | - | - | - | - |

Continued..

| | | | | | |
|---|---|---|---|---|---|
| *Klebsiella pneumoniae* | 32 | - | - | - | - |
| *Proteus mirabilis* | 35 | - | - | - | - |
| *Pseudomonas aeruginosa* | 19 | - | - | 9.5(BS) | 9.5 (BS) |
| *Salmonella typhi* | 35 | - | - | - | - |
| *Staphylococcus aureus* | 30 | - | - | - | - |

**KEY:** (BS) - Bacteriostactic

**Table 17:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled E1, Ethanol/Seed

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 18:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled E2, Ethanol/leaves

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Acinetobacter baumannii* | 19 | - | - | - | 15 |
| *Bacillus cereus* | 14.5 (BS) | - | - | - | - |
| *Escherichia coli* | 25 | - | - | - | - |
| *Klebsiella pneumoniae* | 18 (BS) | - | - | - | - |
| *Proteus mirabilis* | 15.5 (BS) | - | - | - | - |
| *Pseudomonas aeruginosa* | 16 (BS) | - | - | - | - |
| *Salmonella pullorum* | 15.5 (BS) | - | - | - | - |
| *Salmonella typhi* | 30 | - | - | - | 9 |
| *Staphylococcus aureus* | 15 (BS) | - | - | - | - |

**KEY:** (BS) - Bacteriostactic

**Table 19:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled E3, Ethanol/Stems

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Acinetobacter baumannii* | 21 | - | - | - | - |
| *Bacillus cereus* | 17 ( BS) | - | - | - | - |
| *Escherichia coli* | 24 | - | - | - | - |
| *Klebsiella pneumoniae* | 26 | - | - | - | - |
| *Proteus mirabilis* | 15 (BS) | - | - | - | 7 |
| *Pseudomonas aeruginosa* | 15 (BS) | - | - | - | - |
| *Salmonella pullorum* | 17 (BS) | - | - | - | - |
| *Salmonella typhi* | 15 | - | - | - | - |
| *Staphylococcus aureus* | 16 (BS) | - | - | - | - |

**Table 20:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled E4, Ethanol/Roots

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 21:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled E5, Ethanol/SK

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 22:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled W1, Water/Seed

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 23:** Extract labelled W2, Water/leaves

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Acinetobacter baumannii* | 12 | - | - | - | - |
| *Bacillus cereus* | 28 | - | - | - | - |
| *Escherichia coli* | 30 | - | - | - | - |
| *Klebsiella pneumoniae* | 32 | - | - | - | - |
| *Proteus mirabilis* | 32 | - | - | - | - |
| *Pseudomonas aeruginosa* | 6 | - | - | - | - |
| *Salmonella typhi* | 37 | - | - | - | 9 |
| *Staphylococcus aureus* | 35 | - | - | - | - |

**Table 24:** Extract labelled W3, Water/Stems

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 25:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled W4, Water/Roots

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 26:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled W5, Water/Sk

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 27:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled B, Methanol/Sk

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 28:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled B, Methanol/ROOT

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 29:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract Labelled B Methanol/Seeds

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Test isolates* | Result negative for all test isolates | | | | |

**Table 30:** *In-vitro* Antimicrobial Assay on Bacteria Using Extract LabelLed, B Methanol/Stem

| NAME | CONTROL | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| *Staphylococcus aureus* | - | - | - | - | - |
| *Acinetobacter baumannii* | 5.5 (BS) | - | - | - | - |
| *Klebsiella pneumoniae* | - | - | - | - | - |
| *Bacillus cereus* | 6 (BS) | 9.5 | - | - | - |
| *Escherichia coli* | 14 | - | - | - | - |
| *Salmonella typhi* | 12 | - | - | - | - |
| *Proteus mirabilis* | - | - | - | - | - |
| *Pseudomonas aeruginosa* | - | - | - | - | - |

## Discussion

The *Cannabis sativa* extracts used show a variety of antimicrobial potentiality against multidrug resistant bacterial isolates tested. Van *et al*., (1976) studies is in harmony with this. The use of hot water and ethanol as solvents for extracts have additionally been demonstrated to have an inhibitory effect on Gram-negative etiologic agents (Naveed *et al*., 2010).

As shown in this study, n-hexane extracts and a few chloroform extracts of *Cannabis sativa* (A1, A3, A5, D1, D2) are generally active, especially n-hexane extracts of *Cannabis sativa* seed (A1) (Tables 2 to 13). This corroborates with Lone and Lone (2012) investigation which juxtaposed the protein turnout of this plant source and antioxidant effects utilizing acetone extraction as well. A response that depended on the concentration of extracts was reported for *Vibrio cholera*, *P. aeruginosa* and *Candida albicans* susceptibility was also observed in the study. This also shows the medicinal value of *Cannabis sativa* (Lone and Lone, 2012).

This study highlighted a comprehensive antimicrobial potential of *C. sativa*. This corroborates with the report of Sarmadyan *et al*., (2014) on how disk diffusion experiments discovered *Cannabis* extract to have displayed the best antimicrobial responses on selected multidrug resistant bacterial strains. Similarly, Lelario *et al*., (2018), also discovered that the predominant compound of the extracts displayed mild activity against Gram-positive diseases causing organisms.

The observations in this study suggest that Extracts A3, A5, D1, and D2 can be further studied by increasing the concentration and changing the solvent used for extraction. Similarly, Extract A1 is highly effective against test organisms, especially against *Salmonella pullorum* and *Bacillus cereus* where its bactericidal effect is greater than that of the control (Ofloxacin) used. This has some correlation with previous antimicrobial study, the antimicrobial activities of crude aqueous, petroleum ether and ethanolic extracts of the leaves of *C. sativa* were determined. There was conspicuous responses against Gram-positive and Gram-negative bacteria, and fungi (*Candida albicans* and *Aspergillus niger*) for ethanolic and petroleum ether extracts except some aqueous extracts, in correlation with the study of Wasim *et al.* (1995). There was a wide inhibition zone (in diameter) for the *C. sativa* leaves and stem extracts against *Staphylococcus aureus* evaluated.

In consistency with study of Ali *et al*. (2012), *Escherichia coli, Bacillus subtilis, S. aureus, Pseudomonas aeruginosa, A. niger,* and *Candida albicans* screened using appropriate solvent extracts showed some activity. But this is generally, pronounced against Gram-positive bacteria and moderate to no activity against Gram-negative and fungi. Bioactive compounds in *C. sativa* were shown to be responsible for antimicrobial activity of these plant extract preparations using different solvents (Borchardt *et al*., 2008).

Tables 22 to 26 shows low activity of water extracts of *Cannabis sativa.* This is in correlation with a previous report by Jin and Lee (2018) who demonstrated that the low toxic and biodegradability level of *C. sativa* seed oil-water emulsions have valuable applications in the industries and in treating some skin ailments. More studies on *Cannabis sativa* activity are highly recommended including optimum concentration and related physiologic studies on pharmacokinetics and others. Synergistic effect of Stems ethanol/extract -E3 with a distinct bio-active component against *Proteus mirabilis* can also be further studied for scientific clarifications.

## Acknowledgement

We are grateful to members of Environmental Biotechnology and Infectious Diseases Research teams of both Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Nigeria and Department of Chemistry, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria, all health institutions from where samples for this study were collected in Ondo State, Nigeria, for their cooperation as well as the support of the Management of Adekunle Ajasin University, Akungba-Akoko, Nigeria. They made it possible for the work to be carried out at the Center for Infectious Disease Control and Drug Development (CIDCDD), Adekunle Ajasin University, Akungba-Akoko, Nigeria.

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