

ANTIMICROBIAL ACTIVITY OF CANNABIDIOL ON BACTERIA CAPABLE OF FORMING BIOFILM

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Abstract

Some bacterial infections have been in the past years and till date a very tough disease to cure and get rid of with the available antibiotics that can be found in hospitals and pharmacies. This has led to scientist to experiment using many different plant materials to test against such infections to find a total cure of such diseases. One of the plants that have this potential is *Cannabis sativa*. One of the bioactive compounds of *C. sativa* that is suspected to have the potential to treat stubborn bacterial infection is Cannabidiol and also called CBD. The objective of this research is to get pure CBD and use it against stubborn bacterial infections caused by *Staphylococcus aureus* to see if it will be able to treat it. *S. aureus* were

Introduction

Background of the Study

Staphylococcus aureus is one of the maximum large Gram-advantageous bacterial pathogens, inflicting pores and skin infections and in all likelihood tens of thousands and thousands of greater excessive infections international annually (Klevens et al., 2007)

It is a main causative agent in pneumonia and different breathing tract infections, surgical site, prosthetic joint, and

isolated from clinical samples from open wounds and skin infections. The pure CBD was used against the bacteria using disc diffusion method to check if it is susceptible, resistant or intermediate after confirming biofilm formation by the bacterial through 96-well method. Discs impregnated with Penicillin, Ampicillin and distilled water were used as the control. The mean average for Penicillin, Ampicillin and Cannabidiol on ten positive samples were 09mm, 10mm and 18mm, respectively. Using Kirby-Bauer Antibiotic Sensitivity standardized chart, Penicillin and Ampicillin showed that *S. aureus* are resistant to them. While cannabidiol has no standard yet, but 18mm was the mean zone of inhibition recorded now. This could mean that, *S. aureus* are resistant, susceptible or intermediate using pure Cannabidiol as the antibiotic when a standard has been agreed for CBD as a possible antibiotic.

Keywords: Cannabidiol, Biofilm, Susceptible, Ampicillin, Penicillin, *Staphylococcus aureus*

Cardiovascular infections, in addition to nosocomial bacteremia (Tong et al., 2015) *S. aureus* infections, which includes reasonably excessive pores and skin infections, along with abscesses, and wound infections, are typically now no longer existence threatening however can be observed through sizeable morbidity and ache which may be because of their frequency (McCaig et al., 2006) *S. aureus* infections are specifically complex because of often taking place antibiotic resistance in *S. aureus* isolates, amongst which methicillin-resistant *S. aureus* (MRSA) are the maximum important, clinically (Turner et al., 2019)

While sanatorium related MRSA infections are at the decline within the U.S and different evolved nations, that's probably because of accelerated hygiene, improvement of more potent antibiotics and surveillance measures, they're nevertheless at the upward push in poorly evolved nations in Africa (Kourtis et al., 2019)

Resistance to antibiotics like penicillin and others continues to be found in *S. aureus* (Lowy et al., 2023)

S. aureus can display, regularly in blended form, resistance to nearly all to be had antibiotics. Vancomycin stays the antibiotic of ultimate lodge for MRSA infections, with notably vancomycin-resistant traces (VRSA) having took place however now no longer spread, in all likelihood because of the strongly accelerated health value this is imposed through vancomycin resistance genes. However, there are traces which have obtained intermediate resistance to vancomycin (VISA) (McGuinness et al., 2017)

In addition to particular antibiotic resistance, nonspecific antibiotic resistance through biofilm formation performs a function in lots of *S. aureus* infections which can be biofilm-associated (Staphylococcal Biofilms. Microbiol Spectr. 2018) Cannabis plant has many bioactive compounds wherein Cannabidiol (CBD) and Tetrahydrocannabinol (THC) are the maximum usually attention of studies and legislation (Berghamaschi et al., 2011)

The maximum outstanding of the bioactive compounds in *Cannabis sativa* L. is CBD and in evaluation to THC, it has healing residences (Berghamaschi et al., 2011)

CBD is the non psychoactive bioactive compound of *Cannabis sativa* L. and has a small molecule (MW 314 Da) phytocannabinoid that has pentyl-substituted bis-phenol fragrant group (pentylresorcinol) related

to alkyl-substituted cyclohexene terpene ring system (Elsahly et al., 2005)

CBD became first remoted from Minnesota Wild Hemp in 1940, however its shape became now no longer absolutely elucidated till 1963 (Mechoulam et al., 1963)

CBD has been studied in human beings thru inhalation and oral management which can fluctuate from management in rodents, due to the fact it's regularly administered in rodents thru injection or thru oral management wherein each routes can provide exceptional lively blood concentration (Diana et al., 2000)

Researchers have used doses of CBD as much as 1200mg with none critical facet impact (Davies and Bhattacharyya, 2019)

At decrease doses it has physiological results that sell and keep fitness along with antioxidative, anti inflammatory and neuro safety results (Hampson et al., 1998)

Non the less, a few facet results were mentioned for CBD, however in particular in vitro or animal research which encompass decreased fertilization and alteration of mobileular viability (Bergamaschi et al., 2011)

Bacteria have grow to be an increasing number of proof against antibiotics over time which has result in a few nations just like the USA to consult the latest discoveries of the ability impact of CBD on micro organism as a publish antibiotic (Renwick et al., 2018)

Most main pharmaceutical agencies have deserted antibiotic studies and positioned smaller biotech agencies in a economic position, leaving few new therapeutics withinside the medical pipeline (Leopore et al., 2018)

It is genuine for Gram-advantageous infections, in which there were basically no novel molecular training discovered and authorized for medical use due to the fact 1960's, however higher Gram-advantageous

treatment plans are needed, due to the fact resistant Gram-advantageous infections nevertheless purpose great mortality (US Department of Health and, 2019)

Pure CBD became authorized in 2018 through the united states Food and Drug Administration (FDA) and 2019 through the European Medicines Agency (EMA) for the oral management and remedy of epilepsy disorders, Dravet Syndrome and Lennox Crestaut Syndrome (Silvertro et al., 2000)

Pure CBD has been observed to be extensively utilized by the general public of which can be unregulated products, which name for difficulty over pleasant and safety (Hazecamp et al., 2018)

There are many pharmacological residences of CBD, however the one which stood out is its antimicrobial pastime, with a 1996 book through Van Klingerren and Ten Ham, reporting minimal inhibitory concentrations (MICs) for each natural CBD and THC withinside the variety of 1-5Mg ML-1 for Gram-advantageous Staphlococci and Streptococci and did now no longer display pastime towards Gram-negative Escherichia coli, Salmonella typhi (MIC > one hundred µg ML-1) (Mechoulam et al., 1963)

A variety of in advance and next guides have mentioned at the antimicrobial residences of Cannabis extract in place of natural compounds (Iseppi et al., 2019)

CBD became defined to have antimicrobial residences, however became unnoticed till 2008 whilst there has been a brand new locating that assessed the efficiency of Cannabidiol, Tetrahydrocannabinol, Cannabigerol, Cannabichromene and Cannabinol towards six Methicillin resistant Staphylococcus aureus (MRSA) traces, wherein all confirmed MIC to be 0.5 - 2µg ML-1 variety (Appendino et al., 2008)

MATERIALS AND METHODS

Study Location

This study was done in the 300 level microbiology laboratory of Kaduna State University located in Kaduna South. It is located between latitude 10°38'58" N and 10°25'36" N and longitude 7°22'14" E and 7°32'00" E

Sample Collection (Clinical Samples)

Samples from skin infections, wounds, and nostrils were collected from 44 Nigerian Army Reference Hospital Kaduna State. A total of 130 samples were collected, in which 10 samples were tested positive to Staph infection and the remaining were negative and there was contaminations noticed after culturing. It was collected directly from patients using a sterilized cotton swab and inoculated in a nutrient broth which was incubated at 37 °C for 24 hours. Mannitol salt agar (MSA) was used to grow *Staphylococcus aureus*. Some biochemical tests were carried out to confirm that it was *Staphylococcus aureus*. Indole, Catalase, Coagulase, MRVP, Gas, H₂S, and Citrate tests were carried out.

Use of Test Tubes to Assess Biofilm Formation

A total of 15 tubes were used in which, 10 contained the inoculum for the test and the other 5 for the control containing just media (Tryptic Soya Broth).

About 10ml of sterilized media (TSB) was added into all of the 15 tubes. About 5ml of the diluted bacterial culture was added to the 10 test tubes. The tubes were closed and incubated at 37 °C for 24hrs.

After 24hrs, the media is removed from the tubes by pouring and tapping the tubes on a surface for the media to drain out while the tubes are upside-down.

It is then washed with distilled water to remove planktonic bacteria and then allowed to dry for 20 to 30 minutes.

Staining follows. Crystal violet (1mL) was added to all the 15 tubes and incubated for 15mins.

After that, the crystal violet was poured out of the tubes and the tubes were washed with distilled water again and was allowed to dry for 20 to 30minutes. After this, biofilm formation was noticed on the bottom and sides of the test tubes by seeing retention of the stain. While in the control tubes, there was no remaining stain on the tubes after washing with distilled water.

About 96% of ethanol was added into the 15 tubes and incubated for 15mins to solubilize the crystal violet remaining bound to the biofilm for further analysis on a spectrophotometer.

From what's left in the tube, 1mL from it is (solution) was transferred into plastic cuvettes and absorbance was measured at 600nm.

Un-inoculated tubes were used as the negative control and the amount of biofilm formed was read to be 0.39, 0.11 and 0.60 as the weakest, moderate and strongest biofilm formation, respectively.

Testing for Effect of CBD on Bacteria (*Staphylococcus aureus*)

Disk Diffusion Assay also known as Kirby Bauer Standard was be used to test for the effect of the pure CBD on the bacteria isolates.

The susceptibility table by Clinical and Laboratory Standard Institute (CLSI) was used to determine if it is susceptible, intermediate or resistant after measuring the area of inhibition using a ruler.

Isolates were transferred to Mueller-Hinton Agar and the ten plates were labelled for susceptibility testing.

This was done by inoculating the pure isolates on the MHA and using a glass rod spreader to spread evenly on the media aseptically and discs that has CBD already soaked and two other discs are soaked in two other antibiotics (penicillin and ampicillin) of the same dosage as the control, which is not impregnated or soaked with any antibiotics and incubated for 48hours before checking for Zone of Inhibition (ZOI).

RESULTS

Biofilm Formation and Measurement

After five biochemical tests were carried out to identify and confirm the presence of *S. aureus*, the ten positive samples were subjected to biofilm formation using 96-well plate method, but 10 test tubes and 5 control tubes were used as alternatives and were confirmed to have formed biofilm on the sides and bottom of the test tubes by retaining the colour of the crystal violet after the test on the test tubes. It was later measured to see how dense it was using a spectrophotometer at wave length of 600nm.

The table 1 below shows results of the ten test tubes measured using a spectrophotometer.

Table 1. Measurement of Biofilm Formation on Spectrophotometer of *Staphylococcus aureus*.

Biofilm Formation				
S/N	Organism	Weak	Moderate	Strong
1	<i>S. aureus</i>	0.08	----	----
2	<i>S. aureus</i>	0.06	----	----
3	<i>S. aureus</i>	-----	0.12	----

4	<i>S. aureus</i>	0.04	-----	----
5	<i>S. aureus</i>	----	----	0.61
6	<i>S. aureus</i>	0.04	----	-----
7	<i>S. aureus</i>	0.04	----	-----
8	<i>S. aureus</i>	----	0.10	-----
9	<i>S. aureus</i>	0.04	-----	-----
10	<i>S. aureus</i>	0.05	----	-----

Biofilm formation was read as 0.077-0.154 to be weak, 0.154-0.308 to be moderate and above 0.308 to be strong. It is seen on this table that, 0.607 was the only strongest. This was derived using a spectrophotometer.

For table 2, it is showing the ten positive samples of *S. aureus* and their zones of inhibition after penicillin (control), Ampicillin(control) and Cannabidiol (test antibiotic) were used against the bacterial by using disc diffusion method assess if they are resistant, intermediate or sensitive.

Table 2. This table shows the average mean of the zones of inhibitions of all the plates used.

Antibiotic s	Plate 1 (x)	Plate 2 (x)	Plate 3 (x)	Plate 4 (x)	Plate 5 (x)	Plate 6 (x)	Plate 7 (x)	Plate 8 (x)	Plate 9 (x)	Plate 10 (x)	Averag e mean
Penicillin	11	09	11	07	05	12	09	08	09	09	09
Ampicillin	08	10	11	10	07	14	11	11	10	11	10
Cannabidi ol	16	16	18	18	15	17	19	21	19	17	18

According to this table, using the standard for when bacteria is sensitive, intermediate or resistant, it clearly showed that, *S. aureus* is resistant to

penicillin, and ampicillin. As for CBD, as of now, it has no standard we can use to determine if it is sensitive, intermediate or resistant

DISCUSSIONS

It was observed that people who are admitted at the hospital are more prone to diseases caused by *Staphylococcus aureus* due to either weak immune or due to open injuries on the skin (Wertheim *et al.*, 2005). *S. aureus* is mostly found around the walls of the nostrils in 20-40% of a population (Becker *et al.*, 2017). *S. aureus* becomes dangerous to health when there is an opening on the skin that they can easily get access to blood streams and multiply and cause serious challenges (Lowy *et al.*, 1998). These health challenges caused by *S. aureus* has been a great challenge to cure for a long time, this is due to its resistance to antibiotics presently available. *S. aureus* infection has been a major focus in terms of cure and control of the disease by medical practitioners and researchers. Though prevalence of *S. aureus* started decreasing in South Africa from 36% in 2006 to 24% in 2011, which could be as a result of different practices used in against the bacteria (Zervou *et al.*, 2014)

It was noticed when Cannabidiol was used against *S. aureus* that, there was a mean different compared to other antibiotics used, Penicillin and Ampicillin. Though there is no standard data for CBD to show if it is susceptible, intermediate or resistant to *S. aureus*. According to the standard for Penicillin and Ampicillin, it showed that it was resistant to them. The mean, showed, 09mm, 10mm and 18mm, respectively. The same ug of antibiotic discs were used to arrive at this.

The results of the sensitivity of the controls could be related to another done by Raja, Prashanna and Ganga, 2019 Pengov and S. Ceru, 2004 on

the Antibiotic resistance pattern of *Staphylococcus aureus* with reference to MRSA isolates from Pediatric Patients in which some strains of *S. aureus* were found to be resistant when penicillin and ampicillin were used against the bacteria(MRSA). WHO has reported that 64% of MRSA-infected patients are more likely die than non-MRSA-infected patients (WHO, 2018) For CBD, the average zone of inhibition recorded was 18mm which there is no standard to show if it sensitive, intermediate or resistant, because CBD is not yet considered an antibiotic.

CONCLUSIONS

In accordance with the consequences of this study, the subsequent conclusions may be made: In assessment with preceding research no primary variations in sensitivity of *S. aureus* which can be MRSA to antibiotics used as control; penicillin and ampicillin.

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