ARTICLE / INVESTIGACIÓN

Antibacterial effect of Cannabidiol oil against *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and level of toxicity against *Artemia salina*

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Abstract: Acne is one of the most common skin pathologies; one of the causes is *Propionibacterium acnes*, an anaerobic and gram-positive microorganism that lives in the hair follicles of the skin and currently presents resistance to antibiotic-based treatments; this research topic has the purpose of evaluating the antibiotic activity of *Cannabidiol* oil against *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* and the level of toxicity against *Artemia salina*. For the methodology, antibiograms were used by the Kirby-Bauer method, where the concentrations were evaluated: 0,8 %; 0,6 %; 0,4 %; 0,3 % and 0,1 %; Amoxicillin for positive control and Dimethyl sulfoxide (DMSO) for negative control; the percentage of inhibition against *Propionibacterium acnes* and two control bacteria were calculated: *Staphylococcus aureus* and *Staphylococcus epidermidis*. Once the percentage of inhibition was tested, a toxicity study was carried out against Artemia salina to determine its LD50. The *Cannabidiol* oil obtained from the Ecuadorian company was used as the antibiotic agent to be evaluated, and it was found that at a concentration of 0,8%, it presented a percentage of inhibition of 91,2 %; 98,7 % and 93,6 % against *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively, data that do not present a significant difference against Amoxicillin; for the Artemia salina test, an LD50 of 4,8 % was obtained; taking into account that the commercial oil has a presentation of 1,6 % (500 mg/30 mL), it results in a relatively innocuous product. Thus concluding that *Cannabidiol* oil is a very promising antibiotic due to the inhibition percentages presented and low toxicity.

Key words: CBD, antibiograms, bioassay, LD50.

Introduction

The use and abuse of antibiotics, not only in Ecuador but worldwide, is a fashionable and controversial topic; due to the efforts made by professionals, this is a practice that continues to leave in its wake several severe and irreversible consequences. one of them is the bacterial resistance acquired by microorganisms to antibiotics. Several resistances of *Propionibacterium acnes* have been reported over the years, as is the case of Clindamycin and Erythromycin, which were reported in 1979, and later in 1983 the first resistance to tetracycline was reported[10].

It has been reported in patients with severe acne that 70% of them present high biofilm formation and multi-resistance of *Propionibacterium acnes* to various antibiotics. For this reason, it is interesting to use new alternatives. It has been reported in patients with severe acne that 70% of them present high biofilm formation and multi-resistance of *Propionibacterium acnes* to various antibiotics. For this reason, it is interesting to use new alternatives against Propionibacterium acnes; it has been reported in studies[5,11] that *Cannabidiol* works excellent with biofilms of gram-positive microorganisms; therefore, studying the effect of *Cannabidiol* against *Propionibacterium acnes* is promising.

Materials and methods

Cannabidiol oil was obtained from an Ecuadorian company in a 500 mg/30mL presentation. The bacterial strain of *Propionibacterium acnes* ATCC 11827, *Staphylococcus aureus* ATCC 29213, and *Staphylococcus epidermidis* ATCC 14990 were obtained from the Cryobank of the Life Sciences Laboratories of the Salesian Polytechnic University. The manual described by (20) was used as a reference, and the tubes containing the bacterial beads were thawed with a punch to perform the striation in triplicate throughout the petri dish.

The recommendations of (7) were followed to prepare dilutions with oils and dimethyl sulfoxide. Five oil-based dilutions were prepared at concentrations of 0.1 %; 0.3 %; 0.4 %; 0.6 %, and 0,8 %, whose solvent was DMSO; the final volume for each dilution was 5 mL in every amber bottle.

A commercial antibiotic (Amoxicillin) was taken as a positive control, a beta-lactam antibiotic used for both gram-positive and gram-negative bacteria, due to its broad spectrum of bacterial activity[1]. This antibiotic is used for antibiotic testing in the *Staphylococcus* and *Propionibacterium* families because of their sensitivity to its compounds[8,10].

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P. acnes was incubated in TSB medium under anaerobic conditions at 35 °C for 16 hours, for *S. aureus* and *S. epidermidis* were incubated in TSB medium at 37 °C in an incubator.

After the established time passed, it was centrifuged at 350 rpm for 20 minutes, the supernatant was discarded in a beaker with alcohol, the bottom of the bacterial biomass was conserved, sterile saline was added to each tube, and vortexed for 2 minutes until reaching the 0,5 McFarland scale and read in the JASCO V-730 spectrophotometer with the spectra manager TM software until reaching an absorbance of 0,200 at 655 nm, obtaining an inoculum of 106 CFU/mL.

500 µL of bacterial inoculum was taken and dropped in the center of the Petri dish with Muller Hinton[15]. One disc of antibiotic Amoxicillin was placed as the positive control, one blank disk with DMSO as the negative control and 5 blank discs with the respective dilutions from *Cannabidiol* oil, which would be at concentrations of 0,1 %; 0,3 %, 0,4 % 0,6 % and 0,8 % in a volume of 20 µL. Petro dishes with *S. aureus* and *S. epidermidis* were placed in an incubator at 37°C for 24 hours; *P. Acnes* was incubated in anaerobiosis at 35 °C.

When the time of 24 hours in the incubator for *S. aureus*, *S. epidermidis* and *P. acnes* had passed, each Petri dish was checked with a caliper ruler.

The percentage of inhibition of each bacterium concerning each concentration was calculated using the following reference formula (1) from[9].

7 grams of *A. salina* eggs were obtained from a commercial house, 2 g of egg were weighed, hydrated for 30 min with distilled water, then 25 mL of sodium hypochlorite were added (4 replicates); the eggs were recovered and rinsed with distilled water. For the incubation, a 3-liter bottle was used, 1500 mL of 2 % saline water was added, pH 8, temperature 24 °C and constant aeration for 48 hours[22].

To make the emulsions with *Cannabidiol* (CBD) oil, a 1:1 ratio of oil and tween 80 was used as a co-emulsifier used in the cosmetic and food area due to its low toxicity level and according to work carried out by (15) it is considered innocuous with *Artemia*. *Cannabidiol* oil was used to obtain emulsion at 3.2 %; 1.6 %; 0.8 %; 0.4 %, and 0.2 %, with which we worked in test tubes with *A. salina* to determine the LD50.

After 24 hours of incubation, dead nauplii were counted using a NIKON SMZ745 stereoscope, where those that did not show any seconds were considered dead

Results

The percentage of inhibition for *P. acnes* is 91.2% at a concentration of 0.8% of *Cannabidiol* oil, which gives a high percentage of inhibition compared to a commercial antibiotic, Amoxicillin, supporting inhibition compared with a commercial antibiotic, Amoxicillin, supporting the alternative hypothesis showing that *Cannabidiol* oil inhibits *P. acnes*.

A Tukey study showed an essential group of data, in which their averages are not significantly different; the group is formed by the positive control (commercial antibiotic), CBD5 (0.8% *Cannabidiol* oil).

The concentration of *Cannabidiol* oil at 0.8 % has an inhibition percentage of 98.7 %, a value very close to the positive control, which was the commercial antibiotic Amoxicillin.

A Tukey test proved that there is a group of interest where their averages are not significantly different; the group is formed by the control + (antibiotic Amoxicillin) and CBD5 (0.8% *Cannabidiol* oil).

The results show that the percentage of inhibition for S. epidermidis with 0.8 % oil was 93.6 %, affirming the alternative hypothesis on the inhibition of *Cannabidiol* oil against *S. epidermidis*.

The Tukey test shows a critical group where the positive control (Amoxicillin) and CBD5 (0.8 % *Cannabidiol* oil) are grouped. As a result, we would obtain that the 0.8 % *Cannabidiol* oil is similar in inhibition to the positive control (Amoxicillin), supporting the alternative hypothesis that there is at least one concentration that inhibits *S. epidermidis*.

Toxicity test

Dilutions of cannabidiol oil were made at intervals of: 3.2 %; 2.8 %; 2.4 % 2.0 %. In order to determine the lethal dose, a linear regression was performed, and an LD50 of 4.86 % (48 mg/mL) was obtained.

Formatting of Mathematical Components

$$Inhibitory\ effect = \frac{Inhibition\ halo\ diameter}{Positive\ control\ halo\ diameter} * 100 \quad (1)$$

Bacteria	Concentration	X	% inhibition
P. acnes	0,8	1,9	91,2
	Control +	2,0	100

Table 1. Determination of the antibiotic activity of *Cannabidiol* oil against *P. acnes*.

[1] Average halo and percentage inhibition of cannabidiol oil against *P. acnes*

Bacteria	Concentration	X	% inhibition
S. aureus	0,8	1.7	98.7
	Control +	1.8	100

Table 2. Determination of the antibiotic activity of *cannabidiol* oil against *S. aureus*.

[2] Average halo and percentage inhibition of cannabidiol oil against S. aureus.

Bacteria	Concentration	X	% inhibition
S. epidermidis	0,8	1.8	93.6
	Control +	1.9	100

Table 3. Determination of the antibiotic activity of *cannabidiol* oil against *S. epidermidis*.

[3] Average halo and percentage inhibition of cannabidiol oil against S. epidermidis

Discussion

Following the studies of (18) where he mentions that *Cannabidiol* has a potential antimicrobial activity against gram-positive bacteria, such as *P. acnes*, with which using it could be beneficial for the treatment of acne vulgaris. *Cannabidiol* has a potential role as an antimicrobial agent[22]; it was demonstrated through clinical studies that *Cannabidiol* oil acts on sebocytes, thus having an anti-acne function, controlling sebum production, mitigating the inflammatory process and functioning as a bactericidal agent by reducing bacterial proliferation[4,21].

Cannabidiol oil inhibits *S. aureus*; the results obtained by (2) can be compared with those of this work since Cannabidiol, one of the main cannabinoids of the plant, showed potent activity against the strain *S. aureus*.

The results obtained from the test with *S. epidermidis* can be compared with the study conducted by (19), where the mechanism of action of *Cannabidiol* in causing the death of gram-positive bacteria was evaluated due to the ability of this compound to inhibit the release of vesicles from the bacterial membrane; these vesicles are extremely important for cell communication and pathogen-host interaction.

In the negative control of the toxicity test with Artemia salina, saline water was used, and there was no dead individual, so the test is validated as there are no natural factors that can kill the study individuals, as indicated by (13,24); the percentage of mortality in the negative controls did not exceed 10 %. In the positive control, where 96% alcohol was used, it was confirmed as an adequate positive control since the death of the individuals in the study was approved, as indicated by the study of (23).

The use of *Cannabidiol* oil at concentrations from 2 % onwards gradually increases the number of dead *A. salina*[14]. A plant oil, when exceeding an LC50 of 1000 ppm in bioassays with A. salina does not have a high degree of toxicity due to the ability of the nauplii to present a very thin cuticle, which makes them sensitive to toxicants in the medium, which penetrate through the physiological barriers and are rapidly absorbed[6,25].

Conclusions

The valued *Cannabidiol* oil was obtained from an Ecuadorian company, which presents a concentration of 500 mg/30mL. Evaluation by means of the HPLC technique.

Cannabidiol oil showed antibacterial activity with halo averages of 1.8 cm, 1.7 cm and 1.8 cm for *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively, at a concentration of 0.8 %, compared to the control antibiotic (Amoxicillin) with 2 cm of halo, using the statistical analysis it was possible to reject the null hypothesis and accept the alternative since *Cannabidiol* oil inhibits *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* with the proposed concentrations. Likewise, the alternative ideas for the analysis of variance and Tukey are accepted; at least one degree of concentration of *Cannabidiol* oil inhibits the 3 bacteria with a similar effect to Amoxicillin. At the end of the experimental work, it was concluded that the results obtained under the laboratory test show that the use of *Cannabidiol* oil is effective for the control of the mentioned bacteria, and it is a promising field for the possible elaboration of phytoproducts for human use in order to improve and provide all the benefits offered by *Cannabidiol* oil.

For the toxicity bioassay where *Artemia salina* was used, an LD50 value of 4.8 % was obtained, which showed that the commercial *Cannabidiol* oil in a 500 mg/30mL presentation, equivalent to 1.6 %, is a relatively innocuous product at the highest concentration and non-toxic at deficient concentrations. Although at higher concentrations, survival may be negatively affected by swimming problems, the results confirmed the alternative hypothesis that the concentration of *Cannabidiol* oil is directly proportional to the percentage of mortality of *Artemia salina*.

Author Contributions

Conceptualization, Grace Pila and Danny Segarra.; methodology, Grace Pila, and Danny Segarra.; software, Danny Segarra..; investigation, Grace Pila and Danny Segarra.; writing—original draft preparation, Grace Pila and Danny Segarra.; supervision, Marco Cerna; funding acquisition, Grace Pila and Danny Segarra. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

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Data Availability Statement

https://docs.google.com/spreadsheets/d/1mGvmAHt5Q6ZsTLvIysinZDh_uoFFovcHAcjse0yb7ug/edit#gid=816728436

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Conflicts of Interest

The authors declare no conflict of interest.

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