OZONIZED AND NATURAL COMPOUNDS FOR *IN VITRO* *ACINETOBACTER BAUMANNII* TREATMENT

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Abstract

Acinetobacter baumannii stands out as an opportunistic pathogen responsible for Healthcare-Associated Infections. This study aimed to evaluate the efficacy of natural oils in natura and ozonated on A. baumannii mortality. The randomized experiment used the A. baumannii strain and natural oils (palm, canola, and coconut) ozonized and in natura (control group). Minimum inhibitory and bactericidal concentrations and chromatography were employed in the study. Through the ozonized and fresh oils antimicrobial action, the natural oils' effectiveness and the bacteria mortality were evaluated. The data was submitted to the Mann-Whitney and Kruskal-Wallis tests. When comparing the natural oils, ozonized canola oil stood out as the most efficient in destroying the bacteria. The other ozonized oils showed a 90% reduction in the microbial load in the first ten minutes of treatment. For use in natura, palm oil stood out with 85.9% microbial load reduction. According to the results obtained, the antibacterial activity of natural oils is remarkable, showing that they were expressively effective in inhibiting the A. baumannii growth, presenting themselves as a new adjuvant therapy antibacterial drug. The study concluded that the ozonized oils are more efficient in both MIC and MBC when compared between the ozonized and the untreated groups. The bacteria are sensitive to the ozonized oils.

Keywords: *Acinetobacter baumannii*; Vegetable oils; Ozone; Antimicrobial activity; *In vitro* technique.

1. Introduction

Acinetobacter baumannii is a Gram-negative aerobic bacterium that stands out as an important opportunistic pathogen and responsible for Healthcare-Associated Infections (HAIs). Its clinical importance has been associated with high prevalence in epidemics and endemic situations, antibiotic resistance, biofilm-forming in medical devices, and desiccation resistance[1-3].

Thus, this bacterium has shown resistance to antibiotics frequently used in clinical practice[4]. Therefore, it is used as a treatment of the most potent antibiotics, such as carbapenems. However, by using these antibiotics, there is an increase in strains resistant to these drugs, justified by the emergence of β-lactamases[2].

Due to this fact, the use of lipopolypeptides (polymyxin B and polymyxin E) has been referred to as a "last resort." It is increasingly required for *A. baumannii* eradication therapy[5].

Lipopolypeptides during the 1960s were widely used in treatment against *A. baumannii* and *Pseudomonas aeruginosa* infections, being replaced by effective and less toxic drugs, such as cephalosporins and aminoglycosides[5,6]. In the 1990s, with the development of multidrug-resistant strains of *A. baumannii* and *P. aeruginosa*, its use was reinstated, especially in the case of carbapenem-resistant samples. It is important to emphasize that polymyxins' continuous use causes the development of resistant samples of *A. baumannii*, which stands out the resistance rates of 40.7% in Spain and 30.6% in Korea[7,8]. For many countries, polymyxins have been reported as the only option for treating severe infections caused by *A. baumannii*[9].

Besides resistance, antibiotics used against pathogenic microorganisms can induce severe side effects, especially in patients under prolonged treatment. They can also alter the microbiota, which is important for intestinal eubiosis and the whole organism. Antimicrobial agents that increase dysbiosis create an ideal environment for pathogenic microorganism colonization and other infections with potential recurrent episodes[10,11].

As a result of *A. baumannii*'s multidrug resistance to antibacterial drug treatment, and especially the mortality associated with infection by the bacteria concomitant to inadequate therapy, the search for new antimicrobial agents began, among natural products, which contain hundreds of naturally active ingredients in various proportions, eliminates the resistance risk, because microorganisms are not able to adapt to their heterogeneous structures[12].

Ozone is widely recognized as one of the best bactericidal, antiviral, and antifungal agents. It has currently been used as a therapeutic mediator in chronic wounds, ulcers, and ischemic wounds, due to its ability to diminish bacterial infections and heal dermal damage caused by the "oxidative death" effect on microorganisms[13,14].

Ozonated oils have exhibited antimicrobial activity against bacteria and fungi. They can be suggested for use in chronic local infection prevention and treatment, in suitable formulas, and controlled conditions, as an alternative to topical antimicrobials[15,16]. This may be useful to contrast the widespread and indiscriminate use of antibiotics, which has led to bacterial resistance[17].

In this context, the present study aimed to evaluate the antimicrobial activity and ozonated vegetable oils for the in vitro treatment of *Acinetobacter baumannii*.

2. Materials and Methods

2.1. Study design

The *Acinetobacter baumannii* strain was analyzed in the microbiology laboratory in a randomized experimental design study. The treatments for *A. baumannii* consisted of two groups of natural oils, one

group of ozonated and non-ozone oil ("*in natura*" means control group), conducted in the laboratory of Universidade Brasil, Fernandópolis, SP, from August 2019 to February 2021.

2.2. Biological sample and natural compounds

Acinetobacter baumannii ATCC (*American Type Culture Collection*) 17978 strain was used, cultivated in blood agar medium (OXOID®) for 24 hours, and incubated at 37ºC. Three types of natural compounds were used: canola oil (Liza®), coconut oil (Katigua®), and palm oil (Hemmer®).

2.3. Ozonation of the natural compounds

For ozonation, 1 liter of each natural oil was used. Ozone was produced by a generator (Ozon & Life) and an oxygen cylinder. The canola and palm oil were exposed to ozone directly through a diffuser for 4 hours at 25°C, while the coconut oil was ozonized for 6 hours at the same temperature. Ozonation was conducted in a fume hood in a chemistry laboratory according to international safety standards. After ozonation, 0.1 mL of each oil was inoculated in Petri dishes containing *Trypticase Soy Agar* (TSA) (OXOID®), incubated at 37°C for 24 to 48h to verify microbial growth absence. The oil that did not present any microbial colony was considered sterile. The ozonized oils were kept under refrigeration (8ºC).

2.4. Gas chromatography for chemical characterization of ozonized and *in natura* oils

Gas chromatography was used to separate and analyze the chemical mixtures of ozonized and *in natura* canola, coconut, and palm oil. In this process, a gas reservoir with flow rate/pressure control (cylinder containing carrier gas under pressure), a sample injector or vaporizer (block connected to the chromatographic column and the carrier gas feed cylinder to introduce the sample into the column), a chromatographic column and column oven were used. The oils' density was verified by the method of *The United States Pharmacopeial Convention*[18] and to determine the oils' peroxide index, the Cd 8-53 method of the *American Oil Chemists Society*[19] was utilized. According to the *Association of Official Analytical Chemists*, total fatty acid content, acidity, iodine index, and saponification index were determined[20].

2.5. Bacterial inoculum patterning

The bacterial strain was inoculated in *Brain Heart Infusion* (BHI) broth (OXOID®) and incubated for 24 hours in aerobic conditions at 37ºC. Afterward, it was centrifuged (4000 rpm) for five minutes. Then, the supernatant was discarded, and the sediment was resuspended in dimethyl sulfoxide (DMSO) (Synth®) and again submitted to centrifugation. This procedure was repeated five times to remove existing components in the culture medium[21] that could interfere with the minimum inhibitory concentration assessment. Then, the bacterial solution was diluted in DMSO using MacFarland's 0.5 scales, equivalent to a concentration of $1.5x10^8$ colony forming units (CFU)/mL^{-1}.

2.6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) measurement

The bacterial sensitivity test was performed in a 96-well microtiter plate to determine the MIC, following the *National Committee for Clinical Laboratory Standard*[22]. Initially, 50 µL of BHI broth was

dispensed into the wells. In the first row, 50 µL of the oils were added (obtaining the first dilution [50%]). Next, serial dilutions were performed in the columns in the six subsequent wells, removing 50 µL from the well with the highest concentration to the next well, until a concentration of 0.781% was obtained (dilutions 50%, 25%, 12.5%, 6.25%, 3.125%, 1.562%, and 0.781%). A 50 µL aliquot of the bacterial inoculum was then added to each well and incubated for 24 hours at 37°C under aerobic conditions. To determine the MIC, 50 µL of the dye 2,3,5-*Triphenyltetrazolium Chloride* (TTC), which reflects the activity of the dehydrogenase enzymes involved in the respiration process, was added to each well. The MIC was defined as the lowest concentration of the oil capable of inhibiting bacterial growth.

The MBC was measured based on Favre's methodology[23]. It was taken 100 µL of each sample after MIC results, inoculated in blood agar medium, incubated for 24 hours at 37ºC in aerobiosis. Bacterial growth was evaluated, and MBC was considered to be that which showed negative culture or mean of 0.1 CFU. The experiments of MIC and MBC were performed in triplicate.

2.7. Bactericide Kinetics of ozonated and *in natura* oils

The methodology described by Allahghadri[24] was employed. 40 µL of each oil and 5 mL of BHI broth containing a bacterial suspension of 10^6 CFU mL^{-1} were added in tubes. Samples of 0.1 mL were taken every 10 min for 480 min. Samples were immediately washed with sterile phosphate buffer, pH 7.0, centrifuged (10,000 rpm), resuspended in buffer, then inoculated onto BHI Agar, incubated for 24 hours at 37°C. The mechanical colony counter counted CFU. After the colony counting, the microbial load variation was assessed to observe which oil showed the highest negative variation (reduction) in the microbial count. The experiment was carried out in triplicate.

2.8. Data analysis

The data were submitted to a descriptive analysis of the minimum inhibitory and bactericidal concentrations of each of the natural oils according to the treatments (ozonized and *in natura*). The Mann-Whitney test was applied to compare the microbial count and the microbial count variation for the oils evaluated according to the type of treatment (ozonized vs. *in natura*) Kruskal-Wallis test was applied to compare the microbial count and the microbial count variation according to the type of oil. Line graphs were applied to analyze the microbial count according to treatment (ozonated and *in natura*). Statistical tests were applied with a significance level of 5% ($P<0.05$).

3. Results

Acinetobacter baumannii proved to be sensitive to the natural oils used in the study. It was possible to determine the minimum inhibitory concentrations and minimum bactericidal concentrations and verify the oils' physicochemical chromatographic differences (ozonized and *in natura*) when submitted to treatment.

Table 1 shows the MIC and MBC for the ozonated and the *in natura* oils. In this Table, the inhibitory and bactericidal concentrations of the *in natura* oils are higher than the ozonized oils. This result indicates that ozone treatment increases the natural oils' antimicrobial effect since a lower concentration of the oil is

required for the antimicrobial effect to be effectively observed. Among the natural oils evaluated, palm oil is the most noteworthy, showing the lowest minimum inhibitory concentration than the other natural oils.

Table 1. Minimum inhibitory and bactericidal concentrations for each of the oils evaluated in the study.

Natural Oil	Treatment	MIC[1]	MBC[1]
Canola	*in natura*	50%	50%
	Ozonized	25%	25%
Coconut	*in natura*	25%	50%
	Ozonized	12,5%	25%
Palm	*in natura*	25%	50%
	Ozonized	6,25%	12,5%

MIC[1]: minimum inhibitory concentration and MBC[1]: minimum bactericidal concentration.

Table 2 shows the *A. baumannii* microbial counts subjected to the natural and ozonated oils at their respective MBCs. This Table shows that the natural oils did not significantly differ from the *A. baumannii* control (P>0.05). The comparison between the different oils *in natura* also showed no significant differences in microbial counts (P=0.351). A similar result was found when comparing ozonated oils for microbial counts (P=0.298).

Table 2. Mean ± standard deviation (Median) of *Acinetobacter baumannii* submitted to the *in natura* and ozonated oils.

Oils (MBC *in natura*/MBC ozonized)	*in natura*	ozonized	P[1] Value
Canola (50%/25%)	$1,3.10^5 \pm 3,6.10^5$ ($9,4.10^2$)	$1,7.10^3 \pm 2,9.10^3$ ($2,6.10^2$)	0,458
Coconut (50%/25%)	$1,1.10^5 \pm 2,8.10^5$ ($5,7.10^2$)	$2,1.10^5 \pm 5,3.10^5$ ($5,3.10^2$)	0,963
Palm (50%/12,5%)	$1,5.10^5 \pm 3,9.10^5$ ($2,2.10^2$)	$2,0.10^5 \pm 4,6.10^5$ ($2,8.10^3$)	0,115
P[2] Value	0,351	0,298	

P[1] Value referring to the Mann-Whitney test at P<0.05. P[2] Value referring to the Kruskal-Wallis test at P<0.05.

Figure 1 shows the confidence interval for each of the oils according to the treatments, and Figure 2, the confidence interval according to the types of natural oils. The black circles and squares in the Figures indicate the means and medians of the microbial counts.

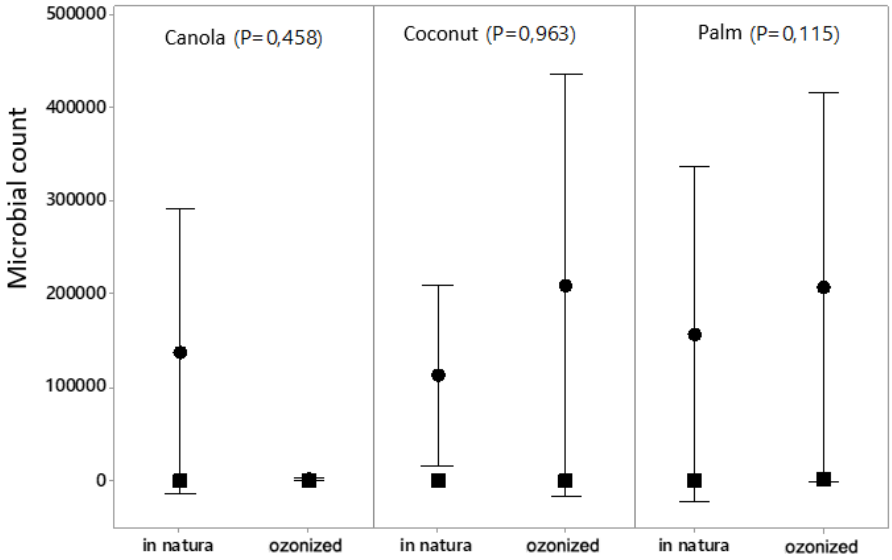

Figure 1. Confidence intervals (95%) of microbial counts according to treatments

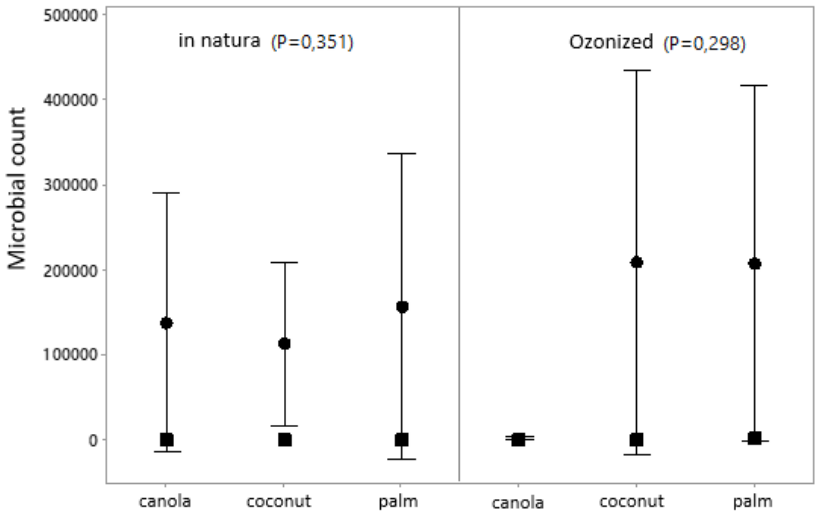

Figure 2. Confidence intervals (95%) of the microbial counts according to the natural oils type.

Figures 1 and 2 show no statistically significant difference in microbial counts according to the treatment and according to the type of natural oil, but it is clear that the ozonized canola oil showed zero microbial counts compared to the other oils studied.

Figures 3, 4, and 5 show each of the natural oils' microbial count behavior according to the treatments' evaluated exposure times.

Figure 3 shows the efficiency of the time of *A. baumannii* inhibition, where its efficiency at zero minutes can be observed for ozonized canola oil compared to the *in natura* oil, whose microbial count at this time was 10^6 bacteria. After 30 minutes, both treatments completely suppressed *A. baumannii*.

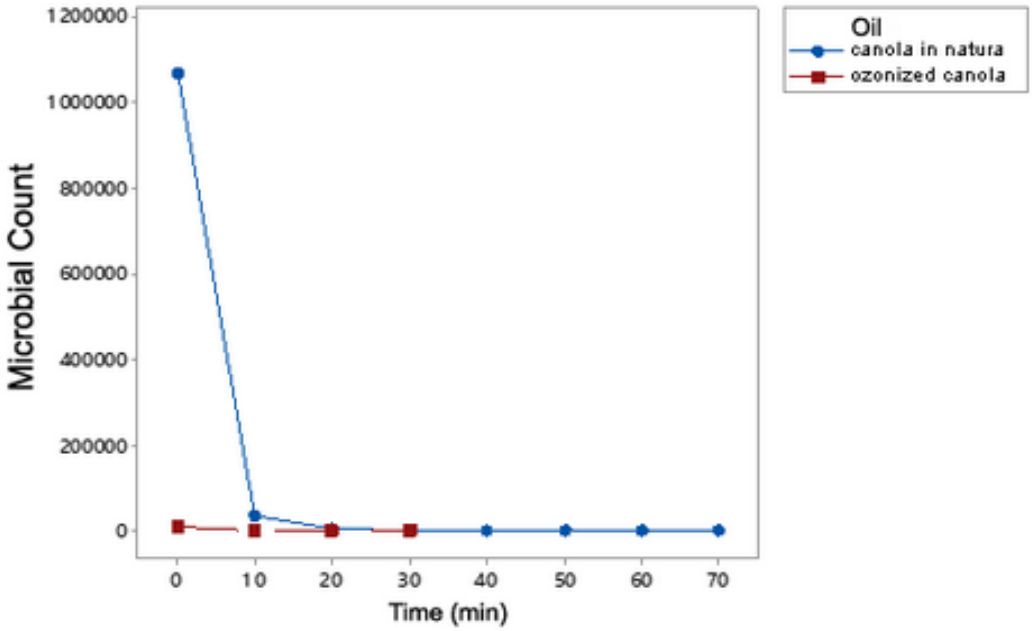

Figure 3. Microbial counts of *Acinetobacter baumannii* for *in natura* and ozonized canola oil according to exposure time.

Figure 4 shows the *Acinetobacter baumannii* microbial count for ozonized and *in natura* coconut oil according to the exposure time. Initially (time zero), an initial bacterial count of 16.10^5 was observed for the ozonized coconut oil. On the other hand, an initial reduction of this bacterium to 10.10^5 was observed for the *in natura* coconut oil. However, after 10 minutes of using both treatments, the inhibition effect is reversed, with the ozonized oil reaching a 1.10^5 inhibition of bacteria, compared to the *in natura* oil (3.10^5 bacteria). The maximum efficiency in controlling bacterial growth was achieved by both oils applied at 40 minutes.

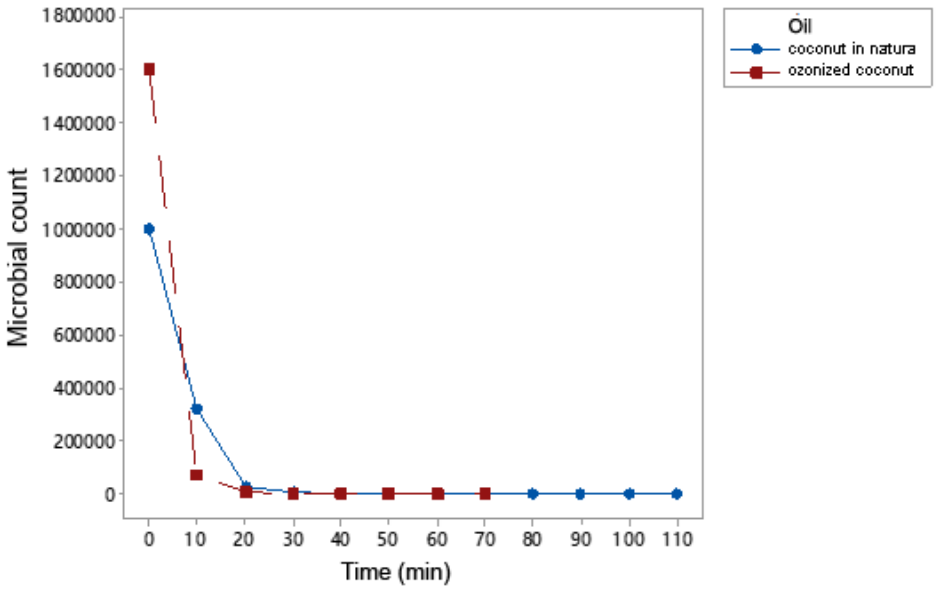

Figure 4. *Acinetobacter baumannii* microbial counts for *in natura* and ozonized coconut oil according to exposure time.

Figure 5 shows the high microbial population for both oil palm, ozonized (13.10^5) and *in natura* (11.10^5). The oil *in natura* extinguished the bacteria at ten minutes, while the ozonized oil allowed the decrease to 1.10^5 of bacteria. At 30 minutes, the maximum inhibition of microbial growth was reached.

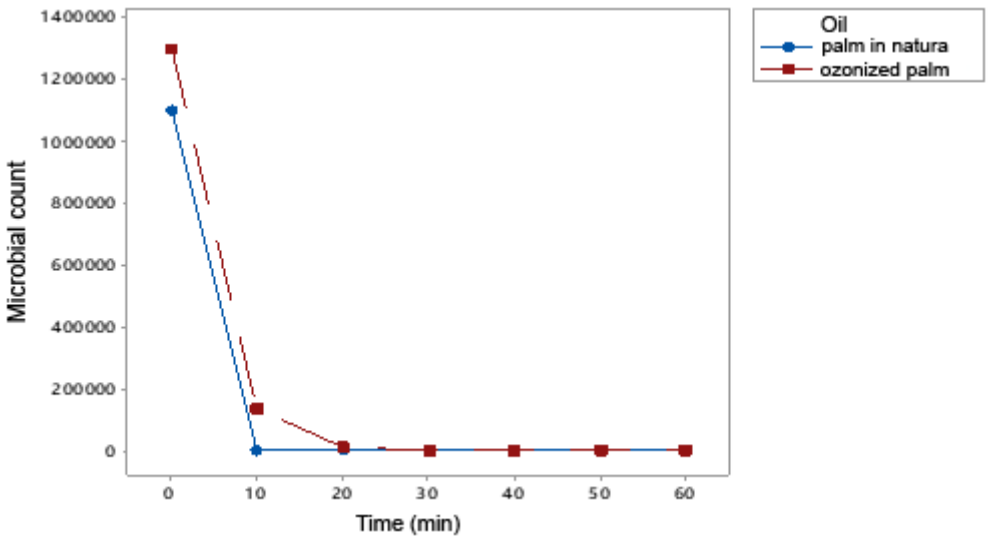

Figure 5. *Acinetobacter baumannii* microbial counts for *in natura* and ozonized palm oil according to exposure time.

Table 3 shows the comparison between the *in natura* and ozonated treatments with a significant difference only in coconut oil (p=0.012). The decrease in the microbial count was significantly higher in the ozonated oil treatment. For the other oils, the ozonated and *in natura* treatments were not significant in decreasing the microbial count (P>0.05).

Table 3. mean ± standard deviation (Median) of the percentage variation (%) of the microbial count in comparison to the natural oils evaluated.

Natural Oils (MBC *in natura*/MBC ozonized)	Treatments		P[1] Value
	in natura	ozonized	
Canola (50%/25%)	-85,5±16,2 (-93,5)	-95,1±5,2 (-96,2)	0,246
Coconut (50%/25%)	-27,4±163,0 (-71,6)	-69,9±66,7 (-93,8)	**0,012**
Palm (50%/12,5%)	-74,7±30,8 (-85,9)	-90,4±6,1 (-91,0)	0,194
P[2] Value	0,058	0,134	

P[1] Value referring to the Mann-Whitney test at P<0.05. P[2] Value refers to the Kruskal-Wallis test at P<0.05.

Figures 6 and 7 show the percentage variation behaviors of the microbial counts according to the ozonated and *in natura* treatments and according to natural oils. The black circles and squares in the Figures indicate the means and medians of the microbial count variation.

Comparing the microbial count variation between ozonized and *in natura* treatments (Figure 6) resulted in a significant difference in coconut oil (P=0.012). Here, the decrease in the microbial count was significantly higher in the ozonated oil treatment. The microbial count reduction compared to *in natura* and ozonized treatments were not significant for the other oils since all P values were greater than 0,05.

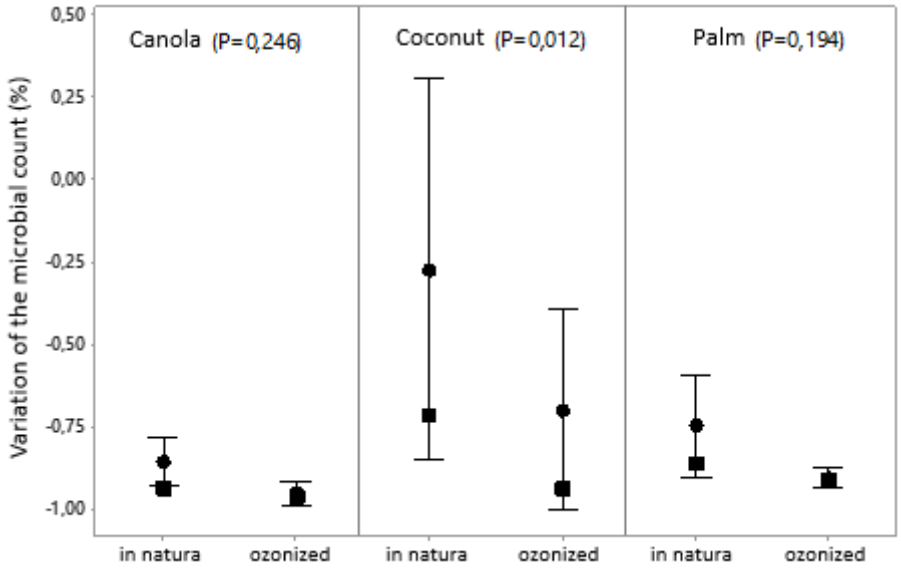

Figure 6. Microbial count percent variation of natural oils according to ozonated treatments and *in natura*.

There was no significant difference when comparing microbial count variation for the different types of natural oils (Figure 7). Despite the absence of a significant difference in the microbial count variation of the *in natura* oils, canola oil showed the greatest decrease in the microbial count (-93.5%), followed by palm oil (-85.9%) and coconut oil (-71.6%). For the ozonized oils, canola oil also exhibited the greatest microbial decrease (-96.2%), followed by coconut oil (-93.8%) and palm oil (-91.0%).

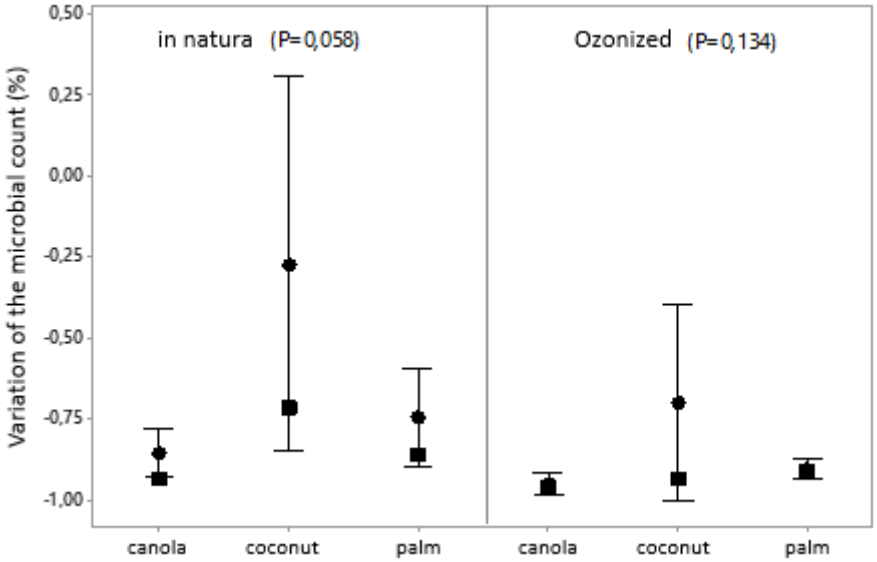

Figure 7. Microbial count percentage variation according to the natural oil type.

The oils properties (palm, coconut, and canola) were also observed, in which the physical-chemical characteristics of the ozonized oils and those without ozone were compared through chromatographic analysis (Figure 8). This Figure shows that palm, coconut, and canola oils that the total fatty acid index for ozonized and non-ozone treatment showed identical results 100g/100g ± 0.02 quantification limits (QL). Also, in the same Figure, the acidity parameter obtained by quantifying the oleic acid was not detectable (less than 0,1 LQ) in both tratment groups.

In Figure 8, the ozonated palm oil density showed a result of 0,892 g/cm^3 ± 0,0002 LQ, while the group without ozone showed values of 0,893 g/cm^3 ± 0,0002 LQ. The analyzed iodine index obtained 51 mgI2/g ± 0,1LQ for the ozonated group, while for the group without ozone, the value found was 55 mgI2/g ± 0,1LQ. The palm oil peroxide index in the ozonated group showed 434,8 mEq/Kg Na2S2O3 ± 0,2 LQ, while it was not possible to detect values for the group without ozone (**ND ± 0,2 LQ). The saponification index of palm oil resulted in 174 mg KOH/g ± 0,1 LQ for the ozonized oil and 186 mg KOH/g ± 0,1 LQ for the one without ozone.

It is observed in coconut oils (Figure 8) that the ozonized group's density observed 0,934 g/cm^3 ± 0,0002 LQ and the group without ozone 0,928 g/cm^3 ± 0,0002 LQ. In the iodine index measurement, the result for ozonated oil was 7,1 mgI2/g ± 0,1LQ, and without ozone was 16,7 mgI2/g ± 0,1LQ. The peroxide index 422,4 mEq/Kg Na2S2O3 ± 0,2 LQ was obtained by the ozonized oil. In contrast, it was impossible to obtain values in the group without ozone (ND ± 0,2 LQ). For the saponification index, a result of 204 mg KOH/g ± 0,1 LQ was obtained for the ozonized oil and 246 mg KOH/g ± 0,1 LQ for the one without ozone.

Canola oils (Figure 8) presents the ozonated oil density was 0,916 g/cm^3 ± 0,0002 LQ and for no ozone was 0,917 g/cm^3 ± 0,0002 LQ. Iodine was observed in ozonated oil with 99,5 mgI2/g ± 0,1 LQ, while without ozone, it showed 110.1 mgI2/g ± 0.1 LQ. The results obtained in the peroxides analysis for the ozonized oil were in the order of 125,7 mEq/kg Na2S2O3 ± 0,2 LQ, in contrast to the group without ozone, whose parameter was not detectable ** ND ± 0 2 LQ. The saponification index for ozonized canola oil was 149,9 mg KOH/g ± 0,1 LQ and no ozone 190,3 mg KOH/g ± 0,1 LQ.

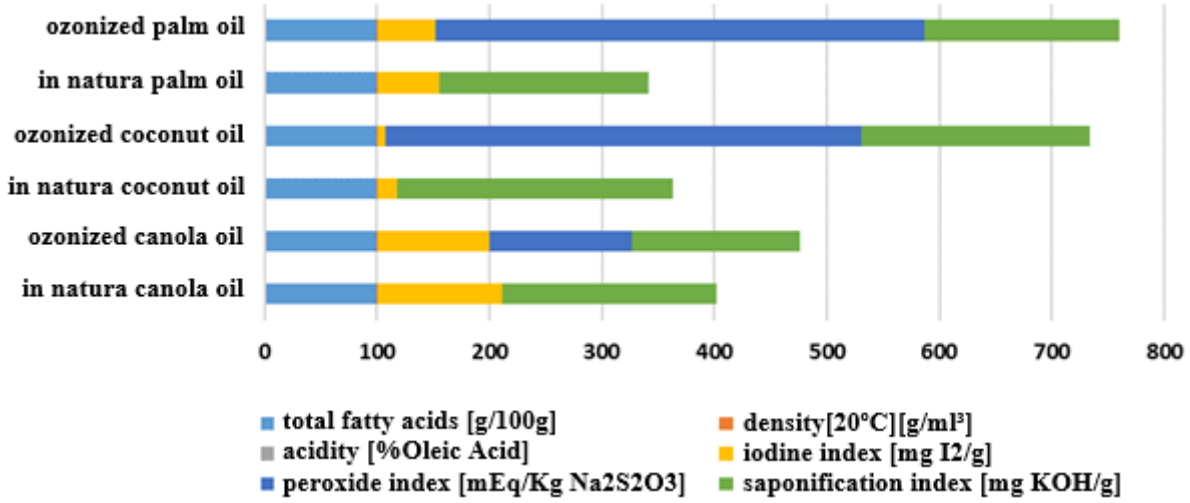

Figure 8. Physicochemical chromatographic differences of natural oils submitted to *in natura* and ozone treatments.

4. Discussion

According to the results (Figures 3 and 4), the microbial count reached zero at earlier times in the ozonated oils when compared to the *in natura* oils. Although there were no significant microbial count differences when comparing the treatments (ozonated and *in natura*), the time to reduce the microbial load to zero was shorter in the ozonated oils. This result indicates that the oil ozonation accelerates microbial

elimination, showing that ozonation has antimicrobial potential. It reached the zero value of microorganisms in shorter times compared to the oil *in natura*. In Figure 5, the microbial reduction time was similar for palm oil.

It is also necessary to consider coconut oil antimicrobial activity (Figure 7) since the ozonation process increased the drop in microbial load from -71,6% (*in natura*) to -93,8% (ozonized). For this oil, ozonation was significantly effective in reducing microbial load.

It is important to emphasize that ozonated and *in natura* oils represent an interesting pharmaceutical approach for treating various human and animal pathologies[25-28].

In some countries, ozonated and *in natura* oils are available for alternative use, characterized as natural and renewable sources, obtained through simple technology, low cost, and broad biological activity with low side effects[29,30].

For more than a century, ozone has been known to be an excellent disinfectant that, however, had to be used for a long time cautiously due to its oxidizing properties. Only in recent decades has it been possible to measure its concentration accurately and permanently incorporate the gas into compounds such as natural oils. Gaseous ozone chemically reacts with unsaturated substrates, leading to therapeutically active ozone derivatives[13,31]. Notably, the pharmacological activity of ozonated oils is dependent on the level of ozonation and the composition of the oil[30,32].

In the oil ozonation process, ozone reacts with carbon-carbon double bonds of unsaturated fatty acids, forming ozonides or 1,2,4-trioxolane rings and peroxides as the primary products responsible for antimicrobial activity and stimulating tissue repair and regeneration properties. Ozonated oils, containing 1,2,4-trioxolane rings formed in an unsaturated fatty acid chain, are considered an active ingredient and a carrier simultaneously, increasing absorption and penetration into the skin[29].

Another relevant consideration is that the ozone antimicrobial properties are due to oxidized compounds, such as hydrogen peroxide, hydroperoxides, aldehydes, and ozonides, formed when polyunsaturated fatty acids present in natural oils come into contact with ozone[33]. In this sense, Captain[34] describes the importance of ozonolysis in different types of oils to discover a broad-spectrum antimicrobial product with greater efficiency for therapeutic purposes. The author points out that the peroxide value should be kept between 300 and 2000 mmol-Equiv/kg. From the results obtained in this research (Figure 8), it was found that the ozonized palm and coconut oils showed values for peroxide 434,8 milliequivalents/kg and 422,4 milliequivalents/kg, respectively, within the recommended range. In comparison, canola oil was lower (125,7 milliequivalents/kg). It should be noted that the ozonized groups showed peroxide formation for all samples, revealing that ozonation promotes the oxidation of grease and causes its deterioration since the values obtained are higher than those recommended by ANVISA[35,36]. Thus, these ozonized oils are incredibly useful in controlling *A. baumannii in vitro*, as observed in Figures 3, 4, and 5.

Similar results were obtained by Hammer et al.[37], when comparing the chemical composition and microbiological activity of ozonized sunflower and olive oils, employing different volumetric methods, liquid chromatography methods, Agar diffusion, microdilution, and macrodilution, for various microorganisms and verified an increase in peroxide and acid values in both oils, being higher in ozonized sunflower oil. The authors state that when peroxide levels are high, ozonized oils have high viscosity,

which hinders access to the iodine bromide reagent's double bonds. For this reason, the iodine value determination is not accurate for this measurement. Of note, in this study, the high peroxide value in ozonated oil was more significant.

Ozone has distinct action mechanisms, depending on the environment where it is applied, being subject to different classifications according to its predominant behavior. Humidity, oxygenation, and temperature significantly affect the ozone action, directly proportional to its effectiveness[38]. As Captain[34] states, since ozonide and peroxide are the main active ingredients, the triozonide is stable and comes into contact with the wound exudate. The same author reports that ozonide and peroxide are the main active ingredients. Triozonide is stable and comes into contact with the wound exudate, generating reactive oxygen species (ROS) and lipid oxidation products. Therefore, this explains the research results, which presented a decrease and zero value of the microbial load (Figure 3, 4, and 5).

Different oils can be ozonated and used for microorganism control; however, olive oil has been one of the most commonly used oils due to its high unsaturated fatty acid content, while coconut oil has high lauric acid and capric acid content. Both oils can heal wounds and reduce infection[39].

Canola oil has been used by Batool et al.[40] for the bactericidal effect evaluation against *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, and Klebsiella pneumoniae*. The results showed inhibitory effects of the oil against *Klebsiella pneumoniae, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa*, but no inhibitory effects against *Staphylococcus aureus*. Analogous antibacterial activity effects were observed for ozonated and *in natura* canola oil against *A. baumannii* (Table 3).

In many countries, palm oil is used for therapeutic purposes in traditional medicine practice. All the plant's parts can be used medicinally. The leaves are squeezed, and the juice obtained is applied to wounds to enhance healing. The sap from this plant is also used as a laxative. The fruit peel ash is used for a soap used for skin infections. Powdered roots are added to beverages for the treatment of gonorrhea, menorrhagia, and bronchitis[41,42]. These therapeutic properties were decisive for palm oil choice in the antibacterial activity evaluation against *A. baumannii*.

In the research of Vijayarathna et al.[43], the in vitro palm oil activity against bacterial, fungal, and yeast *C. albicans* strains was investigated. The findings indicated a broad-spectrum activity of the oil extract, which was more effective against Gram-positive bacteria than Gram-negative bacteria. This occurrence can be explained by the fact that the cell envelope structures differ significantly between Gram-positive and Gram-negative bacteria. Gram-negative bacteria have an outer membrane that surrounds the cell wall, restricting the diffusion of hydrophobic compounds through its lipopolysaccharide shell. The cell wall of Gram-positive bacteria with no such membrane can be more easily permeated[44]. Hence, the palm oil extract constituents can hit various molecular targets, including cytoplasmic membrane, proton motive force, electron flow, and cell content coagulation[25,45].

Regarding extracts and coconut oil, *in vitro* studies have identified antimicrobial activity towards *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella pneumoniae, Proteus vulgaris, Serratia marscens, Salmonella sp, Staphylococcus epidermidis, Micrococcus luteus, Vibrio sp, and Listeria. Monocytogenes*[46,47]. A similar result was observed in Table

3 for ozonated and *in natura* coconut oil against *A. baumannii*, which is evidence of inhibition against this species.

Besides, the extracts and coconut oil's capability to destroy or inhibit Gram-positive and Gram-negative bacteria at low concentrations and minimum contact time establish this plant's secondary metabolites' efficiency as a potential broad-spectrum source of antimicrobial action. For Akinpelu et al.[48], this antimicrobial agent can be used in the treatment of pathogens with multidrug resistance. Another aspect that draws attention through ozone use for clinical therapies is that ozone therapy is also related to skin infection treatment[28], showing that it is possible to use ozone plus natural compounds for microorganism control.

5. Conclusion

It is concluded that the ozonized oils had a more significant reduction in microbial counts when compared to the *in natura* oils; ozonation favored an increase in antimicrobial activity. It is possible to use natural oils (palm, coconut, and canola) to control *A. baumannii*. The palm oil was the most effective in controlling the bacteria, with a MIC of 6,25% and a MBC of 12,5%, requiring 10 minutes for 100% microorganism inactivation.

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DECLARATION OF POTENTIAL CONFLICT OF INTEREST

The authors declare no conflict of interest.

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