Chapter 9

Combinations of Extracts of Propolis and Other Compounds Against Methicillin-Resistant *Staphylococcus aureus*

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66219

Abstract

Staphylococcus aureus is a microorganism of great health risk because of its high resistance to antibiotics. Methicillin-resistant strains (MRSA) are most at risk, especially in hospital patients and children. In recent years, it has been shown that a combination therapy of two or more drugs is more effective than monotherapy traditional. Furthermore, it has also been seen that many natural substances and plant extracts can inhibit the MRSA growth and other microorganisms. However, little has been studied about the combinations of different extracts or extracts combination with other commonly used drugs. The purpose of this work was to evaluate of extracts of propolis with garlic, oregano and ciprofloxacin to inhibit growth of MRSA strains, using isobolographic method. The results showed that combinations of garlic with propolis inhibit the growth of MRSA, but only in small concentrations. High concentrations of these two extracts appear to have an antagonistic effect. Combinations of propolis and oregano show a synergistic effect at any concentration. Finally, the combination of propolis with ciprofloxacin has an antagonistic effect. The action of ciprofloxacin is decreased when was combined with propolis. Health professionals should know this to warn patients when they use a natural resource, especially if a drug is being administered.

Keywords: propolis, garlic, oregano, MRSA, isobolographic

1. Introduction

Staphylococcus aureus is a microorganism of great medical importance because it causes serious damage to health in children and hospital patients. In recent years, they have had a

growing number of *S. aureus* infections having resistance to commonly used antibiotics. These resistant strains are known as "methicillin-resistant" and are identified as MRSA [1]. Between 1997 and 2003, MRSA was the third leading cause of serious infections in hospitals of which 5% were fatal [2]. In Mexico, the Hospital Epidemiological Surveillance Network reported that mortality rates among patients infected with *S. aureus* range from 5 to 70% and mortality rates attributable to MRSA can reach 50%. With data from general, pediatric, university and specialty hospitals, this network reported that in the period 1997–2003, *S. aureus* was third in morbidity and fourth in mortality [2].

They described three mechanisms that explain the resistance of *S. aureus* to β-lactams and others antibiotics:

1. Overproduction of β-lactamase or borderline resistance: Normal staphylococcal penicillinase hyperproduction mediated by plasmids. These strains produce high amounts of enzymes that degrade these antibiotics, resulting oxacillin and methicillin have no effect on them.

2. Modification of penicillin-binding proteins (PBP) corresponds to a minimum modification of PBPs 1, 2, 3 and 4. These proteins are normal molecular weight but have low affinity for β-lactam antibiotics, preventing these are set and fulfill its inhibitory action.

3. Intrinsic methicillin resistance is due to the incorporation into bacterial DNA of a gene mecA. This gene has two regulatory elements (mecR1 and mecI) that control the transcription of the mecA gene responsible for the induction of the synthesis of a binding protein supernumerary transpeptidase penicillin PBP capable of maintaining the integrity of the wall during the growth and division when the normal cellular enzymes are inhibited by antibiotics [3].

Because of this, they have studied various plant substances to inhibit and to control the growth of microorganisms, including propolis (propolis), oregano (*Lippia graveolens*) and garlic (*Allium sativum*). Propolis is a mixture of complex chemical composition containing balms, essential oils, pollen, vitamins, minerals and proteins, substances that confer a variety of biological properties of interest for therapeutic purposes [4]. The antimicrobial properties of garlic have been attributed to allicin that inhibits certain enzymes essential such as cysteine-proteinase and alcohol-dehydrogenase. Oregano has flavonoids, terpenes and phenylpropane derivatives which also confer antimicrobial properties. Each particular substance has proven to be a viable option to control MRSA. However, few studies have been devoted to the combination of these substances to evaluate the synergism in the antibacterial effect.

On the other hand, allopathic treatments used to fight MRSA include some fluoroquinolones such as ciprofloxacin (CPX) and levofloxacin (LVX), which act on DNA gyrase preventing replication. However, in recent years, it has detected an increasing resistance to these antibiotics in several microorganisms including MRSA. An alternative therapy could be the combination of natural extracts or the combination of these drugs such as ciprofloxacin with. There are few studies testing the efficacy of these drugs when combined with extracts commonly used as propolis.

http://dx.doi.org/10.5772/66219

This paper aims to evaluate combinations of extracts of propolis with garlic and oregano extracts and propolis combination with ciprofloxacin to inhibit MRSA in vitro. Microbial resistance tests were made by the macrodilution method, and combinations were evaluated by isobolographic analysis. The isobolographic analysis is a method based on determining the concentration of antimicrobial substance which has 50% of the inhibitory effect (EC50) and presents the advantage of having a greater statistical power than the traditional method to test dose subinhibitory, generally they are 90% of the minimum inhibitory dose (EC90). Furthermore, the isobolographic analysis reveals whether the effect of the combination is synergistic, simple additive or antagonistic.

2. Drugs and resistant strains

Infections due to *S. aureus* may be treated by a wide range of antibiotics. Among those are frequently reported in the literature penicillin, oxacillin, gentamicin, tobromicina, tetracycline, erytromicina, clindamycin, vancomycin, rifampin, ciprofloxacin and linezolid. The fluoroquinolones, such as ciprofloxacin and levofloxacin, have certain advantages over the others because they have very good effect to bacteria that are resistant to aminoglycosides such as penicillin, cephalosporin and tetracycline among others. It is general consensus that methicillin-resistant species such as MRSA are also resistant to aminoglycosides, so ciprofloxacin and levofloxacin are a good alternative. Both ciprofloxacin (CPX) and levofloxacin (LVX) are quinolones of second generation and are characterized by having a good antibacterial activity concentration dependent (or concentration-dependent) against most bacteria G− and against a broad range of bacteria G+. These antibiotics are widely used especially in intensive care units to combat bacteria such as Pseudomonas, Acinetobacter and *S. aureus*, among others [5]. Chemically, CPX is 1-cyclopropyl-6-fluoro-1,4-dihydro-4oxo-7-piperazin-1-alquinilin-3-carboxylic acid which is condensed formula C17H18FN3O3 and 331.35 with a molecular weight g/mol. CPX and LVX as other fluoroquinolones, act by inhibiting DNA gyrase bacteria (topoisomerase II).

Despite the good prospects offered by fluoroquinolones, since its inception, cases of resistance did not take long to appear. The data do not appear to be accurate, the early warning signs to microbial resistance generally occurred when some hospitals around the world announced that Vancomycin, a powerful antibiotic, was unable to fight *S. aureus*. Then, the same thing happened with penicillin in trying to fight *Streptococcus pneumoniae* [6]. After this came the quinolone resistance. Between 1997 and 1999 was conducted a study in six hospitals of Mexico, which were sampled. The incidence of MRSA was 15%, of these isolates showed 10% CPX resistance [7]. Another study indicates that the first case of complete resistance to CPX and norfloxacin was given in 2003 in the Andaman and Nicobar Islands (in the Indian Ocean) by a strain of *Shigella*. In 2007, it reported that the species of *Campylobacter* (commonly combated with CPX) showed a marked increase in the incidence of resistance to flurorquinolonas, whose cause was attributed to these antibiotics are regularly used in poultry regular consumption, such as chicken [8].

Reports of resistance to CPX and LVX in various species appear to have increased since 2010, *Escherichia coli, Salmonella* (which includes not only resistance but also CPX norfloxacin, ofloxacin) and *Clostridium difficile* (which has been particularly resist to LVX) are some examples [9]. As for *S. aureus* is concerned, the first reports of resistance to fluoroquinolones date from early 2000. In a study conducted in Cuba between 2000 and 2001, 284 healthy children hospitalized were sampled. Only 0.35% of healthy children and 2% of those hospitalized were identified as carriers of MRSA. All strains presented the mecA gene and showed marked resistance to tetracycline, penicillin and erythromycin but 7% of them already showed an intermediate sensitivity to ciprofloxacin. For 2005, it is reported in Colombia that of 131 patients diagnosed with conjunctivitis, 30% have confirmed to *S. aureus* as cause. 42% of the isolates showed multidrug resistance and 6% of them showed high resistance to CPX [10]. In other words, in just 5 years, the incidence of resistance to CPX MRSA strains grew alarmingly. For 2007, Callisaya et al. [11] in La Paz Bolivia reported an incidence of 3% CPX resistance in *S. aureus*. It was calculated that, in the past 5 years, 70% of hospital-acquired infections are due to multidrug-resistant strains and that most of them are due to MRSA.

It has been found that combination of two therapies is an excellent tool to combat MRSA. In recent years, these combinations have not only been carried out with various drugs, but is driving the study drugs and combinations of naturally substances as propolis, garlic or oregano and many others, it has been found that extracts of propolis, combined with various drugs have been successful in combating various kinds of pathogens, so research must continue in this direction.

3. Substances with antibacterial effect

It is known that many natural sources from traditional herbal medicine and ancient cultures have a strong inhibitory power against a large number of bacteria. The extracts of leaves, roots, stems or flowers have been used to great effect in this regard. Although only two decades ago they have begun seriously studying these remedies, they have found a large number of substances that are effective as antibiotic. The success of these effects has been attributed to the large number of compounds containing in plants that maybe have a synergistic effect per se. Several studies agree that it is precisely because of the presence of a large number and variety of compounds in such products that microorganisms can hardly get to develop resistance against them.

Propolis is a natural product that has attracted the attention of scientists because it has proven its effectiveness in combating a number of diseases such as cancer, respiratory tract infections, vaginal infections, skin, and promote the strengthening the immune system among others. One aspect that has begun to take in the past 5 years is the combination of these natural products with drugs commonly used in clinical practice or other plants, especially to combat *S. aureus*. Apparently, some of the mechanisms through which works propolis, as is the weakening of the cell wall and inhibition of gene expression, favor a synergism with these drugs reports tests propolis extract blends with antibiotics such as chloramphenicol, gentamicin, netilmicin, tetracycline and clindamycin with very good results have.

3.1. Propolis

Among the products that can be obtained from the hive are wax, honey, royal jelly and propolis. Propolis is a mixture of complex chemical composition containing balms, essential oils, pollen, vitamins, minerals and proteins, substances that confer antimicrobial activity, which is mainly attributable to flavonoids. It is characterized by having 55% aromatic resins and balsams, 30% waxes, 10% essential oils and 5% pollen grains. They have been reported around 38 flavones, 12 benzoic acid derivatives, 14 cinnamyl alcohol derivatives and cinnamic acid, 12 components from alcohols, ketones and phenols, seven terpenes, 11 steroids, few sugars and amino acids [4]. However, its components may vary due its origins, which are: exudates that bees collect from plants, substances secreted by the metabolism of bees or materials that may be added during the preparation of propolis [12]. However, all propolis have strong antimicrobial activity, which has been evaluated on Gram-positive bacteria and Gram-negative bacteria, being more effective on G+ [4].Cinnamic and flavone components of propolis alter membranes and inhibit the bacterial motility. It is believed that synergism can have so some other antibiotics [13]. Flavonoids (quercetin, apigenin, galangin, etc.) and phenolic acids (caffeic, isoferulic, cinnamic and benzoic acid), are toxic to bacteria and, in addition, inhibit the enzymatic activity of hyaluronidase and caffeic acid. The mechanisms of action of propolis depend mainly of flavonoids that bind to biological affinity to heavy metals and polymers, catalyze electron transport phenomena and are working to find free radicals. In order to process propolis, this is mixed with hot water to separate wax, dried by air and dissolved in ethyl alcohol 95% where it is eliminated wax residue, other insects and wood, finally is filtered [12].

3.2. Oregano

Oregano is mainly used to flavor food and as a medicinal plant for respiratory tract problems such as digestive and analgesic [14]. However, oregano was found other uses related to the treatment of more infections. It is known that the essential oil inhibits the growth of fungi, parasites and is an excellent bactericidal against streptococci and staphylococci [15]. In the essential oil of oregano, they have been detected numerous compounds such as flavonoids as apigenin and luteolin, aglycone, aliphatic alcohols, terpene compounds and derivatives of phenylpropane. Among the most common and high concentrations are limonene, caryophyllene, p-cymene, camphor, linalool, pinene, carvacrol and thymol. The antimicrobial activity depends on the chemical composition of the essential oil of oregano, which is related to the species of oregano, geographical conditions, periods of harvesting and extraction method, if however, its activity has been mainly attributed to carvacrol and thymol [16, 17]. Oregano essential oils have antimicrobial effect against certain Gram-positive bacteria and Gram-negative bacteria, such action is due to the effect on the phospholipids of the outer layer of the bacterial cell membrane, causing changes in the composition of fatty acids. Thymol and carvacrol have the high antimicrobial ability on Gram-positive bacteria and in particular on Gram-negative bacteria acting as disintegrators the outer cell wall and inhibit their growth. The hydroxyl group, which possesses both compounds, appears to be responsible for its antimicrobial capacity [18].

3.3. Garlic

Garlic is a plant that belongs to the monocot species of the Liliaceae family of Asian origin, whose medicinal properties have been known since antiquity. Contains an amino acid called alliin (S-allyl-L-cysteine sulfoxide) which is responsible for the characteristic odor and no antimicrobial activity in their natural state, but when garlic is crushed or fermented the aliinase enzyme is released. This enzyme transforms allin to 2-propene sulfonic acid. This compound presents itself antibiotic, antifungal, lipid-lowering, antioxidant and fibrinolytic [19]. Alliin is crystallized from ethanol or acetone dilutions and is stable in aqueous solutions and at high temperatures. When cells are broken, alliin is mixed with allinase and in about 10 seconds all exposed alliin becomes a new group of compounds: allicin and its closest relatives that emit the scent of fresh garlic. However, the importance of this reaction lies in the formation of allicin, which is the compound which has been mainly attributed to the antibacterial power of garlic. Recent studies conducted by numerous researchers have provided a large number of pharmacological evidence to justify its use as antihypertensives, antifungal, antimicrobial, antithrombotic and antihyperglycemic. It has been shown that allicin exhibits antimicrobial activity due to inhibition of RNA synthesis in bacteria [20].

4. Methodology

Twenty-five *S. aureus* strains were collected over a period of 3 months. Biochemical tests were applied to determine genus and species as established by Mac-Faddin [21]. To identify MRSA, strains were tested using oxacillin according to Clinical and Laboratory Standards Institute (CLSI) [22]. Strains spread massively in culture plate and handle samples were taken with the technique "snowball" which was suspended in an Eppendorf tube-containing medium skim milk. They were stored at −80°C until the time of study. *S. aureus* strain ATCC 29213 was used as a positive control, as established by the same CLSI.

4.1. Extraction and formulation of extracts of propolis

Propolis in beekeeper Canatlán region, Durango, Mex (24°35'N, 105°00'W) was collected. Propolis was cleaned manually, separating waxes, vegetable scraps or other insects; 20 g dry propolis was weighed and 100 mL of ethanol was added and allowed to stand for 8 days with occasional stirring, protected from light with aluminum foil covered at room temperature. Thus, the ethanol extract 20% of propolis was obtained (EEP20). Similarly, extract 30% (EEP30) was prepared by weighing 30 g of the same and adding the same amount of ethanol. Then the extracts were filtered through Whatman filter and aliquoted into 50-mL Corning conical tubes. The extracts were kept protected from light and cooling (5–7°C) until the time of the study.

4.2. Collection and formulation of extracts of garlic and oregano

Garlic and oregano were purchased in local supermarkets. The taxonomic classification and identification was carried out at the National Polytechnic Institute-Dgo by D Ph. Ma. Socorro Gonzalez Elizondo. Garlic was cut into cubes of about 1 mm, after 20 g was weighed and was added 100 mL of ethanol. It was allowed to stand for 8 days with occasional stirring, protected from light with aluminum foil covered at room temperature.

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Thus, the ethanolic extract Garlic 20% was obtained (EEA20). Similarly, the extract 30% (EEA30) weighing 30 g thereof by adding the same amount of ethanol was prepared. Then, the extracts were filtered through Whatman filter and aliquoted into 50-mL Corning conical tubes. The extracts were kept protected from light and cooling (5–7°C) until the time of the study. Similarly, the extracts of oregano were obtained, which was cleaned of branches and flowers using only the leaves.

4.3. Isobolographic studies to evaluate synergism

Isobolographic analysis is one way to assess the interaction between two substances or two drugs resulting in the optimal combination of drugs. This method provides a safe way to assess whether substances are stronger by the combination that the application of each separately. This study is performed by a isobologram, which is a graph in rectangular coordinates of substances causing a certain level of effect when applied together. The line joining these two points it is known as "additivity line" and all points on this line, theoretically represent effective doses of the constituents administered jointly [23]. The interaction of the drug in the isobologram can be defined as additive, antagonistic or synergistic. Two drugs interact additively when the effect achieved after administration of both is equal to the sum of the effects that would be achieved if administered separately. When the effect achieved is less than that achieved if the same doses of both drugs were administered separately, it is a case of antagonism. When two drugs are administered and the effect achieved is significantly higher than would be achieved by the sum of doses from each interaction, it is defined as synergy [24]. Furthermore, the method includes a statistical evaluation and determination of the interaction index, as explained later.

The way it was carried out the study of synergism in the combinations was based on the methodology described by Tallarida [25, 26] which is known as isobolographic study. An inoculum of each strain of 105–106 CFU/mL was prepared. Each strain was exposed to seven different concentrations of each extract (25, 12.5, 6, 3, 1.5, 0.7, 0.3% extract v/v) cultivated in broth in tubes 13 × 100 by the macro dilution method [27]. An aliquot of each tube inoculated was also cultivated in Muller Hinton agar and incubated at 36°C for 24 h. After the growth of microorganisms, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determinate as shown in **Figure 1**. The letters AB indicate the antibiotic and size of the bracket is indicative of the concentration of antibiotic.

Concentration of extract broth in which it is detected no turbidity into tubes was established as MIC. The concentration where no growth was detected in plates was established as MBC. An aliquot of the inoculum to be planted plaque was also taken. After appropriate incubation, the inoculum exact population was counted. This is necessary to set the following parameters:

a. Mortality: The difference between population in the inoculum and the populations in plate for each concentrations tested expressed as CFU/mL.

b. Percentage of inhibitory effect: mortality divided among the population of inoculum expressed as a percentage.

c. Dose-effect curve: The effect (%) was plotted (Y) vs. each concentration (dose X) as shown in **Figure 2**.

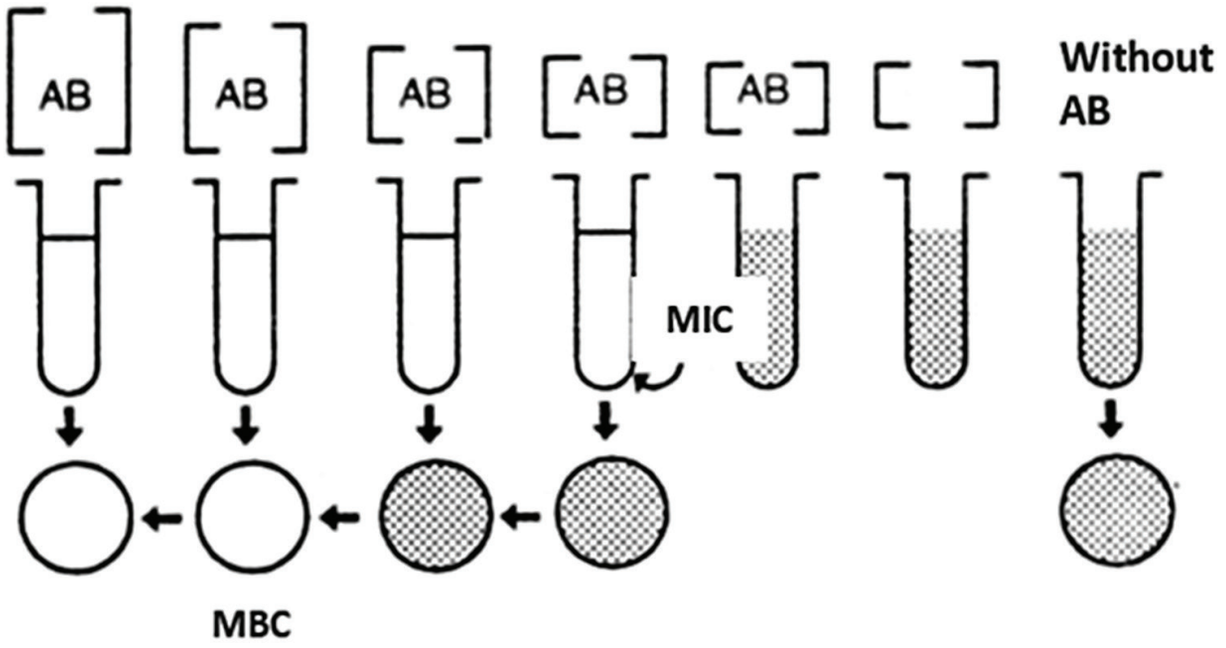

Figure 1. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

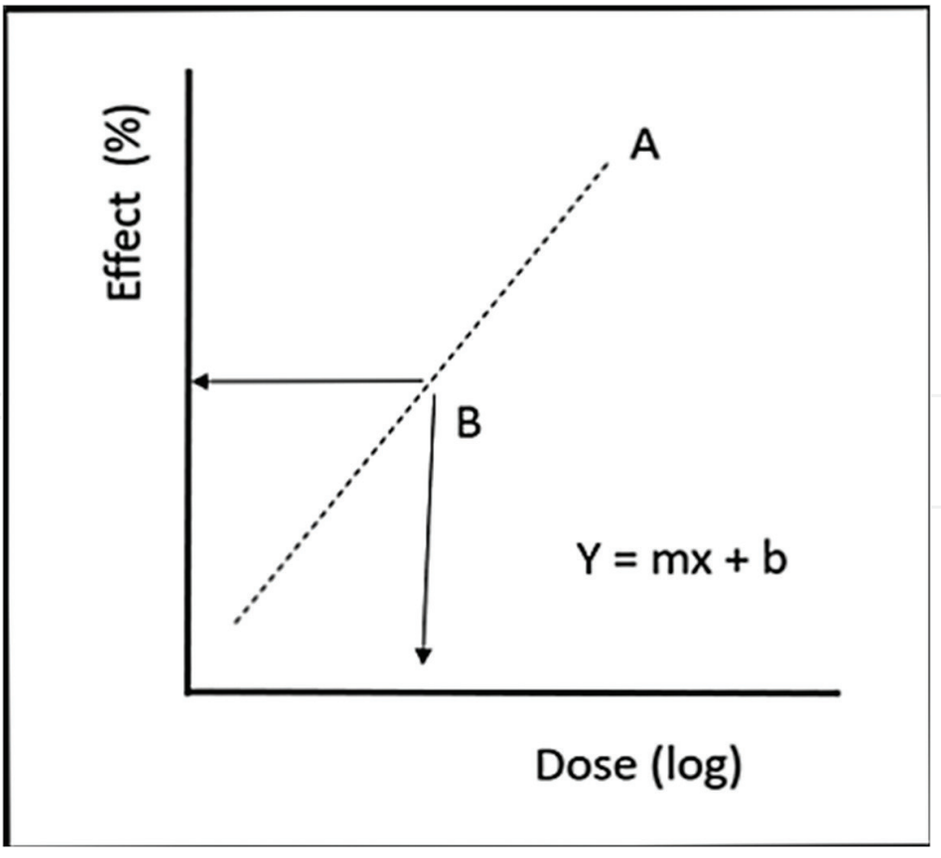

Figure 2. Graphical representation of % effect vs. log dose.

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The dose-effect curve is defined by an equation (Y = mx + b) which allows us to calculate the effective concentration 50 (EC50). This is defined as the dose at which the 50% of effect is achieved, in this case, half of the population counted. Point A in **Figure 2** represents the maximum effect. Point B represents the dose at which 50% is obtained effect for a single drug.

The concentrations that produce a 50% effect is made the graph shown in **Figure 3**. The combinations of the two drugs A and B theoretically produce 50% effect (points a and b) is constructed. However, it may happen that lower concentrations of the drugs A and B combined have the same effect, for example, the point "c". This is a synergistic combination. The point "c" can also be placed above the line of additivity, indicating an antagonistic combination.

Additionally, the significance test or interaction index was performed by the Eq. (1):

$$g = \frac{a}{A} + \frac{b}{B} \tag{1}$$

where,

g = interaction index

A = individual concentration of A

B = individual concentration of B

a = concentration of A in the combination

b = concentration of B in the combination

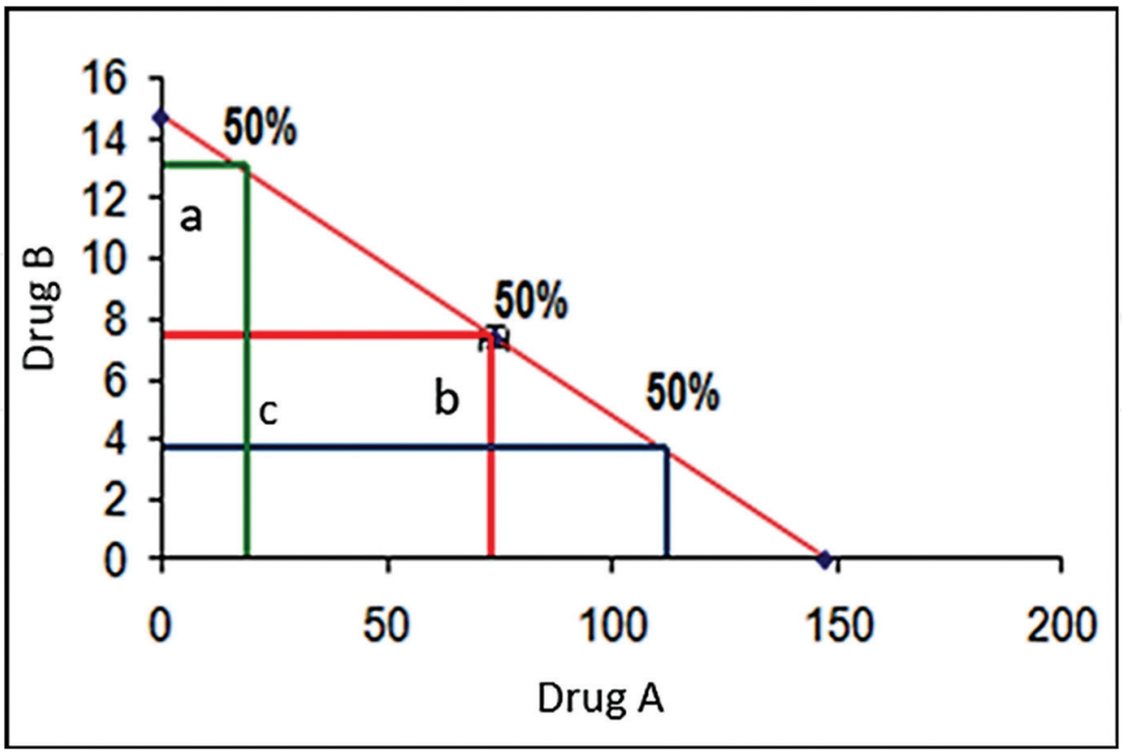

Figure 3. Isobologram.

When g = 1, we have an additive effect, if g < 1, the effect is synergistic, if g > 1, the effect is antagonistic.

Finally, the Student's t test was applied to identify differences between the effects of theoretical and experimental concentrations for each combination applied to five strains MRSA.

Three theoretical combinations for each pair of extracts were tested: propolis-garlic, propolis-oregano and propolis-ciprofloxacin. After that they were inoculated with MRSA strains and the experimental inhibitory effect was determined. With these results, the isobologram was made for each pair of extracts.

5. Results and discussions

5.1. Garlic-propolis combinations

Less inhibitory concentration of antimicrobial in the experimental stage in relation to the theoretical concentration was found. Average was determined as theoretical concentration of garlic and propolis a total concentration of 8.6 ± 0.0 mg/mL to obtain the EC50, however, experimentally a concentration of 5.95 ± 1.19 mg/mL was obtained for the same effect, in other words, a lower amount of extracts combined is required that each individually to obtain the same effect. The interaction index was <1. This indicates a synergistic effect. The statistical test ("t" Student) showed significant differences between the two groups ($p < 0.05$).

The corresponding isobologram is shown in **Figure 4**. The point A shows theoretical garlic and propolis concentrations necessary to obtain a 50% effect (EC50), point B shows the experimental concentration, which is visibly lower, which It indicates a strong synergistic effect.

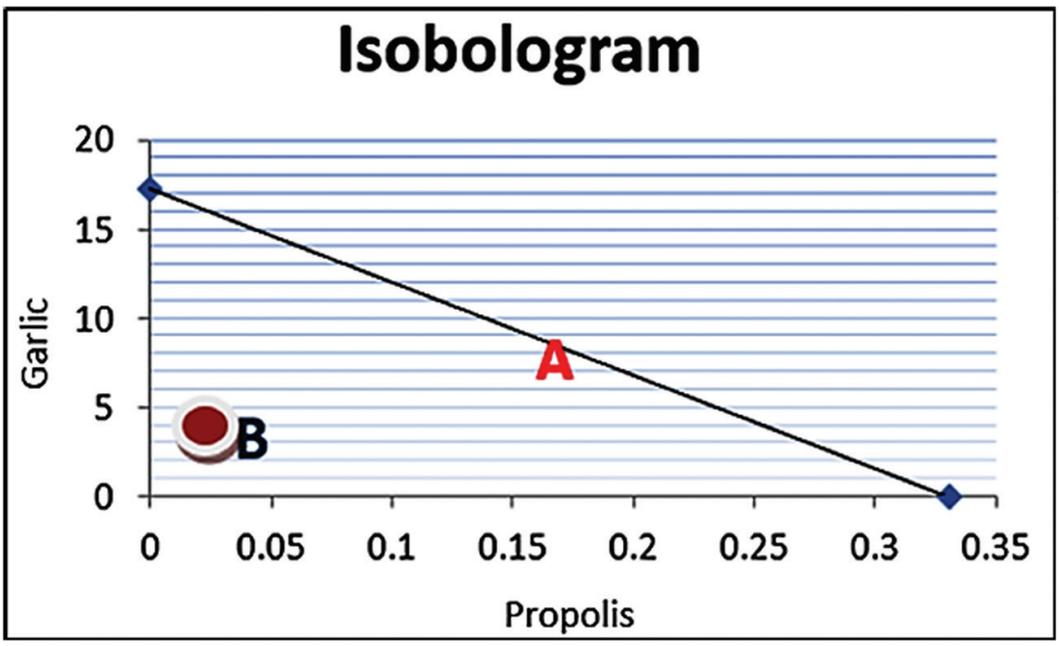

Figure 4. Isobologram combinations garlic-propolis percentage effect.

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Figure 5 shows the colony forming units counted in concentrations EC50 (A) propolis, (B) garlic, (C) garlic + propolis (combination 1), (D) garlic + propolis (combination 2), where the combination 2 is half combination 1. Growth in (C) and (D) is less than that taken in the application of each extract separately, confirming the synergistic effect of the combinations.

In contrast to these results, other combinations tested garlic and propolis showed opposite results. Greater theoretical concentration (17 mg/mL) resulted in an experimental concentration of 60.07 ± 4.8 mg/mL to obtain the same effect. This was verified by determining the interaction rate, which was g = 3.5.

This suggests that increasing concentrations propolis and garlic, their compounds act antagonistically inhibited each other. The cause is not yet well defined but could be a saturation at the same site of action. This should be studied in future. In conclusion, it is recommended to use only diluted combinations of garlic and propolis extracts, for example extracts 20%.

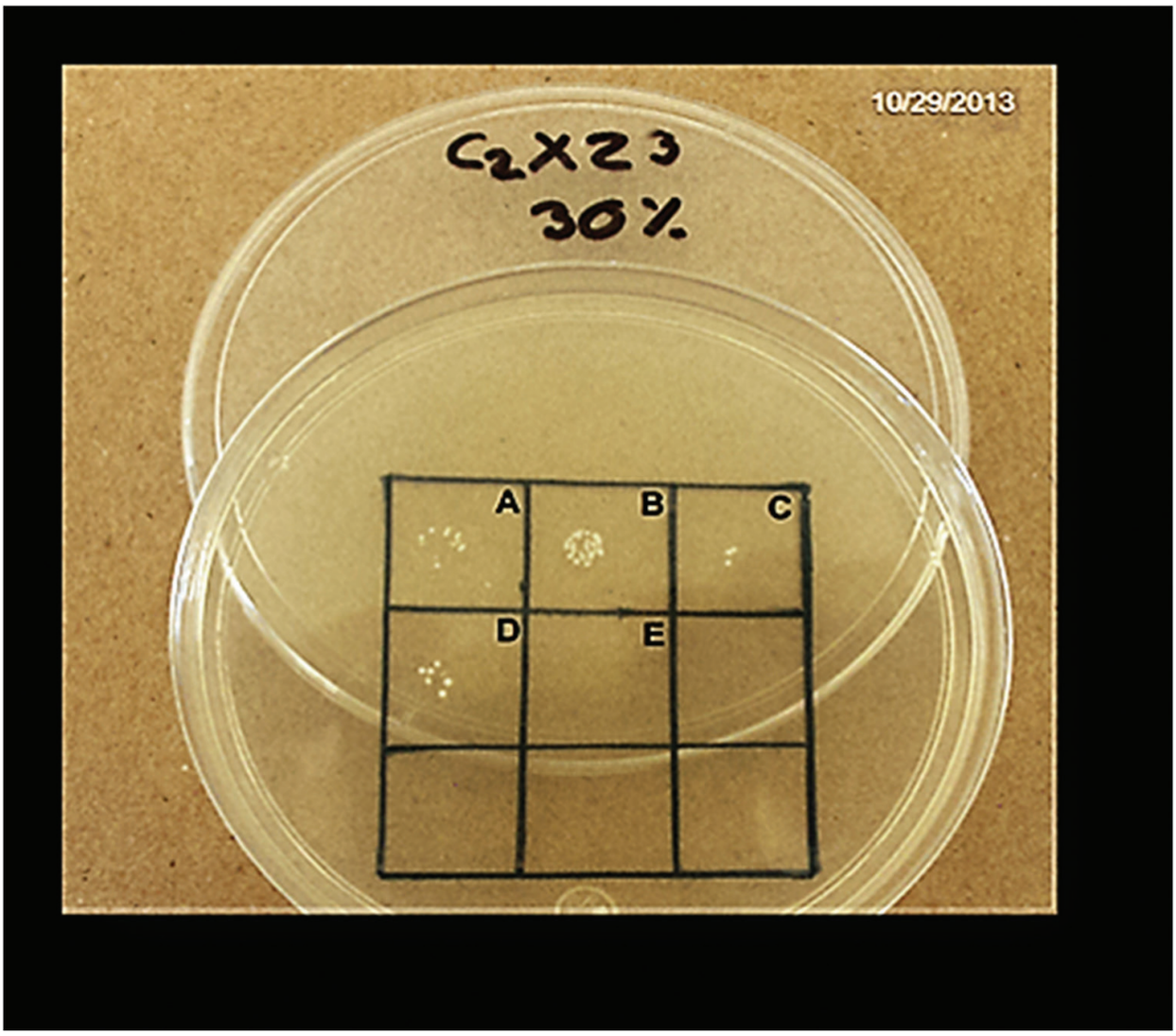

Figure 5. Population counted when grown with theoretical concentrations.

5.2. Oregano-propolis combination

In combination with propolis oregano, lower experimental concentration was also found that the theoretical: 4.9 mg/mL vs. 3.33 ± 0.86 mg/mL. **Figure 6** shows the corresponding isobologram. The interaction index was calculated 0.59 indicating a synergistic relationship. The corresponding Student's t test showed significant differences between treatments.

Unlike garlic, oregano in combinations with propolis shows a synergistic effect always. In fact, combinations of garlic extracts and propolis 30% were more synergistic than any other combination. Chavez et al. [28] had demonstrated the synergistic effect of oregano oil in combination with Gentamicin on E. coli isolates and had suggested that potentiates the effect of oregano other drugs when combined with them. No other reports found on combination of oregano and propolis, but we believe it is a combination that results in a good control of MRSA.

According to the research conducted by Waili et al. [29] on the synergistic effects of propolis extracts with ethyl alcohol on *S. aureus* and our studies agree that potentiates the effect of propolis another drug on *S. aureus* isolates; with the difference that Waili used as a second active substance honey; and this work highlighted the effects of propolis in combination with extracts of oregano.

Chavez et al. [28] showed a synergistic effect between essential oil of oregano and Gentamicin in E. coli isolates using the arithmetic mean of the halos of inhibition, however, we demonstrated a synergistic effect between propolis extracts and extracts of oregano in *S. aureus* applying an isobolographic analysis. In any case, it is shown that extracts of oregano in combination with other drugs or plants potentiates the effect against bacteria and could help fight serious infections in hospitalized patients and children [30, 31]

5.3. Propoleo-ciprofloxacin combination

The theoretical concentration established for ciprofloxacin (CPX) was 9 μg/mL. By combining this with propolis, the inhibitory effect of propolis disappeared. In other words, in 5 of the 7

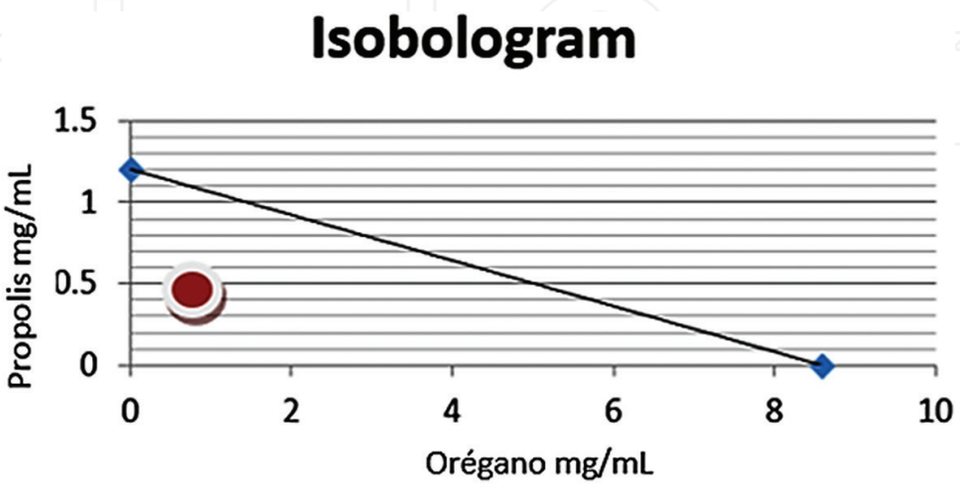

Figure 6. Isobologram oregano-propolis combinations.

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strains studied further growth of MRSA was found in combinations in concentrations of each substance separately applied. This suggests that the propolis is an antagonist of CPX, so the combination of both substances is harmful. In a survey conducted in this study 821 people, 45% said propolis or their products are consumed in respiratory diseases, and 34% of them combined ciprofloxacin or levofloxacin with propolis.

Many studies have reported that *S. aureus* is susceptible to propolis, however, a work published by Wojtyczka et al. [32] showed that propolis has a synergism only when is used in combination with other antibiotics that act on the cell wall and ribosomal functions, but it seems not interact with antibiotics that act on DNA or folic acid biosynthesis. The lack of synergism could also be that both propolis and CPX compete for the same target action: genetic replication and mutually inhibiting effect. However, other studies have reported good results by combining propolis with other drugs: Fernandes et al. [33] reported synergism between extracts of propolis and Chloramphenicol, Gentamicin and Vancomycin among others, but claims to have obtained the same effect between them combined extracts with oxacillin, ampicillin and ofloxacin.

No reports on combinations of garlic-propolis or oregano-propolis were found, therefore, it is difficult to make a comparison and deeper discussion about our results. However, we must make it clear that the basis for further research on alternative medicine to combat MRSA, as our results provide a good foundation for further research feel.

Microbial inhibition studies showed that the presence of *S. aureus* acquired in the community has been increasing, especially in healthy carriers. Its high resistance to CPX and LVX makes potential public health risk. Traditional therapies such as the use of ethanol extracts of propolis are a good alternative combat with the restriction check whether the drug combinations strengthen or inhibit their control.

6. Conclusions

Natural products should be subject to further study and be equally controlled by the health professional to inform the population about the beneficial and nonbeneficial cases. Apparently, the combination of garlic-propolis and propolis extracts of oregano is very effective in combating MRSA in vitro.

Acknowledgements

The authors acknowledge the Faculty of Chemical Sciences received support.

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