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ADVANCES IN NATURAL EXTRACTS USED FOR ANTIBIOTIC-RESISTANT BACTERIA TREATMENT: THE GRAM-NEGATIVE CASES

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ABSTRACT

Background: Infectious diseases are a global problem, the second human cause of death. Infectious diseases caused by pathogenic bacteria have been treated with a high degree of efficacy. However, even when the 20th century was considered the "golden age" of antibiotics, bacteria developed a different resistance mechanism to antibiotics. In 2017, the WHO issued an alert about 12 bacteria with an urgent need to develop new antibiotics. **Aims**: The aim of the present review is to analyze the current knowledge of the antibacterial activity of natural extract-based treatments against the pathogens listed by WHO. **Methods**: A systematic review of the literature in PubMed was performed to search for publications describing the use of natural extracts as antibiotics over bacteria. We focused on the Gram-Negative group. The exclusion criteria consisted of limiting papers on natural extracts tested over the bacteria culture related to eight selected bacteria, according to an alert issued by WHO in 2017, and seven plant extracts. **Results**: All the Gram-Negative bacteria listed in 2017 by WHO have been treated, with different degrees of advance, with some of the plant extracts and plant-based compounds reviewed. In general, the first approach is using inhibition disks applied over the bacterial biofilm in solid culture media. **Discussion**: While Salmonellae and P. aeruginosa have been extensively studied, over N. gonorrhoeae, A. baumannii have been tested with fewer natural extracts. Edible herbs are more often used, as well as artemisa and wine byproducts. In all cases, they are in the early stages of study, not being tested in patients at present. **Conclusions**: Plant extracts and plant-based compounds are effective as antibacterial, with minimal effects on the host cell. Furthermore, they are sustainable, environmentally friendly, and renewable.

Keywords: *natural extract, bacteria, pathogen, Antibiotic-resistant, infectious diseases*.

1. INTRODUCTION

Currently, infectious diseases are the second leading cause of death in the world. The 20th century was considered the golden age of antibiotics, contributing to diminishing morbidity and mortality caused by infections worldwide. However, the massive use and overuse of antibiotics allowed bacteria to acquire resistance to antibiotics. Antibiotic resistance is the ability of pathogens to avoid the mechanism the drug uses against them. Increasing antibiotic resistance (Diallo *et al.*, 2020) in emerging and reemerging bacterial diseases is a public health issue that must be addressed. Epidemiological surveillance programs work together in different countries to diagnose antimicrobial resistance worldwide (Christaki, Marcou, and Tofarides, 2020; Diallo *et al.*, 2020). In 2017, the WHO alerted and called

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researchers to find effective treatments against twelve bacteria, eight of which were Gram-negative. An important approach to finding new and effective treatments for infectious diseases is using plant extracts and natural compounds (Mulat, Pandita, and Khan, 2019). In the present review, we focused our attention on recent literature describing the use of natural extracts to develop new antibiotics against bacterial pathogens. The extracts can be the first step in discovering new active biomolecules or even the antibiotic itself. We summarized recent findings of antibacterial activity extracts and bioactive molecules from aromatic plants, wild plants, and plants used as infusions and byproducts of the wine industry.

2. METHODS

A systematic review of the literature in PubMed was performed to search for publications describing the use of natural extracts as antibiotics over bacteria, collecting and analyzing data. In order to do so, we used the following words/terms in combination: bacteria: (name) AND (natural extract) AND (plant name) AND (antibacterial activity or antibiotic). The exclusion criteria consisted of limiting papers on natural extracts tested over the bacteria culture related to the eight Gram-negative bacteria over the eleven present into an alert issued by WHO in 2017 and seven plant extracts. The search was conducted on papers published until September 2021.

3. RESULTS AND DISCUSSION:

3.1 Results

3.1.1 Antibiotic resistance

Antimicrobial resistance is a global public health crisis that complicates our success in treating bacterial infections. After discovering penicillin, Sir Alexander Fleming warned about the potential risk of underdosing antibiotics, which can lead to antimicrobial resistance. In the last decades, the main problem was the opposite, the overdose of antibiotics, not just in humans but also in animals and agriculture (Laxminarayan *et al.*, 2013). The increasing antibiotic resistance (AR) is a major obstacle in infection management. It has been reported in several countries a high and alarming number of human deaths caused by multidrug resistance: 25.000 deaths in Europe in 2009, according to The European Center for Disease Prevention and Control, 23.000 per year in 2013 just in the USA, according to US Centers for Disease Control and Prevention, and finally 10 million deaths per year are estimated globally by 2050 (Diallo *et al.*, 2020). Each antibiotic can exert its effect at different levels, affecting directly or indirectly essential processes for the bacteria, as shown in Figure 1. Antibiotic resistance can be intrinsic, acquired (involving modifications in their DNA by transformation, transduction, or conjugation), or adaptative (through modulation of gene expression) (Christaki *et al.*, 2020). These modifications can be transient or permanent and allow bacteria to survive in the presence of antibiotics by several mechanisms, including the destruction or modification of the antibiotic by the bacteria, modification of the target (as target mutations, replacement, protection, or overproduction), as well as the reduced permeability or increased efflux of the molecule (Christaki *et al.*, 2020). The emergence of multidrug-resistant bacteria or superbugs is followed closely by international surveillance programs. In 2017, an alert was issued about 12 bacteria requiring urgent antibiotic development due to their increased resistance, being *Acinetobacter baumannii, Pseudomonas aeruginosa* (priority 1), *Staphylococcus aureus, Enterococcus faecium, Helicobacter pylori, Campylobacter spp, Salmonellae, Neisseria gonorrhoeae* (priority 2), *Streptococcus pneumoniae, Haemophilus influenzae* and *Shigella spp* (priority 3). The selected bacteria for the present work were Gram-negative bacteria, excluding form the selection *E. faecium*, *S. aureus,* and *S. pneumoniae*

3.1.2 Natural extracts-action mechanisms

The estimated cost to discover and produce a novel antibiotic is 2,5 billion dollars and can take up to 15 years (DiMasi, Grabowski, and Hansen, 2016), which is why the use of natural extracts or natural compounds with antimicrobial activity is a promising approach. The extracts can be obtained from wild plants, cultivated plants, for instance, aromatic and spices, or byproducts of plants and fruits used in the industry. The extract can be used directly as an additive, like some related foodborne pathogens such as *Salmonella*, or it can be used to search in oral or topical administration on the patient. They are also useful for identifying the molecules(s) responsible for biological activity (Mulat *et al.*, 2019). The identification and isolation of bioactive molecules, such as resveratrol, ursolic acid, chlorogenic acid,

and several polyphenols, among others, allowed researchers to discover the mechanism of action of those compounds. Using polyphenols as an example, the mechanisms of action for their antibacterial activity have not yet been fully elucidated, but some clues suggest their potential clinical use. It is thought that they can interact with the cell wall, cell membrane, and bacterial proteins, altering processes like bacterial adhesion, metabolite, and ion equilibria, inhibiting biofilm formation, or impairing DNA synthesis (A. Silva *et al.*, 2021). They can also interact with covalently and non-covalently bonding with key bacterial proteins, e.g., penicillin-binding proteins, transporter proteins, surface-adhesion proteins, enzymes (including those involved in DNA synthesis), and cell-wall polypeptides. Additionally, some polyphenols can modulate gene expression (A. Silva *et al.*, 2021). For the present work, we selected extracts from aromatic plants, like rosemary [*Salvia rosmarinus* Spenn., Lamiaceae), syn.: *Rosemarinus officinalis* L.], thyme (*Thymus vulgaris* L., Lamiaceae), and oregano (*Origanum vulgare* L., Lamiaceae), wild plants such as jarilla (*Larrea* spp., Zygophyllaceae) and mugwort or artemisa (*Artemisia vulgaris* L., Asteraceae) and some of the industrial interest as yerba mate (*Ilex paraguariensis* A.St.-Hil., Aquifoliaceae) and byproducts of the wine industry from *Vitis vinifera* L. (Vitaceae).

The raw material of the plants can be obtained from several parts, including leaves, tails, roots, the whole plant, the aerial fraction of the plant, and also from the fruits: skin, seeds, or the whole fruit. In the case of *I. paraguariensis*, the commercial presentation ready to use for the preparation of the infusion, called “mate”, is the most commonly used. For *V. vinifera*, we also included wine byproducts and wine as raw material, as resumed in Figure 2. Starting from this material, researchers have been working with extracts, concentrates (only for wine), or Essential Oils (EO).

3.1.3 Bacteria

All the listed bacteria were treated with some of the above-mentioned natural extracts to test their antibacterial activity. It is worth highlighting the great differences in progress: while *Salmonella* and *Pseudomonas* have been extensively investigated, *N. gonorrhoeae*, *Shigella,* and *H. influenzae* have been hardly tested with natural extracts. Differences are also found in the same bacteria, as in the case of *Salmonella*, where not all strains tested with the same extract respond similarly to treatment.

In general, the first approach is the use of inhibition disks. The disk is embedded with the extract or the natural compound in solution and applied over the bacterial biofilm. The inhibition zone is measured concerning the diameter of the area around the disk where the bacteria cannot grow. The solutions can also be added to the liquid or solid culture medium, and growth is monitored by counting the colonies. There are critical parameters to measure when the inhibition is being tested. Minimal Inhibitory Concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibits the growth of the organism. Also, the Minimal Bactericidal Concentration (MBC) provides information on the lowest concentration at or above the MIC required to kill a microorganism. There are also specific parameters, such as MIC_{90}, which indicates the minimum concentration at which 90% of the isolates were inhibited, or IC_{50}, the inhibitory or effective concentration for 50 % of all surveyed isolates of a strain (CLSI, 2020; John E. Bennett, 2020).

Intracellular bacteria need to test their activity once the bacteria invade the host cell. The ideal antibiotic must kill the intracellular bacteria without affecting the cell viability.

The extracts and essential oils tested over each bacteria are summarized in Table 1.

3.1.4 Acinetobacter baumannii

Acinetobacter baumannii is an extracellular, strictly aerobic, nonmotile, Gram-negative coccobacillus. It grows well in conventional culture media. It has been implicated in a wide spectrum of infections, mostly nosocomial. The main anatomical site of colonization is the respiratory tract, which is why it plays an important role in patients in intensive care with mechanical ventilation. Over the past two decades, *A. baumannii* resistance has increased, particularly to beta-lactams, aminoglycosides, fluoroquinolones, and most recently to carbapenems (John E. Bennett, 2020).

Even though *A. baumannii* is at the top of the priority list of WHO, and there is not a significant amount of work on using natural extracts as a specific antibacterial. Among the extracts on which the present review focused, just artemisa was found.

3.1.4.1 Artemisa.

The advances in natural extracts based on artemisa use the aerial part of the plant and/or endophyte of the plant, like *Burkholderia*. The first

reports on this class of extracts consist of a phytosynthesis of silver nanoparticles using an extract from the aerial parts of *A. marschalliana* Spreng. When the nanoparticles were used at a concentration of 100 ppm, the inhibition zones were comparable with those reached with an antibiotic, ampicillin, with 11.45 and 15 mm, respectively (Salehi *et al.*, 2016). The role of silver nanoparticles is mainly the interruption of bacterial membranes. Using the pigment extracted from the endophytes *Burkholderia sp*. WYAT7 isolated from the medicinal plant *Artemisia nilagirica* (Clarke) Pamp, Ashitha *et al.* showed inhibition zones of 14 mm, the smaller zones when compared with other bacteria included in the study, like *Salmonella typhi* or *Klebsiella pneumoniae* (Ashitha, Radhakrishnan, and Mathew, 2020). Working with *A. khorassanica* Podl. (syn.: *A. oliveriana* J.Gay ex Besser) extracts, Fatemi, and col. found a high inhibitory activity against isolated strains of *A. baumannii*, with MICs from 6.25 to 12.5 mg/mL for the aqueous extract and 2.23 to 4.46 mg/mL for the methanolic extract (Fatemi, Sharifmoghadam, Bahreini, Khameneh, and Shadifar, 2020). In the study, they also showed the extracts were able to improve the action of two antibiotics, amikacin and imipenem, when they were used in combination, displaying synergism with MICs from two to ten-fold lower.

3.1.5 Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic, extracellular Gram-negative bacterium that grows very well in a wide variety of culture media, even with few requirements. *P. aeruginosa* is related to superficial infections from which it can cause bacteremia. It is one of the most important pathogens concerning hospital-acquired infections, especially pneumonia associated with ventilatory assistance. It also plays an important role in patients with cystic fibrosis. It presents a resistance mechanism associated with beta-lactamases and carbapenemases (John E. Bennett, 2020).

3.1.5.1 Artemisa.

Two species of artemisa have been tested against *P. aeruginosa, and A. mexicana* Willd. ex Spreng*.,* and *A. nilagirica.* In 1996, twelve plants used in Mexican traditional medicine were used to prepare methanolic extracts and confronted four bacterial pathogens. Among the most effective extracts against *P. aeruginosa,* was the extract of *A. mexicana,* with a MIC of 10 mg/mL (Navarro, Villarreal, Rojas, and Lozoya, 1996). In the case of *A. nilagirica,* Ahameethunisa *et al.* used several extraction solvents and tested all of them over the bacteria. When the inhibition zone was determined, the larger zone was 13 mm and the smaller 8 mm, following the decreasing order according to the solvent used to prepare the extract, hexane > methanol = petroleum ether > ethanol = chloroform > diethyl ether. Interestingly, the methanolic extract showed the lower MIC, being of 32 μg/mL, followed by ethanol, hexane, and diethyl ether with 128 μg/mL and chloroform and petroleum ether with 512 μg/mL (Ahameethunisa and Hopper, 2010). The key difference in the main compounds of the methanolic extracts was the presence of amino acids and tannins. In a recent study, the methanolic extract of *A. nilagirica* was used against seven bacterial and one fungal culture. Among them, the most sensitive was *P. aeruginosa*, which also exhibited a dose-dependent response, with inhibition zones of 16, 17, and 19 mm at 100, 150, and 200 μg concentrations, respectively (Parameswari, Devika, and Vijayaraghavan, 2019).

3.1.5.2 Jarilla.

Several works have shown the capacity of jarilla's extracts to inhibit and/or prevent infections with *P. aeruginosa*. Two papers from Micalizzi's laboratory partially purified proteins of crude aqueous extracts of *Larrea divaricata* Cav. The proteins were used for the production of antibodies in mice. On the one hand, they showed a cross-reaction between proteins of *L*. *divaricata* and proteins of *P. aeruginosa,* specifically cellular and extracellular bacterial proteins. This cross-reaction may indicate antigenic determinants in proteins of jarilla that generate antibodies, and they recognize epitopes that are distributed in proteins of *P. aeruginosa* (C. Sasso *et al.*, 2012). Even more, they also described the capacity of these antibodies to inhibit the hemolytic and proteolytic activity of hemolysins and proteases produced by *P. aeruginosa* (C. V. Sasso *et al.*, 2012). On the other hand, since the antibodies elicited against jarilla proteins of crude extract cross-reacts with cellular and extracellular bacterial proteins of *P. aeruginosa,* Canale *et al.* analyzed the possibility of the cross-reaction with superficial proteins of the bacteria. They found that the antibodies do not just recognize superficial proteins but also opsonize the bacteria, facilitating phagocytosis, which could be crucial for the elimination of the bacteria (Canale, Dávila, Sasso, Pellarín, and Mattar Domínguez, 2018).

3.1.5.3 Oregano.

Several reports have used oregano as EO or extract. For example, Elgayyar *et al.* worked with 12 different commercial EO from herbs and spices used in gastronomy against foodborne pathogens, spoilage bacteria, yeasts, and molds. The most effective was the EO of oregano, with a strong inhibition (zone of 46 mm) over *P. aeruginosa,* and even stronger inhibition against other pathogens such as *Escherichia coli* O:157:H7, *Yersinia enterocolitica* (87 mm) (Elgayyar, Draughon, Golden, and Mount, 2001). In opposition to these results, when Sarac and Ugur prepared their *O. vulgare* subsp. *hirtum* (Link) A.Terracc. EO did not find an inhibitory effect over *P. aeruginosa* even after performing a similar test (inhibition disks) and when they assayed the EO over other Gram-negative bacteria (Sarac and Ugur, 2008). López *et al.* consistently described a weak inhibition with *O. vulgare* EO applied over bacteria and fungus. The Gram-negative bacteria were the most resistant, and *P. aeruginosa* was almost unaffected with concentrations of 6 to 10 % (V/V), showing just a small decrease in colony number with 12% (V/V) of *O. vulgare* EO (López, Sánchez, Batlle, and Nerín, 2007). Interestingly, when a methanolic extract of *O. syriacum* L. was tested, it was the most effective extract to inhibit the growth of *P. aeruginosa,* with a MIC of 2 mg/mL, also showing the lowest MIC for *Candida albicans* and *Staphylococcus aureus* (Assaf, Amro, Mashallah, and Haddadin, 2016). In recent work, researchers found that the antibacterial activity of the oregano extract can be increased by using silver nanoparticles up to 1.3 fold (Meretoudi *et al.*, 2021) when they compared the inhibition zone of the extract versus the extract in silver nanoparticles.

3.1.5.4 Rosemary

Extracts and EO of rosemary have been extensively used as antibacterial. Santoyo and col obtained an EO by supercritical fluid extraction and tested several preparations against bacteria and fungus. The inhibition zones for *P. aeruginosa* were from 19 to 23 mm, similar to the ones obtained for *E. coli*. The MBC was between 1.75 to 2 mg/mL. They also analyzed the composition of the EO and tested the main compounds, being borneol the most effective, with 24 mm of inhibition and an MBC of 1.5 mg/mL (Santoyo *et al.*, 2005). When Sacco *et al.* extracted the non-volatile phenols from rosemary leaves and tested different fractions of the extract, they described a small inhibition (high MBC) for *P. aeruginosa*, with an MBC of 200 μg/mL, in comparison with 130 μg/mL for *S. aureus* (Sacco *et al.*, 2015). Assaf *et al.* also studied *S. rosmarinus* methanolic extracts. They obtained similar results as they showed for *O. syriacum*, MIC of 2 mg/mL, and the combination of both extracts resulted in a MIC of 1+1 mg/mL (Assaf *et al.*, 2016). Comparable results were found with commercial *S. rosmarinus* extract, with a MIC of 6,25 mg/mL and complete biofilm elimination after 5 minutes of incubation with the concentrated extract (200 mg/mL) (J. R. de Oliveira, de Jesus, *et al.*, 2017). In 2018, Gonelimali *et al.* showed that aqueous or ethanolic extracts have no activity against P. aeruginosa, but they were very active against *E. coli* or *S. aureus* (Gonelimali *et al.*, 2018).

3.1.5.5 Thyme

Several *Thymus* species have been tested against *P. aeruginosa* with different effectiveness. EO of *T. kotschyanus* Boiss, and Hohen. and *T. persicus* (Ronniger ex Rech. f.) Jalas were very effective against *S. aureus*, but they did not inhibit the growth of *P. aeruginosa* (Rasooli and Mirmostafa, 2003). However, EO of *T. vulgaris* was the best over eight different EO tested against *P. aeruginosa*, with an inhibition zone of 45 mm and a MIC of 5% (V/V) (N. Silva, Alves, Gonçalves, Amaral, and Poeta, 2013). When Martins *et al.* examined the activity of the extracts with different extraction methods as hydroalcoholic extract, infusion, or decoction, they found a weak halo with the first two methods and a moderate halo with the last one, being in all cases more effective against *E. coli* (Martins *et al.*, 2015). Two interesting works were published in 2017 by Luciane Dias de Oliveira's laboratory. In the first one, they showed the capacity of *T. vulgaris* L. to inhibit the growth of *P. aeruginosa* and prevent biofilm formation (J. R. de Oliveira *et al.*, 2017). The same work described the absence of cytotoxicity and genotoxicity against several cell lines, such as HeLa, MCF-7, and, more importantly, murine macrophages RAW 264.7. In their second publication, they showed that phagocytosis of *P. aeruginosa* by murine macrophages RAW 264.7 was enhanced in the presence of thyme extract, even more than thymol, in contrast to the effect on *S. aureus*, where thymol had higher activity than thyme extract (J. R. de Oliveira, Figueira, *et al.*, 2017). Quite the opposite, Gonelimali *et al.* did not find inhibition of *Pseudomonas*'s growth when they used aqueous or ethanolic extracts of thyme (Gonelimali *et al.*, 2018).

3.1.5.6 Wine byproducts

When Mayer *et al.* (2008) treated *P. aeruginosa* with grape seed extracts, they found a very low activity compared to other bacteria like *H. influenzae* or *S. aureus.* Nevertheless, they

observed a fraction of their purification, composed mostly of monomers like catechin and epicatechin, active only against *P. aeruginosa* but not for any other bacterium studied (Mayer *et al.*, 2008). In the same direction, Cueva and col. treated seven different bacteria with seven phenolic wine compounds and six oenological phenolic extracts, with *P. aeruginosa* being one of the less sensitive treatments (Cueva *et al.*, 2012). Purified compounds like gallic acid or ethyl gallate showed the strongest activity, with an IC_{50} of 205 and 289 μg/mL, respectively. The best grape seed extract was an oligomeric-rich fraction (GSE-O, 21 % flavan-3-ols monomers, and 78 % procyanidins), with IC_{50} of 253 μg/mL (Cueva *et al.*, 2012). Using pressed grape pomace from Merlot and Syrah and different extractions methods, strong inhibitions were obtained against *S. aureus*. However, just one of them was effective against *P. aeruginosa*, the extract from Merlot prepared by supercritical fluid extraction, obtained at 60 ºC and 250 bar of pressure, but not the ones obtained at 200 or 300 bar, or 250 bar and 50 ºC (D. A. Oliveira *et al.*, 2013). Wine samples have been tested against bacteria, as in the case of commercial wines of different grape and origin, used in 2017 by Radovanovic *et al.* They assayed fifteen different wines over six Gram-positive and six Gram-negative bacteria, being the Gram-positive more sensitive. Among the Gram-negative*, P. aeruginosa* resulted the second most sensitive, with 3 Cabernet Sauvignon and one Merlot being the strongest inhibitors, with a MIC and a MBC of 125 μg/mL (Radovanović, Arsić, Radovanović, Jovančičević, and Nikolić, 2017).

3.1.6 Helicobacter pylori

Helicobacter pylori is a highly mobile, spiral-shaped, Gram-negative, extracellular bacteria. It shows slow growth and has complex nutritional requirements. It has a tropism for the gastric mucosa, which is why it plays an important role in gastrointestinal diseases in adults and children. Its treatment is complex, using a wide range of antimicrobials. In recent years, the appearance of resistance to clarithromycin, used in several available therapeutic regimens, has been described (John E. Bennett, 2020).

3.1.6.1 Artemisa

In a big screening performed using extracts from 50 Taiwanese folk medicinal plants, artemisa ethanolic extracts prepared with leaves and stems of *A. argyl* H. Lév. and Vaniot were classified as moderate anti-*H. Pylori*-activity herbs, being 9 of 10 *H. pylori* strains inhibited by their herbal extracts (Wang and Huang, 2005). Jeong *et al.* used artemisa extracts *in vivo* to treat *H. pylori* in infected animals. They supplemented the food of mice with the extract and analyzed their tissues and the presence of molecular markers for the diseases. They found that the extract could rejuvenate H. pylori atrophic gastritis and suppress tumors produced by the bacteria (Jeong *et al.*, 2016). From *Artemisia ludoviciana* subsp. *mexicana* (Willd. ex Spreng.) D.D. Keck aqueous extracts, the reported MIC was 250 μg/mL (Palacios-Espinosa *et al.*, 2021). In the same work, the gastroprotective and anti-inflammatory activities were assessed using different solvents using four different fractions from the liquid-liquid extraction process. The fraction obtained with dichloromethane was the most effective, followed by the ethyl acetate fraction. Finally, they identified 2 compounds responsible for the antibacterial activity: estafiatin and eupatilin (Palacios-Espinosa *et al.*, 2021).

3.1.6.2 Rosemary, jarilla, and oregano

The first report on the use of rosemary extracts to inhibit the growth of *H. pylori* in vitro was published in 1996. Among several plant-based extracts assayed, rosemary extracts of 0.4 % (W/V) displayed inhibition zones of 10 and 8 mm when extracted in water or ethanol, respectively (Tabak, Armon, Potasman, and Neeman, 1996). For jarilla (*L. divaricata*) extracts, leaves and tender branches were used to prepare it with four different protocols: cold extract, infusion, decoction, or simulated digestion, and their MICs against 7 strains of *H. pylori* were determined. The measured MIC values depended more on the strain treated than the extraction protocol. The values were between 0.04 to 0.1 mg/L (Stege *et al.*, 2006). Finally, using *O. vulgare* subsp. *vulgare* and *O. vulgare* subsp. *hirtum* EO, Lesjak and col. found a MIC of 2.0 μL/mL for both EO and *Satureja hortensis* L. (Lamiaceae) EO. When they tested binary or ternary combinations of the EO in different proportions, they found synergistic and additive effects, being *S. hortensis: O. vulgare* subsp. *hirtum 2:1 is* the best combination, with a MIC of 0,5 μL/mL (Lesjak *et al.*, 2016).

3.1.6.3 Thyme

In 1996, Tabak and col. used a series of natural extracts to inhibit the growth of *H. pylori*, finding the thyme extract the most powerful (Tabak *et al.*, 1996). The first approach was using inhibition disks, and based on those results, they tested different species of thyme: *T. citriodorus* Schreb., *Coridothymus capitatus* (L.) Rchb. f. and *T. vulgaris*. Among them, *T. vulgaris* showed the

strongest inhibition, even stronger than nalidixic acid, cotrimoxazole, sulfamethoxazole, or colistin sulfate. Moreover, when the extract was added to a liquid medium, the bacterial growth was completely inhibited at 3,5 mg/mL (Tabak *et al.*, 1996). Another specie of thyme used was *T. kotschyanus* Boiss. and Hohen., tested against 70 isolated *H. pylori* strains from children 4-15 years old. They found that 66 % of the isolates were sensitive to extracts of *T. kotschyanus* (Farahnaz Nariman, Fereshteh Eftekhar, 2004).

3.1.6.4 Wine byproducts

The extracts are often prepared from different grape varieties, and also, the main isolated bioactive molecule, resveratrol (3,5,4'-trihydroxystilbene), is used. Starting from wine, Mahady and Pendland prepared a wine concentrate by drying and treating the *H. pylori* and adding it to the culture media. They found an MIC_{50} of 50 μg/mL, while the resveratrol solution assayed showed an MIC_{50} of 12,5 μg/mL (Mahady and Pendland, 2000). The same laboratory also prepared 2 new extracts: a Pinot Noir wine concentrate and a second extract by dissolving 1500 ml of red wine in 3 L of methanol, concentrating under reduced pressure, filtering, and collecting both the filtrate (alcohol soluble) and the particulate matter (alcohol insoluble). Only the concentrate and the alcohol insoluble fractions were active, with MIC of 50 and 25 μg/mL, respectively (Gail B. Mahady, Ph.D., Susan L. Pendland, Pharm.D, and Lucas R. Chadwick, 2003). Extracts from whole grapes (variety Colorino, Sangiovese, and Cabernet Sauvignon) were tested against 2 different strains of H. pylori. The determination of the MBC showed the Colorino grapes as the most active, with a MBC of 1.35 and 3.57 mg/mL for the strains G21 and 10K, respectively, after 24 hours of incubation. In the same period, Sangiovese and Cabernet Sauvignon grapes extracts showed activity only against G21 strain, with an MBC of 4 mg/mL (Martini *et al.*, 2009). When they used the main compounds of the extract, resveratrol showed the best activity against both strains, G21 and 10K (MBC 68 and 84 μg/mL, respectively), while myricetin, gallic acid, and quercetin showed MBCs three to four times higher (Martini *et al.*, 2009).

3.1.7 Campylobacter spp

The genus *Campylobacter* comprises small, mobile, extracellular Gram-negative bacilli with a curved morphology. They are aerobic and microaerophilic, with important nutritional requirements for their growth. It is most frequently found to cause gastroenteritis in humans. It is not usually treated, but erythromycin is the most widely used antibiotic when the condition is severe. There are species of *Campylobacter* that are resistant to beta-lactams. With the wide administration of fluoroquinolones, resistance to this group of antimicrobials is observed (John E. Bennett, 2020).

3.1.7.1 Artemisa

In 2011, Castillo *et al.* published the results of a big screening of plant extracts and their activities against *Campylobacter jejuni* and *Campylobacter coli*. (Castillo, Heredia, Contreras, and García, 2011). Their work showed *A. ludoviciana*'s extracts as one of the most powerful against the bacteria. With larger inhibition zones and lower MIC (4-4.5 mm and 0.5-0.6 mg/mL, respectively), they also found a strong reduction of *C. jejuni* and *C. coli* at the cell surface of Vero cells when they pre-incubated the bacteria with the extract of artemisa. Furthermore, their results confirmed a strong inhibition of the activity of the best-characterized *Campylobacter* toxin, the cytolethal-distending toxin (Castillo *et al.*, 2011). Unfortunately, they did not continue the research.

3.1.7.2 Oregano and thyme.

Both extracts have been used comparatively in several works, although the first report used thyme only. Ethanolic extract and post-hydrodistillation residue were assayed against *C. jejuni*, reaching MICs of 1.25 and 0.625 μg/mL, respectively (Šikić Pogačar, Klančnik, Bucar, Langerholc, and Smole Možina, 2016). Both extracts reduced the cell adhesion by up to 35 % compared to the control, and they were more effective when the extract was pre-incubated with the cells, and the bacteria were added later. In comparative research, Ozogul and col. found the oregano and thyme extracts are the most active against several foodborne pathogens, including *C. jejuni* (Ozogul, Kuley, Ucar, and Ozogul, 2015). In both extracts, carvacrol was determined as the main compound, and the authors indicated the molecule responsible for its antibacterial activity. Carvacrol activity has been reported, inhibiting motility without affecting their growth or flagella, and abolishing infection of epithelial cells (van Alphen, Burt, Veenendaal, Bleumink-Pluym, and van Putten, 2012).

3.1.7.3 Rosemary

An interesting difference between the most commonly used methods, diffusion disks and agar dilution, was found while investigating rosemary activity against *C. jejuni*. The researchers used four commercial rosemary

extracts, two oil-soluble and two water-soluble extracts. The inhibition zones showed very weak activity for the water-soluble extracts, with a MIC of 40 mg/mL (Klancnik, Guzej, Kolar, Abramovic, and Mozina, 2009). However, when they used the extract in liquid culture media, they found a MIC of 5 mg/mL for the water-soluble extract and 0.625 mg/mL for the oil-soluble extract. The author explained the difference by arguing a potentially weaker diffusion or other antagonistic effects of the tested substances in *Campylobacter* solid medium and, consequently, higher MICs in agar. Interestingly, they also tested two of the most bioactive molecules in the rosemary extracts, carnosic acid and rosmarinic acid, with MICs of 5 and 1.25mg/mL, respectively (Klancnik *et al.*, 2009). In a later work, using rosemary EO, Ozogul and col found a weak inhibition of the rosemary EO against *C. jejuni*, compared with the effect of other 11 EO or the effect of the rosemary EO against other bacteria (Ozogul *et al.*, 2015).

3.1.7.4 Wine byproducts

Castillo and col performed the screening. *Vitis vinifera* grape extracts showed small inhibition zones when applied to the bacterial cultures (Castillo *et al.*, 2011). Nevertheless, later work showed the capacity of grape extracts to inhibit the growth of *C. jejuni*. Using the waste of wine production, grape seed, and skins of Pinot Noir variety, the MIC was 1.25 mg/mL, and when using commercial resveratrol, it was even lower, 0.313 mg/mL (Klančnik *et al.*, 2017). They also described a change in the morphology of the bacteria, from spiral to nonspiral or short bacillary forms. Also, the attachment to cells was diminished by up to 20 % when they treated the cultures with very low concentrations of extract (50 μg/mL).

3.1.8 Neisseria gonorrhoeae

Bacteria of the species *Neisseria gonorrhoeae* have a cocci-like morphology, and are Gram-negative, strictly aerobic, and facultatively intracellular. They have strict nutritional demands for their growth that hinder their growth. It presents tropism for the simple columnar epithelium in the urethra, endocervix, rectum, pharynx, and anus. It is the causal agent of gonorrhea, the second most prevalent sexually transmitted infection in the world. Over the years, *N. gonorrhoeae* has developed a wide variety of resistance mechanisms that have caused a delicate situation due to the few available antimicrobial treatment options based on ceftriaxone and azithromycin (John E. Bennett, 2020).

N. gonorrhoeae is the least tested with natural extracts from the WHO list. Besides some publications with garlic extracts, the only work with extracts we listed uses one of the main compounds found in grape and wine extracts, resveratrol.

3.1.8.1 Wine byproducts.

In 2001, Docherty et al assayed the activity of resveratrol on the growth of six pathogenic bacteria, including *N. gonorrhoeae* and *N. meningitidis*. The results obtained were promising, with an inhibition of 50 % of the growth with 25 mg/L and a total inhibition with 75 mg/L for *N. gonorrhoeae* while for *N. meningitidis* were 100 and 150 mg/L respectively (Docherty, Fu, and Tsai, 2001). Unfortunately, the research group did not pursue the investigation.

3.1.9 Salmonellae

The genus *Salmonella* is made up of intracellular motile Gram-negative bacilli belonging to the family Enterobacteriaceae. They are facultative anaerobes that are easily grown in conventional culture media. They are associated with gastrointestinal conditions and a systemic condition called typhoid fever. In both cases, the interaction of the bacteria with the intestinal mucosa is required. They are also associated with bacteremia and vascular infections. Antimicrobial treatment is usually reserved for typhoid fever, while gastroenteritis requires fluid replacement and symptomatic treatment. The use of fluoroquinolones must be carried out according to the antibiogram results due to the appearance of strains resistant to these antimicrobials (John E. Bennett, 2020).

3.1.9.1 Artemisa

Several species of *Artemisia* have been tested against *Salmonella*, with a high spectrum of results. For example, while *A. argyi* has no antibacterial effect against 17 species of *Salmonella*, while it shows strong activity against *Listeria monocytogenes* (Militello *et al.*, 2011) *A. tournefortiana* EO showed potent antibacterial activity against 20 isolated strains of *S. enteritidis* with a MIC range of 1.95-3.9, 3.9-31.2 and 3.9-31.2 μg/mL, for the hydroalcoholic, aqueous, and hexane extracts respectively (Khosravani, Soltan Dallal, and Norouzi, 2020). In a recent study, Meng-Ting Yang and col. found antibacterial activity against *S. enterica*, working with three variants of *A. argyi* and three variants of *A. indica* Willd., with MICs from 1.56 to 25 μg/mL

(Yang *et al.*, 2020). They also showed the activity of six main compounds of the EO, with the best results reached when carveol or α-elemene were used. Furthermore, carveol was suggested to play a role in bacterial membrane destruction.

3.1.9.2 oregano

Several *Origanum* species have been used against *Salmonella*, with the dried leaves or the fresh plant, for the extract preparation. In a large screening of EOs from different sources, Hammer and col found MICs of 0.5 and 0.12 % (V/V) for *O. majorana* and *O. vulgare,* respectively (Hammer, Carson, and Riley, 1999). *O. vulgare* ethanolic extract showed similar inhibition zones to gentamicin, with 18 and 19 mm of diameter, respectively, with a MIC of 78.13 μg/mL (Oniga *et al.*, 2018). Obtaining the extracts through hydrodistillation from manually harvested *O. majorana*, Ed-Dra *et al.* found inhibition zones from 15.3 to 18.5 mm working with 8 different *Salmonella* species, with MIC and MBC of 1% (V/V) for all the 8 strains (Ed-Dra *et al.*, 2020). Differences also can be found regarding the culture method of the herbs, as was shown for *O. ehrenbergii* Boiss. wild versus cultivated. The antimicrobial activity of the EOs against *Salmonella enterica* serovar *Typhimurium*, was tested, with the wild herb essential oil showing superior efficacy (Barbour *et al.*, 2014).

3.1.9.3 Thyme and rosemary

These aromatic herbs have been used to prepare EOs and tested against several *Salmonella*'s strains. They were included in the screening of Hammer *et al.*, showing similar MIC, higher than 2 % (V/V) (Hammer *et al.*, 1999). Similar results were shown for rosemary, with a MIC of 2 % (V/V) and moderate activity, with inhibition zones ranging from 8.1 to 10.3 mm (Ed-Dra *et al.*, 2020). When they are used in parallel, there is always higher activity in the thyme EO, independently of the incubation temperatures, as was shown with four different strains of *Salmonella* (Al-Nabulsi *et al.*, 2020), with a difference of 3 times when comparing the inhibition zones. In the case of *T. hirtus* subsp. *algeriensis* Boit. and Reut., was also found to have a moderate difference in their antibacterial activity depending on their growth region in Tunisia, were the herb cultivated in Kairouan (lower altitude, minor thermal amplitude) have the lowest antibacterial activity (Fatma, Mouna, Mondher, and Ahmed, 2014). The authors found a correlation between the strongest activity of the EO and their antioxidant activity, also related to their higher content of monoterpene alcohol.

3.1.9.4 Wine byproducts

Extracts from seeds, skins, and grape pomace have been tested for their capacity for *Salmonella* growth inhibition. Working with grape skin extract obtained from red grapes *Vitis labrusca* L. (Isabel varietal), Aiub *et al.* demonstrated a variable activity over different strains when the extract was pre-incubated with *S. typhimurium* strains TA97, TA98, TA100, and TA102, with a survival up to 32 % using 10 μg/ml over TA102 strain (Aiub *et al.*, 2004). A major concern is the putative genotoxicity that those compounds can induce; in the study, they showed no mutagenic effect over *Salmonella* strains or Balb/c 3T3 fibroblasts (Aiub *et al.*, 2004). Using skin and seed extract, the antibacterial effect over *Salmonella poona* was dose-dependent, being more efficient with the Gram-positive *Bacillus cereus* (Serra *et al.*, 2008). The authors also studied olive extracts, which did not affect *S. poona*. Starting from the same material, seeds, and skins of black grapes, Lluis *et al.* found a strong growth inhibition over *S. typhimurium* strains TA1537 and TA98, with a dose of 0.05 mg. In comparison, a dose of 5 mg was less effective in TA100 (Lluís *et al.*, 2011), highlighting that different strains respond differently to the treatments. The same work showed that a dose of 5000 mg/Kg did not produce an acute toxic effect on rats, and there was no mutagenic effect on mammalian erythrocytes. A recent study used grape pomace extracts to test the IC_{50} over *S. enterica* subsp. *enterica*, with an IC_{50} of 0.752 % (V/V), showing that grape marc was even richer in bioactive compounds than skin extracts compared with previous works. In a comparative study, the seeds extract of three different grape varieties, Hasandede, Emir, and Kalecik Karasi, were tested using agar diffusion disk technique in *S. enteritidis* and *S. typhimurium* cultures. The diameter of the halo of inhibition was higher when 10 % (V/V) was used, showing small differences among the grape varieties, ranging from 21.7 up to 27.7 mm being the most effective Kalecik Karasi for *S. typhimurium* and Emir for *S. enteritidis* (Baydar, Sagdic, Ozkan, and Cetin, 2006). By using Ohmic Heating to prepare grape pomace extracts in concentrations of 1 mg/ml on diffusion disks, a moderate halo formation was found when citric acid or methanol was used in the extraction. However, no halo was generated with water or lactic acid as solvents (Coelho *et al.*, 2021), showing the importance of the solvent used for the extraction.

3.1.9.5 Yerba mate

The extracts of yerba mate are generally

prepared from commercial presentations. Gonzalez-Gil and col. reported a high antibacterial activity against *Salmonella* when they added the extract to bacterial suspensions (Gonzalez-Gil *et al.*, 2014). They found a MIC of 7.4 mg/mL, and the extracts were active against *S. enteritidis* 13A, *S. tiphymurium* DT104, and *S. senftenberg*. Once again, the solvent used for the extraction is important in their posterior biological activity, as shown for methanolic and ethanolic extracts over *S. enteritidis* (Martin *et al.*, 2013), where the ethanolic extract produced higher inhibition zones and a lower MIC (3.13 mg/ml). The researchers related the higher activity with the highest total phenolic content obtained with the ethanolic extraction. Similar MICs were obtained from water and acetone:water extracts for different Salmonella strains, ranging from 1.56 to 6.25 mg/mL (El-Sawalhi, Fayad, Porras, Fayad, and Abdel-Massih, 2021).

3.1.10 Haemophilus influenzae

Haemophilus influenzae is an extracellular immotile Gram-negative coccobacillus. It is found mainly in the upper respiratory tract. They are aerobic and have some nutritional requirements for their development. It is related to symptoms of otitis media, community-acquired pneumonia, meningitis, exacerbations of chronic obstructive disease, and respiratory infections and can cause invasive conditions such as septicemia. In addition, beta-lactamase-producing strains can complicate treatment with ampicillin and amoxicillin (John E. Bennett, 2020).

3.1.10.1 Artemisa

For treating *H. influenzae*, researchers of China used *A. asiatica* Nakai ex Pamp. EO. Supercritical CO_2 fluid extraction technology was used to extract the essential oil from powdered leaves. The EO proved to have maximum growth inhibition against *H. influenzae*, tested by disc-diffusion method with an inhibition zone of 24.5 mm, MIC of 1.9 mg/ml, and MBC of 4.5 mg/mL, a result close to other antibiotics (e.g., streptomycin and penicillin). Furthermore, examination of the growth curve showed that the EO- treated cultures exhibited a rapid decrease, in contrast to the control, which showed a fast increase (Huang, Qian, Xu, and Huang, 2018).

Another method used to test the bactericidal effect was the leakage of macromolecules (proteins, RNA and DNA) through the cell membrane due to its damage, which was confirmed. Furthermore, the scanning electron microscope observed deformed cell morphology and shrunken cells (Huang *et al.*, 2018).

3.1.10.2 Thyme

Ács and col used six commercial EO, including *T. vulgaris*, against respiratory tract pathogens such as *H. influenzae.* Two methods tested the antibacterial activity of commercial EO: broth macrodilution test to determine MIC and MBC, and vapor phase test to measure MIC produced by the volatile compounds only (Ács *et al.*, 2018). They found a MIC and MBC of 0.11 and 0.22 mg/mL, respectively, for the EO, with a difference in the volatile compounds, where the MIC was 25 µL of EO/L of airspace volume (Ács *et al.*, 2018).

3.1.10.3 Wine byproducts

In 2008, Mayer *et al.* reported the antibacterial activity of grape seed extracts. In their work, extracts from powdered grape seeds were obtained by Aquasolv and by microwave-assisted extraction (MAE). First, the extracts were fractionated on a column, resulting in four fractions (P1-P4). Afterward, fraction P2 yielded fractions S1 and S2 after eluting with different volumes of ethanol.

The raw MAE extracts had some antibacterial activity against *H. influenzae*. Fractions P1+S1 showed moderate activity, while P3+S2 showed high activity. Fraction P3 (250 µg/ml at a dilution of 1:20) proved to be active against *H. influenzae* and other bacterial strains; this fraction contained oligomeric units of catechin and epicatechin (procyanidins). In contrast to fraction P1, which has mostly monomers, and did not prove any activity against them (Mayer *et al.*, 2008).

3.1.11 Shigella spp.

Shigella species are nonmotile intracellular Gram-negative rods. They are the causal agent of shigellosis, an invasive condition that affects the colon and rectum, producing epithelial invasion with significant fluid and electrolyte losses. The usual treatment for gastrointestinal symptoms is fluid replacement. In moderate to severe cases, antibiotic therapy can be instituted. High resistance levels to ampicillin, trimethoprim-sulfamethoxazole, and fluoroquinolones have been demonstrated (John E. Bennett, 2020).

3.1.11.1 Artemisa

Extracts from two different species of *Artemisia* have been reported as effective growth inhibitors, *A. nilagirica* and *A. dracunculus* L. In the

first case, data originated from a large screening using extracts of *A. nilagirica* prepared with different solvents and examined against 11 clinical and 4 phytopathogens causing human illness and damage to major crops. *Shigella flexneri* showed a moderate inhibition zone, from 8 to 10 mm, reaching the larger zones when the extracts were prepared with methanol or chloroform (Ahameethunisa and Hopper, 2010). When the MIC was determined, one of the less sensitive bacteria was *S. flexneri*, with a MIC from 128 to 512 μg/mL. In the second case, extracts of *A. dracunculus* showed inhibition zones over cultures of *Shigella* RSHI only when prepared with methanol but not with chloroform or acetone (Benli, Kaya, and Yigit, 2007). The researchers showed the effect on the bacteria by Electron Microscopy, finding a difference in the morphology of the treated cells, which appeared shrunken with a loss of cytoplasm and depression of the cell walls.

3.1.11.2 Oregano, rosemary, and thyme

Among the three aromatic herbs, oregano is the most investigated so far. In 2003, the three were included, among other 14 species and herbs, for testing against two strains of *S. sonnei* and two strains of *S. flexneri*. In this work, the herbs and species were not extracted but were added directly to the culture media, 1 %(W/V), before their sterilization. The three herbs inhibited the growth of all tested strains except *S. flexneri* CIP. The MIC was determined for all the herbs, 0.5% (W/V). Intriguingly, they found big differences in the inhibition capacity depending on the culture media among MH broth and MH agar (Bagamboula, Uyttendaele, and Debevere, 2003). Using a different species of oregano, *O. libanoticum* Boiss., and preparing the extract with the whole plant, Barbour *et al.* showed strong inhibition of *S. dysenteriae*, reaching 99,9% of inhibition when the disks were loaded with 20 μL of extract. Interestingly, when the disks were loaded with 10 μL of extract, the inhibition was 11.1 % (Barbour *et al.*, 2004). Another specie tested was *O. minutiflorum* O. Schwarz and P.H. Davis; their extracts were prepared with different solvents. In all cases, the extracts showed important antioxidant activity. Regarding the antibacterial activity, it was used in diffusion disks against five different bacteria, obtaining the biggest inhibition zones for *S. sonnei* RSKK 878, independently of the solvent used. When comparing solvents, the descendent order in function of their inhibition zone was *n*-hexane, acetone, ethyl acetate, ethanol, methanol, suggesting the more bioactive molecules are non-polar (Oke and Aslim, 2010). In the same sense, the lowest MIC was achieved with *n*-hexane extracts (125 μg/mL).

3.1.11.3 Wine byproducts

Radovanovic *and col.* inhibited bacterial growth using different crude commercial wines. By using wines of several varietals from Bosnia, Montenegro, Serbia, and Macedonia and adding wine samples to the culture broth, they were able to inhibit the growth of several Gram-negative and Gram-positive bacteria. Nonetheless, *S. sonnei* was the least sensitive among the Gram-negative bacteria (Radovanović *et al.*, 2017).

3.2 Discussion

Bacterial infections can be the origin of diseases with a variety of symptoms and consequences that can lead to death in humans, other animals, and plants. Currently, infectious diseases are the second leading cause of death in the world.

Although the 20th century was considered the golden age of antibiotics and helped to reduce morbidity and mortality caused by infections worldwide, the increasing antibiotic resistance of various pathogenic bacteria has changed the situation and has become necessary for the development of new antibiotics.

Among the strategies to discover new molecules effective against bacteria, using natural extracts is a promising approach to finding bioactive compounds or even an extract or essential oil as a therapeutic agent.

In this review, we have highlighted the latest advances in this field in relation to Gram-negative bacteria, which were included in a 2017 alert from WHO. There is a wide range of results, from wild plants to herbs used in gastronomy to byproducts of the wine industry, including the wine itself.

The classical and conventional methods need to be enriched with new technologies such as nanotechnology, biotechnology, and cellular and molecular biology to improve their ability to penetrate the cell wall, their activity, their targeting, and their delivery.

The natural extracts are active, sustainable, environmentally friendly, and renewable, in line with the latest trends in industrial development. Replacing classical solvents with natural deep eutectic solvents would help to reduce the environmental impact. It will be necessary to continue research, including the mechanism of action, activity at the molecular

level, toxicity towards the host cell, and clinical phases in humans.

4. CONCLUSIONS

The emergence of multidrug-resistant bacteria and the emerging and reemerging infectious diseases are important public health problems and big challenges for researchers. Multidisciplinary approaches are necessary to find a solution.

Plant extracts and plant-based compounds are effective as antibacterial, with minimal effects on the host cell. Furthermore, they are sustainable, environmentally friendly, and renewable. They even can increase the added value of industrial processes like wine and infusions elaboration, as well as juice production, using byproducts of the elaboration itself.

The natural extracts and their bioactive compounds can be used alone or in combination with traditional treatments. In all cases, they must be validated *in vitro* and *in vivo* and pass all 5 phases successfully to be used in humans. Most of the reports involved *in vitro* assays and some of them have been tested in eukaryotic cells or mammals to discard adverse effects. They sometimes achieved the patent to be used (Guglielmi, Pontecorvi, & Rotondi, 2020).

Of the bacterial strains alerted by WHO, *N. gonorrhoeae and A. baumannii* are the least tested with natural extracts. On the other side, *Salmonellae*, and *P. aeruginosa* are the most studied. Nevertheless, *P. aeruginosa* been shown to have a very low susceptibility to the natural extracts treatments; even more, there are contradictory results about this issue.

Some foodborne pathogens have been tested not just isolated *in vitro* but also in the food they normally infect, as in the case of *Salmonellae*. In those cases, the extract has an extra requirement, and it must not affect the typical flavors of the food.

Identifying new molecules with antimicrobial activity, improving their purification, and understanding the mechanism of action of natural compounds are essential for finding effective treatments for untreatable infections.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the selected references.

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5.4. Competing Interests

The authors declare that they have no competing interests.

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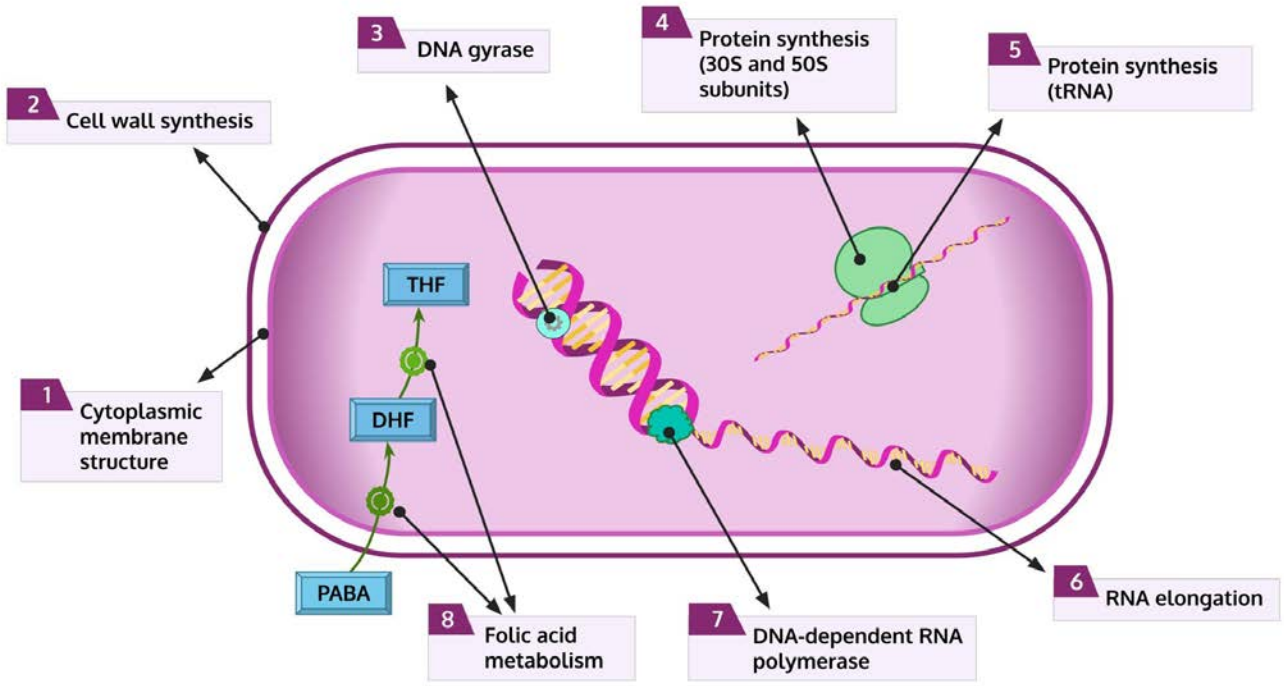

Figure 1. Action mechanisms of antibiotics

1: Changes in permeability or damage to the cytoplasmic membrane. 2: Inhibition of cell wall synthesis. 3: Inhibition of DNA gyrase. 4: Reversible inhibition of protein synthesis by subunit binding (30S or 50S). 5: Inhibition of protein synthesis by prevention of t-RNA binding to the A site. 6: Formation of a stable complex with DNA and RNA elongation prevention. 7: Inhibition of DNA-dependent RNA polymerase. 8: Inhibition of dihydropteroate synthase and dihydropteroate reductase

Figure 2. Raw material for extracts and essential oil preparation. The starting material for the extracts and EOs is, in general, stems, leaves (natural or dried leaves) and for *Vitis vinifera*, seeds, fruit skin, whole fruit, and also byproducts of the wine industry as well as the wine itself.

Table 1. Extracts and EOs used for the bacterial treatment. References: 1: artemisa, 2: jarilla, 3: oregano, 4: rosemary, 5: thyme, 6: wine, 7: yerba mate. +: used, -: not used

Bacterial strain	Extract-Essential Oil						
	1	2	3	4	5	6	7
Acinetobacter baumannii	+	-	-	-	-	-	-
Pseudomonas aeruginosa	+	+	+	+	+	+	-
Helicobacter pylori	+	+	+	+	+	+	-
Campylobacter spp	+	-	+	+	+	+	-
Neisseria gonorrhoeae	-	-	-	-	-	+	-
Salmonellae	+	-	+	+	+	+	+
Haemophilus influenzae	+	-	-	-	+	+	-
Shigella spp.	+	-	+	+	+	+	-