Biotecnia
Journal of biological and health sciences
http://biotecnia.unison.mx
Universidad de Sonora
ISSN: 1665-1456

Review

Bacterial resistance in diarrhea and tea tree oil as a potential alternative treament: a review

Resistencia bacteriana en diarrea y aceite esencial de arbol de té como potencial tratamiento: revisión

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ABSTRACT

Bacterial diarrhea is a global health concern, particularly in developing countries like Mexico, where high morbidity and mortality rates persist, especially in children under five years of age. While antibiotics like ciprofloxacin, ceftriaxone, and azithromycin are effective, increasing bacterial resistance has led to the search for alternatives. Tea tree essential oil (TTEO) has been proposed as a potential treatment, but research, especially *in vivo*, remains limited due to oil composition variability and a lack of standardized protocols. This review compiles current data (2000-2024) on the epidemiology, diagnosis, treatment, and antibiotic resistance of critical diarrhea-causing bacteria (*E. coli*, *Shigella* spp., *Campylobacter* spp., and *Salmonella* spp.) and evaluates TTEO's antibacterial potential. *In vitro* studies show its bactericidal and bacteriostatic effects, while *in vivo* studies assess its therapeutic impact on animal models. In conclusion, TTEO holds promise as an alternative or adjuvant to antibiotics for treating bacterial diarrhea. However, further *in vivo* studies are required to confirm its efficacy and optimize its clinical application.

Keywords: Diarrhea, Antibiotics, Antibiotic-resistant, Essential oils, Tea tree.

RESUMEN

La diarrea bacteriana es un problema de salud pública mundial, especialmente en países en desarrollo como México, donde persisten altas tasas de morbilidad y mortalidad, sobre todo en niños menores de cinco años. Aunque los antibióticos como la ciprofloxacina, ceftriaxona y azitromicina son efectivos, el aumento de la resistencia bacteriana nos ha forzado a buscar alternativas. El aceite esencial de árbol de té (TTEO) ha sido propuesto como tratamiento potencial, pero la investigación, especialmente *in vivo*, es limitada debido a la variabilidad en la composición del aceite y la falta de protocolos estandarizados. Esta revisión recopila datos actuales (2000-2024) sobre la epidemiología, diagnóstico, tratamiento y resistencia a antibióticos de bacterias clave que causan diarrea (*E. coli*, *Shigella* spp., *Campylobacter* spp. y *Salmonella* spp.), y evalúa el potencial antibacteriano del TTEO. Los estudios *in vitro* muestran sus efectos bactericidas y bacteriostáticos, mientras que los estudios *in vivo* evalúan su impacto terapéutico en modelos animales. En conclusión, el TTEO tiene potencial como una alternativa o complemento a los antibióticos para tratar la diarrea bacteriana, pero se necesitan más estudios *in vivo* para confirmar su eficacia y optimizar su aplicación en la práctica clínica.

Palabras clave: Diarrea, Antibióticos, Resistente a los antibióticos, Aceites esenciales, Árbol de té.

INTRODUCTION

One of the leading health problems worldwide is malnutrition and infection. Both conditions have been associated with high morbidity and mortality rates in children and adults, resulting in intestinal alterations and a negative impact on nutrient absorption (FDA, 2023). In addition, the indiscriminate or inappropriate use of antibiotics in animals and humans has contributed to the increased bacterial resistance to multiple drugs (van den Bogaard, 2000; Bouarab-Chibane, 2019). Pathogen bacteria such as *Escherichia coli*, *Shigella* spp., *Campylobacter* spp., and *Salmonella* spp. (Hirose and Sato, 2011) are on the list of the highest epidemiological surveillance worldwide, causing mild symptoms to severe diseases (WHO, 2017). Thus, they negatively impact on the host's nutritional status and are associated with alterations in the intestinal mucosa, resulting in a low absorption capacity of nutrients (Fagundes-Neto and Affonso-Scaletsky, 2000).

Natural alternatives with antibacterial properties have been sought, and they may be an appropriate adjuvant for conventional antibiotics (Calo *et al*., 2015). Essential oils (EOs) of some plants have shown antibacterial properties *in vitro*, producing inhibition percentages ranging from deficient (13 %) to very satisfactory (96 %) against some pathogenic bac-

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Received: February 27, 2023
Accepted: October 8, 2024
Published: November 7, 2024

teria (Kon and Rai, 2012; Chouhan *et al*., 2017). In addition, EO's components can exert antioxidant effects (Miguel, 2010; Yang *et al*., 2019), which may positively impact the host's nutritional status since they act as functional foods for both animals and humans (Firmino *et al*., 2020).

Search strategy

This review was conducted by retrieving data from reputable scholarly databases, including Google Scholar, PubMed, Springer, Science Direct, and Wiley Online Library. The search used keywords pertinent to the efficacy of tree tea essential oil against *Shigella*, *Salmonella*, *Campylobacter*, and *Escherichia coli*, encompassing both *in vitro* and *in vivo* evaluations at the gastrointestinal level. The inclusion criteria for selected studies encompassed publications from 2000 to 2023. Furthermore, an investigation into plant extracts' safety profile and compositional characteristics demonstrating efficacy was undertaken to provide a holistic perspective on their potential applications.

***Shigella* spp.**

Shigella is a Gram-negative bacterium from the Enterobacteriaceae family and causes approximately 125 million diarrhea cases and 160,000 deaths annually, and a third of this figure are children. One of the important clinical aspects of *Shigella* is that even a low infectious dose is associated with aggressive watery diarrhea or bloody diarrhea (Baker and Chung-The, 2018). In addition, this bacterium is the third most isolated pathogen by clinical laboratories in the United States. *Shigella* is subdivided into four major species: *Shigella dysenteriae*, *Shigella boydii*, *Shigella flexneri*, and *Shigella sonnei* (Bowen, 2018).

Shigellosis is a disease that occurs once the bacteria settle in the intestine. *Shigella* spp. can tolerate the acidic pH of the stomach, colonizing the digestive tract, generating pores in the membrane of epithelial cells, and invading their cytoplasm. *Shigella* spp. can then multiply in adjacent cells without moving through the extracellular medium (Barrantes-Jiménez and Achí-Araya, 2009). Although all *Shigella* spp. can cause bloody diarrhea, *S. dysenteriae* can produce Shiga toxin, which causes hemolytic uremic syndrome, associated with blood in the urine and, occasionally, clots in blood vessels of the kidneys (Lampel, 2012). To prevent this infection, hand washing, food care, precautions in drinking water (especially in developing countries), use of alcohol-based sanitizers, and avoiding fecal exposure in the sexual act are more often recommended (Bowen, 2018). On the other hand, special attention must be paid to fulfill those measures, particularly to children under five years old. In addition, when an infant is infected, special care must be taken so that there is no person-to-person transmission, especially if the child's diaper must be changed (CDC, 2023c).

To diagnose properly, a doctor must suspect shigellosis based on its usual symptoms such as pain, fever, and watery or bloody diarrhea. Subsequently, confirmation is carried out with serological or molecular methods (Lampel, 2012).

***Salmonella* spp.**

Salmonella is a Gram-negative bacterium belonging to the Enterobacteriaceae family. *Salmonella* strains can cause two types of illnesses: typhoid fever and non-typhoid salmonellosis. Although both cause fever, the former is more severe and has a higher mortality rate (Hammack, 2012). However, globally, non-typhoid salmonellosis is one of the four leading causes of diarrheal illness, with around 153 million cases of gastroenteritis and 57,000 deaths (Hunter and Francois-Watkins, 2018). There are 2,500 serotypes of the two main species, *Salmonella bongori*, and *Salmonella enterica*, the second being the most relevant clinically and in public health (WHO, 2019).

Most *Salmonella* strains are pathogenic due to their ability to invade and survive in host cells. When *Salmonella* enters the digestive tract, it can penetrate epithelial cells by injecting effectors through the membrane into the cytoplasm, or some adhesion systems, detected in strains such as *S. enterica* (Shu-Kee *et al*., 2015). Within these systems, adhesins, invasins, exotoxins, and endotoxins have been identified to ensure its survival in low pH. These properties give specificity to the different serotypes to adapt to the host, promoting the advancement of the disease (Jajere, 2019).

Therefore, since it has been reported that salmonellosis is the most significant foodborne infection worldwide, its prevention is a concern for public health (Jajere, 2019), so risk prevention is focused on primary production to food transportation (WHO, 2019). In addition, the usual safety measures, such as hand washing and precautions when in contact with farm or domestic animals, are recommended. On the other hand, greater caution is recommended in children under five years old, who are the most susceptible to salmonellosis (Hunter and Francois-Watkins, 2018).

Regarding diagnosis, coprological analysis is the selection technique for timely detection. Similarly, this can be detected in cultures from samples of blood, urine, abscesses, and cerebrospinal fluid (Hunter and Francois-Watkins, 2018; CDC, 2022b). In addition, in countries such as the United States, it is important that the laboratories responsible for clinical diagnosis report to the Center for Disease Control and Prevention (CDC) for proper epidemiological surveillance (CDC, 2019).

***Campylobacter* spp.**

Campylobacter is a Gram-negative, spiral S-shaped bacteria belonging to the family Campylobacteriaceae. It causes diarrhea and is one of the most prevalent enteric pathogens in developing and developed countries. In the United States, 1.3 million are infected every year (CDC, 2023a). Although the disease by this bacterium is considered mild, in populations under five years of age, it may be fatal (WHO, 2020), where *Campylobacter jejuni* is the most common agent found associated with diarrheal disease, in Argentina (30.1 %), Peru (23 %) and Colombia (14.4 %) (Fernández, 2011).

C. jejuni is competitive in the intestine of the host, taking DNA from the environment, allowing recombination between strains, and favoring its genetic diversity (Young *et al*., 2007), which allows it to adapt to adverse acidic and aerobic environments. Through flagella and union factors, *C. jejuni* can attach to epithelial cells of the intestine, colonizing the host's intestine and causing severe diarrhea, fever, and blood in the stool (Dasti *et al*., 2010).

There are no vaccines to prevent infection, but prevention is achieved by applying basic hygiene recommendations, such as hand washing, correct cooking of food, and proper treatment of contaminated water (Geissler *et al*., 2018). In addition, some organizations emphasize disease control with timely hygiene measures at all stages of the food chain, from farms to homes (WHO, 2020).

The diagnosis of *C. jejuni* is made with unique isolation and growth conditions due to its microaerophilic characteristics. The stool or rectal samples are inoculated in a selective medium, and the culture is kept at an ideal temperature (42 °C) for 72 h, with 5 % oxygen (Foley, 2012). On the other hand, microscopic visualization has been recommended, where the spiral S shape of the bacterium can be observed to establish an opportune diagnosis. A relevant issue is that laboratories analyze the sample in no more than 2 hours, taking advantage of the unique bacterial characteristics (Geissler *et al*., 2018).

Escherichia coli

E. coli is a group of Gram-negative bacilli and facultative anaerobes belonging to the Enterobacteriaceae family (Vila *et al*., 2016). *E. coli* strains are predominant in the intestinal microbiota of mammals and, specifically, in humans, showing a prevalence higher than 90 % (Denamur *et al*., 2021). At the epidemiological level, the diarrheagenic *E. coli* (DEC) subgroup is the leading cause of diarrhea, a condition that kills infants in developing countries (Hebblestrup, 2014). Around 6 DECs have been identified, including Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Shiga-toxigenic *E. coli* (STEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (O'Reilly *et al*., 2018).

Diseases associated with DEC had a 30–40 % prevalence in Asia, the Middle East Africa, Central America, South America, and Mexico, especially within children populations (Gomes *et al*., 2016; O'Reilly *et al*., 2018). In Mexico, these bacteria represent the top pathogens that cause diarrhea in children, with 30.9 %, followed by *S. enterica* (11.4 %), *Shigella* spp. (10.8 %), and *Campylobacter* spp. (5.6 %) (Rios-Muñiz *et al*., 2019). For example, EPEC has been known to cause 17 to 19% of childhood diarrhea (Vidal *et al*., 2007). On the other hand, ETEC and EAEC, which produce "traveler's diarrhea," affect both children and adults, with prevalences ranging from 5.1 to 12.2 % in Northwest Mexico. The rest of the DEC seems less frequent, with prevalences ranging from 0.2% to 1.4 % (Rios-Muñiz *et al*., 2019).

The pathogenesis of DEC depends on the pathotype and the specific strains. However, most of them mainly affect the small or large intestine, with an incubation period ranging from 8 hours to 10 days, mainly causing watery diarrhea (Gomes *et al*., 2016; O'Reilly *et al*., 2018; Rios-Muñiz *et al*., 2019). Specifically, ETEC, EIEC, EAEC, and STEC adhere to the intestinal mucosa, releasing enterotoxins and cytotoxins that promote inflammation, triggering diarrhea (Vidal *et al*., 2007; O'Reilly *et al*., 2018). In addition, EPEC adheres to the intestinal mucosa, causing a flattening of villi and inflammatory changes, leading to conformational changes and reduction of hydrolysis and absorption of nutrients (Morales-Cruz and Huerta-Romano, 2010). Finally, although little is known about its pathogenicity, DAEC is believed to form a diffuse adherence that induces protruding structures protecting its colonies and allowing disease development (O'Reilly *et al*., 2018).

Some strategies have been proposed to prevent infection by DEC, among which general hygiene in food preparation, hand washing, and food cooking (62.6 °C) stand out. In addition, it is recommended to avoid consuming foods such as raw milk and dairy products and unpasteurized juices (CDC, 2023b). This type of prevention may be impractical for humans since there are different pathogenic strains with different transmission routes. Therefore, the most widely used strategy worldwide is the epidemiological surveillance of specific strains that can contaminate food and infect humans. This strategy includes the detection of potential hazards of specific groups such as STEC, estimation of risk of food contamination, counts of viable organisms to trigger the disease, and timely diagnosis (Newell and La Ragione, 2017).

A diagnosis of EPEC can be carried out using a coprological analysis to confirm the presence of these bacteria. However, diagnostic tests that only detect a subset of STEC, known as enterohemorrhagic *E. coli* (EHEC), and recently for ETEC, are usually performed in most hospitals due to its prevalence and clinical importance (O´Reilly *et al*., 2018). In the case of EHEC, detection of specific physiological markers produced by the EHEC O157:H7 strain are performed since this is the primary bacterium of this pathotype. Similarly, some tests to detect ETEC are performed by culturing the sample in selective media, such as eosin agar and methylene blue, followed by serotyping or the use of molecular techniques for serotype identification (Morales-Cruz and Huerta-Romano, 2010; Newell and La Ragione, 2017).

Conventional treatment

The usual treatments for gastrointestinal diseases caused by these bacteria, especially diarrhea, are based on fluid and electrolyte replacement (WHO, 2020b). However, in some cases of immunocompetent patients with bloody diarrhea, patients who are immunocompromised, or in contact with another infected patient, antibiotic treatment is recommended (Shane *et al*., 2017). The prescription of these drugs varies depending on different factors, such as the patient's age or the pathogenic bacteria that causes the disease (Cohen *et* *al*., 2017). Among the most important and the first choices to treat infections by *E. coli* are azithromycin, ceftriaxone, and ciprofloxacin.

Azithromycin is a second-generation broad-spectrum macrolide antibiotic. Guidelines for treating gastrointestinal diseases, especially diarrhea, recommend this antibiotic to treat *Campylobacter*, *Shigella*, and DEC infections (Shane *et al*., 2017). Its primary mechanism of action is based on bacterial protein synthesis by interfering with their 50S ribosomal subunits (Parnham *et al*., 2014). On the other hand, effective doses vary depending on age, reaching doses of 500 mg (Cohen *et al*., 2017). However, this antibiotic is not recommended in patients with STEC, especially children, since it can cause hemolytic uremia syndrome, characterized by kidney damage, hemolytic anemia, and thrombocytopenia (Tarr *et al*., 2018).

Ceftriaxone is a third-generation cephalosporin prescribed for treating *Salmonella* and *Shigella* infections (Lamb *et al*., 2002). The main action of this antibiotic lies in its ability to inhibit the synthesis of mucopeptides in the bacterial cell wall. In addition, it has been shown that ceftriaxone establishes bonds with the enzymes responsible for cell wall synthesis and cell division, such as carboxypeptidases and endopeptidases, which cause bacterial cell death. Doses range from 20-50 mg/kg for standard treatment, and up to 80 mg/kg in the case of severe infections (Schleibinger *et al*., 2015).

Finally, ciprofloxacin is a fluoroquinolone effective against *Salmonella*, *Shigella*, and *E. coli* (Shane *et al*., 2017; Hunter and Francois-Watkins, 2018). The antimicrobial mechanism is based on inhibiting the DNA gyrase enzyme, a DNA topoisomerase involved in bacterial cell replication. It causes bacterial DNA breaks and suppresses the division of the target cell (Campoli-Richards *et al*., 1988; Ojkic*et al*., 2020). Doses range from 20 mg/kg/day in children to a maximum of 500-1000 mg in adults for 3-5 days (Shane *et al*., 2017; Cohen *et al*., 2017). However, in recent years, the loss of effectiveness of antibiotics against bacteria was reported.

Antibiotic resistance

Drug-resistant diseases currently cause 700,000 deaths per year, and estimations are that by 2050, there will be around 10 million deaths per year (WHO, 2022b). Although antibiotics are the drugs of choice for treating gastrointestinal illnesses caused by bacteria, antibiotic resistance is an increasing problem. This natural phenomenon is observed in some medications, but their indiscriminate use in humans and animals has aggravated this problem (CDC, 2023c).

It has been reported that *Shigella* spp. strains have acquired resistance to antibiotics, including ciprofloxacin (8.9 %) and ceftriaxone (9.3 %) (Puzari *et al*., 2018; Hussen *et al*., 2019). On the other hand, *Campylobacter* spp. strains have developed resistance with prevalences of 45% and 89.9 % to azithromycin and ciprofloxacin, respectively, at their usual doses (Yang *et al*., 2019). In the case of non-typhoidal *Salmonella*, a resistance increase from 12.3 % to 19.2 % in children has been reported in China within a 4-year period (Wu *et al*., 2021). Finally, different *E. coli* strains have presented resistance of up to 14.2, 9, and 20 % to ciprofloxacin, ceftriaxone, and azithromycin, respectively (Eltai *et al*., 2018; Xiang *et al*., 2020).

The WHO and CDC have developed several recommendations and strategies, among which, are the prescription of antibiotics only when necessary, and investing in the development of new antibiotics and vaccines, which are the ones that stand out (WHO, 2022e; CDC, 2023c). Recently, work has been done on developing these new strategies, among which the tea tree essential oil (TTEO) can be found. This essential oil has been proven effective *in vitro* and *in vivo* to inhibit the growth of some bacteria. It may be an alternative or natural adjuvant in treating human and animal bacterial infections (Chouhan *et al*., 2017).

Tea Tree essential oil as alternative treatment

Antimicrobial compounds and preservatives have delayed food spoilage (Perricone *et al*., 2015). EOs have been an alternative since ancient times, gaining relevance in recent years for their antimicrobial properties. In addition, studies have confirmed their different antioxidant and anti-inflammatory properties (Dagli *et al*., 2015). EOs are volatile secondary metabolites produced by plants and are responsible for their aromatic properties (Bakkali *et al*., 2008). In general, EOs are liquid, volatile, and soluble compounds in lipids and organic solvents, which are obtained from different parts of plants (e.g., flowers, seeds, leaves, grouts, and bark) through different extraction techniques (Aziz *et al*., 2018). Among their components stand out the terpenoid and non-terpenic compounds raised by the phenylpropanoid pathway of eugenol, cinnamaldehyde, and safrole (Dhifi *et al*., 2016). The concentration of these components depends on different factors, such as the source of extraction, geographical location, season, and maturity of the plant from which they are extracted (Dagli *et al*., 2015).

TTEO, obtained from *Melaleuca alternifolia*, has been used for almost 100 years in countries such as Australia, due to its properties as a complementary and alternative medicine (Carson *et al*., 2006). This EO contains oxygenated cyclic monoterpenes and hydrocarbons, such as terpinen-4-ol, ɣ-terpinene, α-terpinene, and 1,8-cineol (Dagli *et al*., 2015). Among them, the predominant is terpinen-4-ol (30-40%), to which most of its bioactivities are attributed (Groot and Schmidt, 2006).

TTEO has shown some *in vitro* antimicrobial properties, for example, as antifungal, especially against *Candida albicans* the leading cause of vaginal infections, with minimum inhibitory concentrations (MIC) ranging from 0.06 to 8.0 %. TTEO seems to alter the properties of the membrane and inhibit the respiration of the fungus at MICs from 0.25 % to 1.0 % (v/v) and reaching minimal bactericidal concentration (MBC) (Carson *et al*., 2006). Also, TTEO has shown some effect against viruses that cause herpes, which is better when combined with eucalyptus EO (Gavanji *et al*., 2016). On the other hand, *in vitro* studies show that TTEO reduce by 50 % of the growth of protozoa such as *Leishmania major* and *Trypanosoma brucei*, and it has inhibited all *Trichomonas vaginalis* at 300 mg/mL (Carson *et al*., 2006).

In summary, TTEO exhibits various antimicrobial properties *in vitro*, supporting its potential application in treating infections caused by various microorganisms. However, it is essential to consider that results from *in vitro* studies may not always translate directly to clinical efficacy, highlighting the need to explore these effects in animal models.

Tea tree oil against bacteria: *in vitro* and *in vivo* assays

Regarding antibacterial properties, concentrations from 0.78 to 50 mg/mL have been tested against bacteria that cause urinary tract infections. This demonstrates the TTEO *in vitro* and *in vivo* ability to reduce bacterial load (Loose *et al*., 2020). Table 1 summarizes some studies reporting their MBC and MIC *in vitro* against *E. coli*, *Shigella* spp., *Campylobacter* spp., and *Salmonella* spp. On the other hand, *in vivo* assays with TTEO (1000 mg/kg), resulted in an increased count of *Lactobacillus* colonies within the cecal contents in Partridge Shank chickens (n = 144) at 50 days of supplementation. Terpinen-4-ol, the primary constituent of TTEO, has been identified to possess selective antimicrobial properties against intestinal pathogens *in vitro*, which results in the modulation of the cecal microbiota composition through the enhanced *Lactobacillus* population because of TTEO supplementation (Qu *et al*., 2019).

Additionally, TTEO as a dietary supplement, at concentrations ranging from 50 to 150 mg/kg in broiler chicken diets, exhibited notable effects. A significant enhancement in daily weight gain (7% approximately) was observed as a reduction in both morbidity and mortality rates (Bakkali *et al*., 2008). Also, the same concentrations described above (50 mg/kg to 150 mg/kg) of TTEO supplementation resulted in an increased average daily feed intake and a tendency of daily gain in weanling piglets (n = 120) after 21 days (Aziz *et al*., 2018).

Table 1. *In vitro* effect of Tea Tree essential oil on different bacteria associated with diarrhea.
Tabla 1. Efecto *in vitro* del aceite esencial de Árbol de Té sobre diferentes bacterias asociadas a la diarrea.

Bacteria	Inhibition zone (mm)	MBC (µL/mL)	MIC (%)	Reference
Shigella spp	-	-	0.25	Harkenthal *et al*., 1999
Campylobacter spp	26.7-30	-	0.001	Kureckci *et al*., 2013
Salmonella spp	37.4	4	-	Swamy *et al*., 2013
E. coli	17.0-35.03	4	0.03-0.5	Bučková *et al*., 2018; Kureckci *et al*., 2019

MBC: Minimum Bactericidal Concentration; MIC: Minimum Inhibitory Concentration.

This study suggested that the passage of these compounds through the gastrointestinal tract might not affect the native microbiota of an organism; however, information is limited, and additional studies exploring its effects in different living organisms may provide more precise information.

On the other hand, the consumption of TTEO encapsulated with n-hexane lactulose, gum Arabic, and maltodextrin for 28 days seems to modulate the microbiota in weanling pigs (n = 144) and reduced the *E. coli* in the intestine and diarrhea episodes (Wang *et al*., 2021). However, despite the evidence about its effective activity against *Shigella*, *Salmonella*, *Campylobacter*, and *E. coli*, additional *in vivo* studies are still required to test the efficacy of a TTEO oral administration against these bacteria in infected animal models.

Some studies administer TTEO orally for other purposes in different animal models. It was observed that the consumption of 0.2 mg/kg of TTEO (60 days) added to the diet of goats (n = 24) improved intestinal immune function (Lv *et al*., 2022). Likewise, using TTEO integrated into the chickens' diet also improved immune function (Abo Ghanima *et al*., 2021). Therefore, these findings indicate that the treatment's potential effects go beyond its antimicrobial activity.

While existing studies have investigated the antibacterial properties of TTEO, a critical research gap exists in understanding its efficacy through oral administration against specific gastrointestinal pathogens in infected animal models. *In vitro* studies have demonstrated TTEO's ability to reduce bacterial load. However, a comprehensive exploration of its effectiveness *in vivo* against critical bacteria such as *Shigella*, *Salmonella*, *Campylobacter*, and *E. coli* must be explored. Current research indicates positive outcomes, such as increased *Lactobacillus* colonies in Partridge Shank chickens and notable effects on growth rates and morbidity in broiler chickens and weanling piglets when supplemented with TTEO in their diets. Nevertheless, the passage of TTEO through the gastrointestinal tract and its impact on native microbiota remains misunderstood. Additionally, despite encapsulation studies showing promise in modulating microbiota and reducing *E. coli* in weanling pigs, more research needs to be done on the oral administration of TTEO, specifically targeting other bacteria in infected animal models. Bridging this gap will provide valuable insights into the practical efficacy and safety of TTEO as an oral therapeutic intervention against gastrointestinal pathogens, informing potential applications and advancing our understanding of microbial communities' impact on different organisms.

Mechanisms to support the TTE biological activities

The mechanisms proposed to explain the TTEO effects are diverse and will depend on the doses used and the analyzed pathogen characteristics. It is suggested that modifying the cell membrane structure is most important, reducing the membrane potential (Swamy *et al*., 2016). In addition, it has been reported that TTEO can affect potassium ion channel homeostasis and interfere with glucose-dependent respiration, affecting bacterial membrane integrity, e.g., *E.*

coli (Yadav *et al*., 2016). Also, TTEO can improve intestinal development, cytokine secretion, gene expression of tight junction proteins, and Notch2 signaling to some extent, surpassing the effect of some antibiotics. The integrity of tight junctions is a crucial indicator of intestinal well-being, and any disturbance in their structure and function is often linked to intestinal stress injury. Perturbations in critical tight junction proteins, including occlusions, claudins, ZOs, and MUC2, can result in augmented intestinal permeability and compromised nutrient transport. In addition, the disruption of tight junctions has been implicated in developing inflammatory bowel disease, irritable bowel syndrome, and infectious diarrhea (Dong *et al*., 2019).

On the other hand, previous works have report the toxicity of TTEO in epithelial-like cells (Hela), at IC50 of 2.7 ± 0.07 g/L (Carson and Riley, 1995). Likewise, toxicity tests were also reported in ICR male mice using nano TTEO emulsions in agreement with the guidelines drawn up by the Organization for Economic Co-operation and Development. In this context, it was shown that TTEO nanoemulsions showed lower toxicity (oral LD_{50} 1656 mg/kg) compared to TTEO alone (oral LD_{50} 854 mg/kg). Similarly, the antibacterial activity against *Salmonella typhimurium* and *E. coli* has been tested *in vitro*, concluding that TTEO can be used as a potential oral antimicrobial agent (Wei *et al*., 2021). Emulsions have been proposed as a strategy to make efficient use of TTEO. This system allows for the encapsulation and protection of bioactive compounds in droplets, with sizes ranging from micrometers to nanometers (Singh and Pulikkal, 2022).

The mechanisms underlying the effects of TTEO are multifaceted and contingent, on both the administered doses and the specific characteristics of the studied pathogen. These findings endorse TTEO's suitability as a potential oral antimicrobial agent. Moreover, the adoption of emulsions as a delivery strategy for TTEO is proposed, presenting an efficient means to encapsulate and safeguard bioactive compounds, offering potential applications in antimicrobial interventions.

Challenges in the development of treatments based on essential oils

Effective implementation of essential oil-based antibacterial treatments faces multifaceted challenges that require careful attention in development and clinical application. The variability in the composition of these oils raises questions about the identification of essential compounds, and determining optimal concentrations for consistent antibacterial activity. Furthermore, addressing technical challenges in formulation and administration, along with the need to achieve clinical acceptance, completes the picture of challenges that must be overcome to fully exploit EO's therapeutic potential in treating bacterial infections.

On the other hand, regulation of the consumption of essential oil-based products varies significantly depending on the jurisdiction and the specific purpose of the product. In general, EOs are commonly used in dietary supplements and cosmetic products, and regulations cover aspects such as labeling, allowable concentrations, and safety requirements. When used for medicinal purposes, some countries may subject these products to more rigorous regulations. Regulation on alternative therapies can be diverse, from limited to more detailed, depending on local laws. Safety and toxicity are key considerations, with regulatory agencies evaluating the safety of EO and setting limits to protect consumers. Additionally, in the marketing of dietary supplements, specific regulations regarding content and health claims may apply. Given the constant evolution of the EO industry, consumers and manufacturers should stay informed about local regulations and consult with relevant authorities to ensure regulatory compliance, empowering them with the knowledge to make informed decisions.

The acceptance of products based on EO is diverse and depends on several factors. In recent years, there has been a rise in the popularity of these products, driven by increasing attention towards natural and alternative approaches to wellness. Acceptance may be influenced by factors such as knowledge and education about the associated benefits and risks, personal experience with positive results, cultural and traditional attitudes towards natural medicine, and the general perception of effectiveness. However, acceptance may also vary depending on individual perceptions of alternative medicine and preference for more conventional approaches.

Considering EO as an oral treatment for gastrointestinal bacterial infections involves several key factors. Although *in vitro* studies suggest antimicrobial properties, the transition to clinical efficacy requires further investigation. Local regulations and safety should be considered, as some EOs can be toxic in large quantities in clinical practice. The diversity of gastrointestinal infections and the variability in efficacy against different pathogens are important to evaluate. Finally, using EOs in this context requires not just consideration but also careful evaluation and medical supervision to ensure safety, efficacy, effectiveness, and consideration of individual circumstances, thereby reassuring patients and healthcare professionals about the thoroughness of the treatment process.

CONCLUSIONS

As described, public health concerns about antibiotic-resistant bacteria are growing worldwide. So, essential oils may offer an alternative or adjuvant solution to establish new treatment strategies. Essential oils such as TTEO can potentially be used to treat gastrointestinal diseases associated with bacteria, because of their excellent antimicrobiological properties. However, the effects reported in the literature are primarily *in vitro* studies. So, further studies in animal models are required to investigate the TTEO properties.

ACKNOWLEDGMENTS

The author thanks CIAD and CONAHCYT for supporting this review.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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