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Evaluation of the Anti-Adherent Potential of the Essential Oil of *Matricaria chamomilla* L. (Asteraceae) Against Food-Contaminating Pathogenic Strains

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ABSTRACT

Foodborne illness is one of the world's most frequent public health problems. From this perspective, the present study's objective is to evaluate the antibacterial and anti-adherent activity of the essential oil against strains of *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae,* and *Staphylococcus saprophyticus*. For the assays, the slant-tube technique was used to determine the Minimum Inhibitory Adherence Concentration (MIC) to glass in the presence of 5% sucrose. The experiments were performed in duplicate. From the results obtained regarding the anti-adherent activity, it was found that the essential oil was able to inhibit the adherence of *Staphylococcus saprophyticus* at a ratio of 1:2, while the chlorhexidine digluconate 0.12% prevented adherence at a ratio of 1:8, possessing greater anti-adherent activity against *S. saprophyticus* than the tested oil. However, from the *in vitro* assay with *E. coli*, it was observed that the essential oil showed greater anti-adherent activity against this pathogen when compared to 0.12% chlorhexidine digluconate, which inhibited in the proportion of 1:2, while the essential oil of *M. chamomilla* inhibited in the proportion of 1:4. In the test performed with *Klebsiella pneumoniae*, it was observed that Oe inhibited biofilm formation at a 1:1 ratio, and no inhibition was observed with the use of chloredixin. Regarding *Enterobacter cloacae*, it was found that both *M. chamomilla* essential oil and chlorhexidine digluconate were not able to inhibit biofilm formation at the concentrations tested. In summary, the essential oil demonstrated an anti-adherent potential against foodborne strains of *Staphylococcus saprophyticus*, *Escherichia coli*, and *Klebsiella pneumoniae*.

INTRODUCTION

Foodborne diseases (FOBD) are one of the most frequent public health problems in the world. They are caused by the ingestion of water and food contaminated by etiological agents, mainly pathogenic microorganisms. According to the UN, every year, millions of people worldwide fall ill and many die after ingesting contaminated food. In the period between 2000 and 2019, 14,030 outbreaks of ATD were reported in Brazil, most of them resulting from infections caused by bacteria and their toxins (Brasil, 2019).

Foods, in general, are rich sources of nutrients, which when in conjunction with intrinsic factors, such as pH and humidity, favor bacterial proliferation, and proliferation can cause both health and economic losses for industries due to deterioration (Flores; Melo, 2015). There are more than 250 types of ATD in the world, and most of them are infections caused by bacteria and their toxins (Brasil, 2019). Such factors, along with consumer demand for natural products and microbial resistance to conventional drugs (Abad *et al.*, 2007), reinforce the need for studies regarding the *in vitro* antimicrobial activity of essential oils and their contribution to food preservation.

Among the pathogenic bacteria constantly identified in outbreaks involving foodborne diseases, *Staphylococcus* spp. and pathogens of the Enterobacteriaceae family, such as *Escherichia coli*, *Enterobacter* spp, and *Klebsiella pneumoniae* that although they integrate the normal human microbiota, the external addition of these microorganisms, usually causes disharmony in the organism, resulting in infectious pictures (Feitosa *et al.*, 2017; Melo *et al.*, 2018; Pereira *et al.*, 2017; Silva *et al.*, 2018). Both *Staphylococcus* spp. and *E. coli* are addressed in ANVISA's (2013) Manual of Basic Procedures in Clinical Microbiology for the Control of Healthcare-Related Infection, either because they can cause severe infections and/or present resistance to routinely used antimicrobials, as is the case of *Staphylococcus saprophyticus*, which is resistant to novobiocin.

The impact of antibiotic resistance (AR) is growing to dangerously high levels in all parts of the world (WHO, 2018). According to the Pan American Health Organization (PAHO, 2018), RA is currently one of the biggest global threats to health, food safety, and development. In addition, several studies have reported that the ability of these bacteria to associate in the form of biofilm, either on physical or biological surfaces, is a mechanism that contributes to increased resistance to antimicrobials/sanitizers used in clinical routines and industries (Pasternak, 2009; Rosa *et al*., 2017; Scherrer; Marcon, 2016).

The growth of biofilms in food preparation environments can facilitate food contamination and hinder the sanitation process. According to Nesse *et al.* (2014) facilities where there is food processing, the biofilm acts not only as a reservoir of potential pathogens that can favor cross-contamination between products but also as an environment for the dissemination of virulence genes, resistance, and transmissibility of etiological agents that can cause diseases to consumers.

Given the growing incidence of pathogens that are multi-resistant to conventional drugs, the importance of the search for new alternatives is emphasized. The use of natural products obtained from medicinal plants has become a viable possibility in the fight against these microorganisms, given the pharmacological properties already registered, such as antimicrobial, anti-adherent, antifungal, antioxidant, and among others (Aquinoa *et al.*, 2017; Costa *et al.*, 2017; Santos *et al.*, 2015). In this sense, due to the broad biological activity presented, these plants could become a possible source for the synthesis of new drugs.

Matricaria chamomilla L. is an herb, popularly known as chamomile and widely spread as a medicinal plant, belonging to the Asteraceae Family. Species belonging to this family are found in practically every environment in the world. It is a family that stands out for its high economic, ornamental, and medicinal importance, since it includes not only various food and ornamental species (e.g. sunflower) but also species of medicinal value, such as *M. chamomilla* (Judd, *et al* 2009).

Chamomile is a plant with roots in traditional European medicine, and today it is officially included in the pharmacopeias in several countries. The spread of its use has probably occurred due to the presence of several active ingredients, such as alpha-bisabolol, flavonoids, apigenin, and other substances of pharmacological character (Sharifi-Rad *et al.*, 2018). Studies have shown that these constituents of chamomile essential oil have several properties, such as antimicrobial activity provided by bisabolol and flavonoids, and anxiolytic and sedative properties provided by apigenin (Madrigal-Santillán *et al.*, 2014).

Under such a perspective, the present work aimed to evaluate the anti-adherent activity of *Matricaria chamomilla* essential oil on the cell viability of pathogenic food contaminating bacteria, such as *Escherichia coli*, *Staphylococcus saprophyticus, Enterobacter cloacae,* and *Klebsiella pneumoniae*.

MATERIALS AND METHODS

In vitro tests

Study site

The laboratory tests were performed at the Microbiology Laboratory of the Federal University of Campina Grande, Center for Health and Rural Technology, in the city of Patos, in the sertão region of Paraiba.

Test substance

The essential oil of *Matricaria chamomilla* L. was purchased from the company QUINARÍ® (Ponta Grossa - PR). For the pharmacological assays, the substance was solubilized in DMSO and diluted in distilled water. The concentration of DMSO (dimethylsulfoxide) used was less than 0.1% v/v. The project followed the rules of CGEN - Genetic Heritage Management Council, registered in the SISGEN platform under protocol number A3DD7F.

Bacterial Species and Culture Media

In the present study, for each experiment, we used one strain of *Escherichia coli* EC46, one strain of *Staphylococcus saprophyticus* SA45, one strain of *Enterobacter cloacae* ECI41, and one strain of *Klebsiella pneumoniae* KP104. All are clinical strains, belonging to the Microbiology Laboratory of the Academic Unit of Biological Sciences, and were isolated from food contaminated with the respective bacterial species. The strains were maintained on Muller-Hinton (MH) agar at 4°C. For the experiment, together, the strains were obtained from overnight cultures in MH at 37°C and diluted in sterile saline solution to obtain the final concentration of approximately 1.5 x 108 colony forming units per mL (CFU/mL), adjusted by turbidity comparing with the 0.5 tubes of the McFarland scale (Bona *et al,* 2014).

Determination of the Minimum Inhibitory Adherence Concentration (MIC)

The Minimum Inhibitory Adherence Concentration (MIC) of the essential oil was determined in the presence of 5% sucrose, according to Albuquerque *et al.* (2009) with modifications, using corresponding concentrations of the pure essential oil up to dilution 1:1024. Both *E. coli, E. cloacae, K. pneumonia*, and *S. saprophyticus* strains were grown at 37°C in Mueller Hinton broth (DIFCO, Michigan, USA), then 0.9 ml of the subculture was dispensed into test tubes, and then 0.1 ml of the solution corresponding to the oil dilutions was added. Incubation was done at 37°C for 24 hours with tubes tilted at 30º. The reading was done by visual observation of the adherence of the bacteria to the walls of the tube, after shaking the tube and/or adding fuchsin dye to the tube wall. The assay was performed in duplicate. The same procedure was performed for the positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate Palmolive Company, New York, USA). CIMA was considered the lowest concentration of the agent in contact with sucrose which prevented the bacteria from adhering to the walls of the glass tube.

RESULTS AND DISCUSSION

From the present study, the Minimum Inhibitory Adherence Concentration (MIC) of the essential oil of *M. chamomilla* L., against *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus saprophyticus* strains, according to the different concentrations suggested in the methodology, was evidenced.

From the study performed, it was observed that the essential oil was able to inhibit the adherence of the *Escherichia coli* strain at a ratio of 1:4, while the 0.12% chlorhexidine digluconate prevented adherence at a ratio of 1:2, demonstrating that the essential oil showed greater anti-adherent activity against *E.coli* than the chlorhexidine digluconate, as can be seen in table 1.

Table No. 1: Minimum Inhibitory Adherence Concentration (MIC) in µg/mL of Matricaria chamomilla L. essential oil and 0.12% chlorhexidine digluconate, against food-contaminating Escherichia coli.

Substance / **Strain**	***Escherichia coli* EC46**
Essential oil and *Matricaria chamomilla* L.	1:4
Chlorhexidine digluconate and 0,12%	**Fonte:** Autores, 2021. 1:2

Chlorhexidine digluconate is a very powerful synthetic antiseptic, and precisely due to its high efficacy, it is usually the reference standard compound for studies that aim to elucidate the potential of possible antimicrobial substances (Gunsolley, 2006; Kluk *et al*., 2016).

In line with the present study, in an assay performed by Albuquerque *et al* (2010), it was also possible to observe an anti-adherent effect of chamomile extract, on dental biofilm microorganisms, such as the bacterial species *Streptococcus mutants* (ATCC 25175), *Streptococcus sanguinis* (ATCC 10557), and *Lactobacillus casei*, thus proving the anti-adherent potential of the plant species under study.

The anti-adherent potential of *M. chamomilla* L. essential oil against *E. coli* is promising, since chlorhexidine digluconate, which is the main chemical agent used as an adjuvant for biofilm control, may present adverse effects and was shown to be less effective than the essential oil tested.

Lima *et al.* (2021), when evaluating the anti-adherent potential of *Lavandula hybrida* Grosso oil against *Escherichia coli* strains, also presented results that corroborate with the present research, in which they elucidated the pathogen's sensitivity to this natural agent, which was more effective than chlorhexidine digluconate, as reported in this research on the essential oil of *Matricaria chamomilla*.

In a study by Santos *et al.* (2005), the presence of *K. pneumoniae* was found in 80 percent of the samples of enteral foods prepared in a homemade way. In this context, Straccialano *et al*. (2016) isolated samples of salad in nature, sold in restaurants and fast foods, not only strains of *K. pneumonia* but also *Enterobacter* sp., reinforcing the relationship between these pathogens as foodborne diseases.

According to Arnold *et al.* (2012), by synthesizing the enzyme known as *Klebsiella pneumoniae* carbapenemase (KPC), which is responsible for inactivating a large number of antimicrobial drugs, the transmissibility of strains of *K. pneumonia* through food may infer in pathologies difficult to treat through traditional antimicrobial agents, making it necessary to search for alternative therapies. In this sense, it reinforces the potential of natural products as anti-adherent agents, and as an alternative in the control and prevention of infections and biofilm formation by *Klebsiella pneumoniaee.*

Moreover, from the present study, we also observed greater anti-adherent action of *M. chamomilla* essential oil against *Klebsiella pneumoniae* strain, when compared with chlorhexidine digluconate, since the 0.12% chlorhexidine digluconate could not inhibit biofilm formation on the wall of the glass tube in the concentrations tested, while the essential oil of *M. chamomilla* inhibited at a ratio of 1:1, as shown in Table 2.

Table No. 2: Minimum Inhibitory Adherence Concentration (MICC) in µg/mL of Matricaria chamomilla L. essential oil and 0.12% chlorhexidine digluconate against Klebsiella pneumoniae, a food contaminant.

Substance / Strain	***Klebsiella pneumoniaee* KP104**
Essential oil and *Matricaria chamomilla* L.	1:1
Chlorhexidine digluconate and 0,12%	-

Source: Authors, 2022.

In line with this, when evaluating the anti-adherent potential of *Lavandula híbrida* Grosso essential oil against strains of *Klebsiella pneumoniae*, Souza et al. (2019) evidenced in their research that La vandula oil was also able to inhibit the adhesion of the microorganism to the glass tube wall.

Staphylococcus saprophyticus is a bacterium that can be transmitted by ready-to-eat foods, as is the case of *S. saprophyticus* isolated from food, and it is generally resistant to antibiotics used in clinical routine, as evidenced in the work of Freitas *et al.* (2019), in which they identified a prevalence of *S. saprophyticus* in a food matrix in 28% of the samples studied, with some isolates being multidrug-resistant. Furthermore, there are reports of *S. saprophyticus* strains in eggshells exposed in popular trade, as well as in poultry meat and sausages such as salami (Barcelos *et al*.,2017; Charmpi *et al*., 2020; Sommers *et al.* 2017).

In the present study, it was observed that *M. chamomilla* essential oil was able to inhibit the adherence of *Staphylococcus saprophyticus* at a ratio of 1:2, while 0.12% chlorhexidine digluconate prevented adherence at a ratio of 1:8, possessing greater anti-adherent activity against *Staphylococcus saprophyticus* than the tested essential oil, evidenced in Table 3.

Table No. 3: Minimum Inhibitory Adherence Concentration (MICC) in µg/mL of Matricaria chamomilla L. essential oil and 0.12% chlorhexidine digluconate, against food-contaminating Staphylococcus saprophyticus

Substance / Strain	***Staphylococcus saprophyticus* SA45**
Essential oil and *Matricaria chamomilla* L.	1:2
Chlorhexidine digluconate and 0,12%	1:8

Source: Authors, 2021.

In addition to the anti-adherent potential of the essential oil of *Matricaria chamomilla*, L. against *S. saprophyticus* evidenced in the present work, aljanaby (2018) reported in his research about the *in vitro* antibacterial activity of the aqueous extract of *Matricaria chamomilla*, the antibacterial activity of the extract against strains of *S. saprophyticus* isolated from the urinary tract of pregnant women with infection. Besides *M. chamomilla* L. other vegetables, such as the species *Coriandrum sativum*, have already shown antibacterial action in work performed by Zangeneh *et al.* (2018), on *Staphylococcus saprophyticus*. In this context, it is undeniable that the use of natural products is very promising in the fight against bacterial adherence and growth, as shown in the literature.

Another important microorganism with pathogenic potential transmitted through food is *Enterobacter cloacae*, having already been isolated from foods such as artisan cheeses, raw bovine milk, and green juices, as well as on surfaces of commercial food establishments (Alves *et al.*, 2015; Braga *et al.*, 2020; Duarte *et al*., 2020; Lázaro *et al.*, 1999). According to Mezzatesta, Gona & Stefani (2012), *E. cloacae* can be responsible for various bacteremia of the lower respiratory tract, as well as endocarditis, and other pathologies. A high frequency of resistance of the bacterium to amoxicillin, ampicillin, cefoxitin, first-generation and broad-spectrum cephalosporins was reported in a study by Davin-Regli (2015), thus reinforcing the need to search for alternative therapies to treat diseases caused by this microorganism.

Based on the proposed methodology, it was not possible to evidence anti-adherent activity of both *M. chamomilla* essential oil and 0.12% chlorhexidine digluconate for the *Enterobacter cloacae* strain ECI41food contaminant, as shown in Table 4.

Table No. 4: Minimum Inhibitory Adherence Concentration (MICC) in µg/mL of Matricaria chamomilla L. essential oil and 0.12% chlorhexidine digluconate against Enterobacter cloacae, a food contaminant.

Substance / Strain	***Enterobacter cloacae* ECI41**
Essential oil and *Matricaria chamomilla* L.	-
Chlorhexidine digluconate 0,12%	-

Source: Authors, 2022.

Pequeno *et al.* (2018) when testing the anti-adherent effect of *Matricaria chamomilla* hydroalcoholic extract in reducing biofilm of *C. Albicans* and *Enterobacter cloacae in vitro*, observed that there was a significant reduction of *Enterobacter cloacae*, differing with the results exposed in this study, since the essential oil of *M. chamomilla* essential oil was unable to inhibit the formation of *Enterobacter cloacae* biofilm on the glass tube wall, as was 0.12% cloredixindigluconate.

Among the diverse range of natural products employed in alternative therapies (e.g. aromatherapy), essential oils are described as products with great potential for therapeutic, pharmacological, and food preservative purposes (Santos *et al.*, 2018). Such potential is verified in a study conducted by Santurio *et al* (2007), who, when evaluating the antimicrobial activity of the essential oils of oregano and thyme, common herbs in Brazilian cuisine, obtained expressive antibacterial results against serovars of enteric Salmonella, a microorganism widely associated with STDs.

Studies such as that of Tavares *et al.* (2014), have already shown that it is feasible to use an edible topping plus essential oils, such as *Origanum vulgare* (Oregano), to promote an increase in the shelf life of the food, without altering its sensory characteristics, due to the antibacterial potential of the essential oil.

Santos *et al.* (2018), prepared an Edible Coating based on alginate containing OEs of *Cinnamomum cassia* and *Myristica fragrans*, and it was verified an extension in the shelf life of apples that were coated by this RC, for about 15 days, has resulted in the decrease in the browning index and reduction of 3 log cycles of *E. coli*, a pathogen associated with fecal contamination and consequently the STD's, thus demonstrating the inhibitory potential of these natural products against microbial growth that causes deterioration/contamination of food.

In this context, besides the anti-adherent potential against food contaminating bacteria evidenced in this research, the hydroalcoholic extract obtained from the plant species under study, also showed antimicrobial activity against other microorganisms, as exposed in research conducted by Haluch *et al*. (2020), which showed positive results against strains of *Candida albicans*, and is in line with the analysis performed by Sima *et al.* (2020), about the active peptide MCh-AMP1 of chamomile, in which the results showed cell death of *Candida albicans* ATCC 10231.

Thus, studies such as those mentioned above, show the antimicrobial potential of essential oils in combating microorganisms that contaminate food, which can generate both economic losses and the health of consumers, Besides ethnopharmacological research also report the popular use of medicinal plants by traditional communities in fighting diseases, as is the case of herbs such as thyme, oregano and also chamomile, reinforcing the need for studies such as those conducted in this work, for better elucidation of the antibacterial potential of chamomile essential oil (Messias *et al*. 2015; Nunes, 2019).

CONCLUSION

In summary, through the experiments performed in this study, according to the values obtained for the Minimum Inhibitory Adherence Concentration, it is concluded that the essential oil presents an anti-adherent potential against three of the four bacterial strains used in this study. However, further studies are needed to clarify the potential of *Matricaria chamomilla* L. essential oil anti-adherent against the cell viability of other bacteria of medical importance that contaminate food and are related to outbreaks of ATD.

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