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ACTIVIDAD INHIBITORIA DEL EXTRACTO DE BARBASCO (Bonellia sprucei) Y ACEITE ESENCIAL DE ANÍS ESTRELLADO (Illicium verum) COMO DESINFECTANTES PARA SUPERFICIES EN CONTACTO CON ALIMENTOS CRUDOS

INHIBITORY ACTIVITY OF BARBASCO EXTRACT (Bonellia sprucei) AND STAR ANISE ESSENTIAL OIL (Illicium verum) AS DISINFECTANTS FOR FOOD-CONTACT SURFACES

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RESUMEN: La contaminación microbiológica de superficies en contacto con alimentos representa un riesgo significativo para la inocuidad alimentaria, especialmente durante la manipulación y procesamiento de productos crudos. Es por ello que, se necesitan otras alternativas de desinfección que sean eficaces y sostenibles para reducir el uso de compuestos químicos convencionales. El objetivo principal fue realizar una evaluación de la actividad inhibitoria del extracto de barbasco (Bonellia sprucei) y del aceite esencial de anís estrellado (Illicium verum) como desinfectantes para superficies que tengan un contacto directo con los alimentos crudos. No obstante, hubo una formulación de tres tratamientos con diferentes concentraciones de extracto y aceite esencial: T1 (0,05 % – 0,95 %), T2 (0,03 % – 0,97 %) y T3 (0,01 % – 0,99 %), y se analizaron mediante pruebas de difusión en disco e hisopado de superficies frente a Escherichia coli y Staphylococcus aureus. Por otro lado, con los análisis de las propiedades físico-químicas del tratamiento con mayor capacidad antimicrobiana se procedió a comparar su eficacia con un desinfectante comercial. El resultado final evidencio que el tratamiento T3 presentó la mayor capacidad inhibitoria frente a ambos microorganismos. Y con el resultado del análisis físico-químico demostró un perfil de tensoactivos totales de 92,2 %, material activo valorable de 8,1 %, alcalinidad libre de NaOH de 0,8 % y un pH de 4,0. En la comparación con el desinfectante comercial, T3 mostró igual eficacia frente a S. aureus y menor eficacia frente a E. coli. Se concluye que la formulación T3 constituye una alternativa viable para la desinfección de superficies en contacto con alimentos, con especial efectividad frente a microorganismos gram positivos.

Palabras clave: *Barbasco, desinfectante, aceite esencial, actividad antimicrobiana, anís estrellado*

ABSTRACT: Microbiological contamination of food-contact surfaces represents a significant risk to food safety, particularly during the handling and processing of raw products. Therefore, effective and sustainable disinfection alternatives are needed to reduce the use of conventional chemical compounds. The main objective of this study

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was to evaluate the inhibitory activity of barbasco extract (Bonellia sprucei) and star anise essential oil (Illicium verum) as disinfectants for surfaces in direct contact with raw foods. Three formulations with different concentrations of extract and essential oil were developed: T1 (0.05%–0.95%), T2 (0.03%–0.97%), and T3 (0.01%–0.99%). Antimicrobial activity was assessed using disk diffusion and surface swabbing tests against Escherichia coli and Staphylococcus aureus. Additionally, the physicochemical properties of the formulation with the highest antimicrobial capacity were analyzed and its efficacy was compared with a commercial disinfectant. The results showed that treatment T3 exhibited the highest inhibitory activity against both microorganisms. Physicochemical analysis of T3 revealed a total surfactant content of 92.2%, an active matter content of 8.1%, free NaOH alkalinity of 0.8%, and a pH of 4.0. When compared with the commercial disinfectant, T3 demonstrated equivalent efficacy against S. aureus and lower efficacy against E. coli. It is concluded that formulation T3 constitutes a viable alternative for the disinfection of food-contact surfaces, with particular effectiveness against Gram-positive microorganisms.

Keywords: *Barbasco, disinfectant, essential oil, antimicrobial activity, star anise*

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INTRODUCTION

Food safety and security remain among the main risk factors for public health and for the sustainability of modern agri-food systems. One of the principal risks associated with the loss of safety is the microbiological contamination of surfaces that come into direct contact with raw foods during the stages of handling, processing, and storage. These surfaces, when not subjected to adequate cleaning and disinfection processes, can act as reservoirs of pathogenic microorganisms, favoring cross-contamination and the transmission of foodborne diseases (1,2).

The World Health Organization has estimated that foodborne diseases affect hundreds of millions of people annually worldwide, with deficiencies in hygienic-sanitary practices being one of the determining factors in their occurrence (3). In this context, bacteria such as Escherichia coli and Staphylococcus aureus are widely used as indicator microorganisms of contamination on food-contact surfaces. E. coli is mainly associated with fecal contamination and severe gastrointestinal conditions, while S. aureus stands out for its capacity to produce heat-stable toxins responsible for food intoxications, even when the food has been subjected to thermal processes (4–6).

Traditionally, the food industry has resorted to the use of synthetic chemical disinfectants, such as chlorinated compounds, quaternary ammonium compounds, and alcohols, owing to their high antimicrobial efficacy. Nevertheless, the continuous use of these agents has been related to adverse effects, among them the generation of chemical residues, negative environmental impact, corrosion of surfaces and equipment, as well as the development of microbial resistance (7,8). These limitations have driven the search for natural, safe, and sustainable alternatives that allow

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adequate levels of hygiene to be maintained without compromising human health or the environment.

In recent years, various studies have demonstrated that plant extracts and essential oils possess bioactive compounds with antimicrobial properties, among which flavonoids, saponins, phenolic compounds, and terpenes stand out. These secondary metabolites exert their action mainly through the alteration of the bacterial cell membrane, the inhibition of essential enzymes, and interference in metabolic processes fundamental for microbial survival (9–11). Owing to these characteristics, products of plant origin have aroused growing interest as potential disinfectant agents in the food industry.

Barbasco (Bonellia sprucei) is a plant native to tropical regions of South America, traditionally used by local communities for its biological properties. Previous research has reported the presence of saponins and other compounds with surfactant and biocidal activity, which suggests its possible application as an antimicrobial agent (12,13). On the other hand, star anise essential oil (Illicium verum) is widely recognized for its high content of anethole and other aromatic compounds with demonstrated antimicrobial activity against Gram-positive and Gram-negative bacteria (14,15).

Despite the scientific evidence that supports the individual antimicrobial activity of barbasco extract and of star anise essential oil, the information available on their combined use as disinfectants for surfaces in contact with raw foods is limited, especially in the Ecuadorian agro-industrial context. This knowledge gap justifies the need to evaluate alternative formulations that integrate both compounds and allow their real efficacy against indicator microorganisms of contamination to be determined.

In this sense, the general objective of the present research was to evaluate the inhibitory activity of barbasco extract (Bonellia sprucei) and of star anise essential oil (Illicium verum) as disinfectants for surfaces in contact with raw foods. As specific objectives it was proposed: to formulate different treatments with variable concentrations of both compounds, to evaluate their antimicrobial activity against Escherichia coli and Staphylococcus aureus, and to compare the efficacy of the most effective treatment with a commercial disinfectant. The hypothesis of the study establishes that the combination of barbasco extract and star anise essential oil presents significant antimicrobial activity comparable to that of a conventional chemical disinfectant.

MATERIALS AND METHODS

This study adopts an experimental approach, of quantitative type, with a descriptive and comparative scope. The study was carried out under controlled conditions according to the standards, with the objective of evaluating the antimicrobial activity of barbasco extract (Bonellia sprucei) and of star anise essential oil (Illicium verum) as disinfectants for surfaces in contact with raw foods (1,2).

For this reason, plant material was used: barbasco (Bonellia sprucei), which came from local suppliers. The plant material was subjected to a process of selection, washing with potable water for the elimination of impurities, and drying at room temperature, avoiding direct exposure to sunlight. Subsequently, the barbasco was ground and subjected to a maceration process (3) using an appropriate solvent, with the purpose of

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obtaining the extract. The resulting extract was filtered to eliminate solid residues and stored in sterile containers until its use.

Star anise essential oil (Illicium verum) was acquired from a certified supplier, guaranteeing its purity and quality. This oil was conserved in airtight containers, protected from light and at room temperature, until its employment in the formulation of the treatments (4).

For the preparation of the disinfectant, three experimental treatments were formulated, combining barbasco extract and star anise essential oil at different concentrations. Treatment T1 was formulated with 0.05 % barbasco extract and 0.95 % essential oil; treatment T2 with 0.03 % extract and 0.97 % essential oil; and treatment T3 with 0.01 % extract and 0.99 % essential oil. Each formulation was homogenized by constant agitation until uniform solutions were obtained, which were stored in properly labeled sterile containers.

The evaluation of antimicrobial activity was carried out against strains of Escherichia coli and Staphylococcus aureus, selected for their importance in food safety (5). The disk diffusion method was employed to determine the inhibitory capacity of the treatments. For this purpose, bacterial suspensions adjusted to a concentration equivalent to the McFarland standard were prepared, which were uniformly seeded onto nutrient agar plates. Sterile paper disks impregnated with each treatment were placed on the plates, including a commercial disinfectant as positive control. The plates were incubated at 37 °C for 24 hours and antimicrobial activity was evaluated by measuring the inhibition zones.

Additionally, a microbiological evaluation was performed on food-contact surfaces by the swab method. The surfaces were treated with the developed formulations and subsequently sampled to determine the microbial reduction achieved by each treatment (7). The results were expressed in colony-forming units.

The treatment that presented the highest antimicrobial activity was subjected to physicochemical analyses, evaluating parameters such as pH, free alkalinity expressed as NaOH, titratable active matter, and total surfactant content, following methodologies established in current technical standards.

The data obtained were analyzed by descriptive statistics, using means and standard deviation to compare the efficacy of the treatments and of the commercial disinfectant (8).

RESULTS

The estimation of the efficacy of the natural barbasco extract in combination with star anise essential oil as an alternative sanitizing agent for surfaces in contact with raw foods was carried out by means of a set of microbiological tests and rigorous statistical analyses. For this purpose, three experimental treatments were formulated, denominated T1, T2, and T3, which varied in the proportions of barbasco extract and star anise essential oil, keeping the remaining components of the formulation constant. This strategy made it possible to evaluate objectively the influence of the relative

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concentration of the active compounds on the antimicrobial capacity of each treatment (1,2).

Table 1. Microbiological evaluation of the different proposed treatments by disk diffusion.

Treatment	Inhibition zone (mm) *S. aureus*	Inhibition zone (mm) *E. coli*
T3	24.80C	23.00C
T2	14.00B	12.00B
T1	6.60A	5.11A
p-value	0.0001	0.0001
S.E.	0.730	0.841

The main evaluation of antimicrobial activity was carried out by means of the disk diffusion test, a technique widely used to determine the sensitivity of microorganisms to antimicrobial agents (3,4). The results obtained evidenced clear and consistent differences among the treatments evaluated. Against Staphylococcus aureus, Treatment 3 presented the highest inhibitory capacity, reaching an average inhibition zone of 24.80 mm. This value reflects a high and sustained antimicrobial action, superior to that observed in the other treatments. Treatment 2 showed an intermediate response, with an average zone of 14.00 mm, while Treatment 1 registered the lowest efficacy, with an average inhibition diameter of 6.60 mm. Analysis of variance confirmed that the differences observed among the treatments were statistically significant, with a p-value lower than 0.0001, which indicates high reliability of the results obtained.

When analyzing the response against Escherichia coli, a behavior similar to that recorded for S. aureus was observed, although with slight variations in the magnitude of the inhibition zones. Treatment 3 again stood out as the most efficacious formulation, reaching an average zone of 23.00 mm, which evidences a notable inhibitory capacity against this Gram-negative bacterium. Treatment 2 presented moderate inhibition, with an average zone of 12.00 mm, while Treatment 1 showed a limited response, with an average value of 5.11 mm. The statistical analysis again yielded a p-value of 0.0001, confirming significant differences among the treatments. The calculated standard error values for both bacteria were low, which suggests adequate experimental precision and good repeatability of the measurements performed.

The joint analysis of the results obtained by the disk diffusion test made it possible to identify a clear trend in the behavior of the formulations evaluated. An inverse relationship was evidenced between the concentration of barbasco extract and the antimicrobial efficacy of the compound disinfectant. As the proportion of barbasco decreased and the concentration of star anise essential oil increased, a significant increase in inhibitory capacity was observed, particularly in Treatment 3. This behavior suggests that the bioactive compounds present in star anise essential oil, such as anethole and other aromatic terpenes, play a predominant role in the biocidal action of the formulation. At the same time, higher concentrations of barbasco extract could be partially interfering with or antagonizing said effect in the system evaluated (6,7).

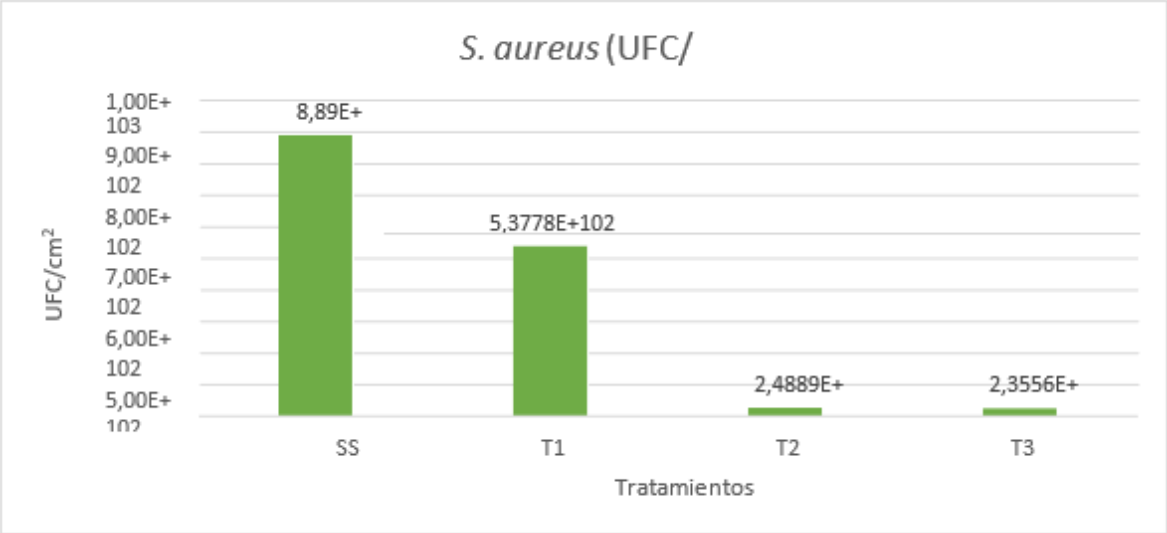
In addition to the in vitro tests, microbiological evaluations were performed by the swab method on surfaces in contact with raw foods (1), with the objective of analyzing the efficacy of the treatments under conditions closer to a real application. In the case of

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Staphylococcus aureus, the initial count on untreated surfaces was 8.89 × 10^2 CFU/cm^2, a value that reflects a significant microbial load. After application of the disinfectant treatments, a variable reduction was observed according to the formulation used. Treatment 1 managed to reduce the microbial load to 5.37 × 10^2 CFU/cm^2, evidencing limited efficacy. In contrast, Treatments 2 and 3 showed a much more pronounced reduction, reaching final values of 2.48 × 10^1 and 2.35 × 10^1 CFU/cm^2, respectively. These results confirm the high capacity of Treatment 3 to reduce the presence of S. aureus on surfaces, coinciding with what was observed in the disk diffusion test.

Figure 1. Swab analysis of the surface prior to the disk diffusion test for S. aureus.



For Escherichia coli, the initial count on untreated surfaces was 4.67 × 10^2 CFU/cm^2. After application of Treatment 1, the bacterial load was reduced moderately, reaching a value of 3.13 × 10^2 CFU/cm^2. However, Treatments 2 and 3 achieved a practically total elimination of recoverable bacteria, recording final counts of 20 CFU/cm^2 in both cases. These results are particularly relevant, since they demonstrate that, although E. coli presents greater intrinsic resistance owing to its outer membrane structure (5), the formulations with higher content of star anise essential oil were capable of reducing the microbial load to minimum detectable levels.

Once the formulation with the best antimicrobial performance had been identified, corresponding to Treatment 3, its physicochemical properties were characterized in order to verify compliance with the criteria established in the INEN 2985 Standard for surface disinfectants (8). The analysis revealed that the total surfactant content reached a value of 92.2 %, which indicates adequate detergent capacity and favors the action of the product on the treated surfaces. Likewise, the biodegradability of the surfactant was greater than 90 %, which suggests a lower environmental impact and greater compatibility with sustainability criteria. The titratable active matter was quantified at 8.1 %, remaining below the maximum permitted limit of 10 %, which guarantees the efficacy of the product without compromising user safety. Free alkalinity, expressed as sodium hydroxide (NaOH), was 0.8 %, a value below the maximum permitted limit, which indicates a low risk of corrosion and safe handling. Finally, the pH of the formulation was determined at 4.0, locating it within the range established by the

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standard and favoring the stability of the active compounds, as well as the antimicrobial activity of the product.

Table 2. Physicochemical parameters of the disinfectant treatment with the best antimicrobial response.

Parameters	Barbasco and star anise disinfectant	Parameters established by NTE INEN 2985 (2015)
Total surfactants	92.2 %	≥ 90 %
Titratable active matter	8.1 %	10 %
Free alkalinity as NaOH	0.8 %	1.0 %
pH	4.0	3.5 – 10

The final phase of the results analysis included a microbiological comparison between Treatment 3 and a commercial disinfectant of common use for food-contact surfaces. Against Staphylococcus aureus, the commercial disinfectant presented an average inhibition zone of 24.20 mm, a value very close to that obtained with the natural formulation. Statistical analysis by Student's t-test did not evidence significant differences between the two products, which indicates that Treatment 3 possesses efficacy comparable to the commercial disinfectant for the control of Gram-positive bacteria. Nevertheless, when evaluating the response against Escherichia coli, the commercial disinfectant showed a superior inhibition zone, reaching 26.60 mm, while Treatment 3 presented a lower value. The statistical analysis confirmed a significant difference in favor of the commercial product, which suggests that the natural formulation might require additional adjustments to enhance its action against Gram-negative bacteria.

Table 3. Comparative analysis between the commercial disinfectant and the best treatment.

	S. aureus	*E. coli*
t-Student	-0.866	3.674
p-value	0.7940	0.0003

Taken together, the results obtained allow the conclusion that Treatment 3 positions itself as a promising natural disinfectant, with high efficacy against Gram-positive pathogens, adequate physicochemical properties, and competitive performance versus commercial products, which supports its potential application as a sustainable alternative in the disinfection of food-contact surfaces.

DISCUSSION

The results obtained in the present research evidence that the combination of barbasco extract (Bonellia sprucei) and star anise essential oil (Illicium verum) constitutes a viable alternative for the disinfection of surfaces in contact with raw foods, especially when adequate proportions of both components are employed. The variation in the concentrations of the natural agents evaluated made it possible to demonstrate that antimicrobial efficacy does not depend solely on the presence of both compounds, but on the balance between them, which proves decisive for maximizing their biocidal action.

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In this sense, Treatment 3, characterized by a lower proportion of barbasco extract and a higher concentration of star anise essential oil, showed the highest inhibitory capacity against Staphylococcus aureus and Escherichia coli. This behavior suggests that the bioactive compounds present in the essential oil play a predominant role in the antimicrobial activity of the formulation. Various studies have indicated that metabolites such as anethole, estragole, and limonene possess a marked affinity for bacterial cell membranes owing to their lipophilic nature, which allows them to alter the permeability of the lipid bilayer, provoke the exit of essential intracellular components, and, finally, induce cell death (1–3).

A relevant aspect observed in this study is that the increase in the concentration of barbasco extract, present in Treatments 1 and 2, did not translate into greater antimicrobial activity. On the contrary, the overall efficacy of the formulation decreased. This effect could be associated with a possible antagonistic interaction between the compounds of barbasco and those of star anise essential oil when the former is found at higher concentrations, or else with a dilution effect of the more potent active principles of anise. Similar findings have been reported in investigations where the inadequate combination of plant extracts reduces the expected biocidal activity (4,5).

When contrasting these results with the scientific literature, coherence is observed with studies that report high efficacy of star anise essential oil against Gram-positive bacteria, with inhibition zones comparable to those obtained in this research (6). However, it is particularly relevant that, unlike other works where E. coli showed resistance to anise extracts, Treatment 3 achieved significant inhibition of this Gram-negative bacterium. This effect could be attributed to the high purity of the essential oil employed and to the specific synergy reached in the formulation evaluated, which allowed partial overcoming of the outer membrane barrier characteristic of this bacterial group (7).

From the physicochemical point of view, Treatment 3 presented characteristics that reinforce its potential application in the food industry. The high biodegradability of the surfactant responds to the growing need for more environmentally friendly products, constituting an advantage over conventional synthetic disinfectants, which tend to be persistent and to generate contaminating residues (8). Likewise, the acidic pH of the formulation not only complies with the limits established by current regulations, but also contributes to potentiating antimicrobial activity, given that many pathogens present limited growth under acidic conditions (9). The low free alkalinity, for its part, reduces the risk of corrosion and favors the conservation of work surfaces.

Finally, the comparison with the commercial disinfectant made it possible to contextualize the performance of the developed product. The absence of significant differences in the inhibition of S. aureus positions Treatment 3 as an effective alternative for the control of Gram-positive bacteria. Nevertheless, the greater efficacy of the commercial product against E. coli highlights the structural complexity of Gram-negative bacteria and the need to optimize natural formulations that improve the penetration of their active compounds, while at the same time maintaining an ecological and sustainable approach (10).

CONCLUSIONS

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The formulation of Treatment 3, composed of 0.01 % barbasco extract and 0.99 % star anise essential oil, proved to be the most efficacious among the alternatives evaluated, achieving significant inhibition zones of 24.80 mm against Staphylococcus aureus and 23.00 mm against Escherichia coli. This superior antimicrobial activity is attributed mainly to the high concentration of star anise essential oil, rich in compounds such as anethole and limonene that destabilize bacterial membranes. It is concluded that there exists an inverse relationship in the interaction of the components, where higher concentrations of barbasco extract, present in the other treatments, negatively affect the biocidal potency of the mixture, suggesting that efficacy rests predominantly on the active principles of star anise.

The analysis of the physicochemical properties of Treatment 3 confirmed its suitability and safety as a disinfectant agent, fully complying with the parameters established by the NTE INEN 2985 standard. The product presents a high biodegradability of 92.2 %, which reinforces its profile as a sustainable and ecological alternative. In addition, its acidic pH of 4.0 and low free alkalinity guarantee not only chemical stability and the potentiation of antimicrobial activity, but also the protection of the treated surfaces against corrosion and safety for the user, validating its potential industrial application.

When comparing the proposed natural disinfectant with a commercial product, it was determined that Treatment 3 possesses statistically equivalent efficacy against Gram-positive bacteria such as S. aureus, positioning itself as a viable and competitive alternative. However, against Gram-negative bacteria such as E. coli, the commercial disinfectant maintained statistical superiority, probably owing to synthetic ingredients designed to penetrate the complex outer membrane of these microorganisms. This indicates that, although Treatment 3 is effective, its formulation might require adjustments or additional natural adjuvants to maximize its spectrum of action against Gram-negative pathogens and to reach levels of efficacy identical to those of traditional chemical disinfectants in this specific bacterial group.

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