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Study Of The Antibacterial Activity Of Extracts And Oil Of Uvilla (Physalis Peruviana L) On Strains Of Escherichia Coli, Salmonella Spp And Arcobacter Spp In Ecuador

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ABSTRACT

Resistance of pathogenic bacteria to multiple antimicrobials has become a global threat to health and food safety. This research determined the antimicrobial activity of extracts and oils of Physalis peruviana, where it was established as study factors: extracts of parts of the plant (leaves and stem) and oils of the uvilla berry (using soxhlet); for antimicrobial activity, Escherichia, Salmonella and Arcobacter strains belonging to the microorganism bank of the State University of Bolivar were used. After the analysis, the stem extract showed a better inhibitory effect against Escherichia coli strains with a halo diameter of 14,67 mm, a value very close to that produced by streptomycin and penicillin; with respect to the Salmonella strains, the best effect was presented by the oil of the Physalis peruviana berry with 14 mm, in this case, the antibiotics for clinical use presented values greater than 20 mm. Finally, for Arcobacter the results were not encouraging, being only the extract of the leaves the one that presented activity of 3,5 mm, well below that established to be considered an antibiotic agent. Therefore, it is concluded that the analyzed uvilla extracts and oils are effective against γ proteo bacteria, but not against ε proteo bacteria.

Keywords: Antimicrobial activity, extracts, oils, Physalis peruviana, Escherichia, Salmonella, Arcobacter

1. INTRODUCTION

Food contamination results in diseases that constitute a health problem worldwide, because they are one of the main causes of death; in addition, it generates significant losses in industries (Orberá, 2014).

The first estimate of the global burden of foodborne diseases shows that almost 1 in 10 people get sick each year from eating contaminated food, 420,000 die as a result of these diseases. Africa and South-East Asia regions have the highest burden of foodborne illness (FAD) (World Health Organization, 2016; Ministerio de Salud Pública, 2015). Worldwide, around 250 causative agents of ETAs have been described, among the most prominent we find bacteria, viruses, fungi, parasites, prions and toxins. Some of these show a high incidence, such as E. coli, Salmonella spp, Staphylococcus aureus, Clostridium spp, Listeria

monocytogenes and some species of fungi, among others, which are present in different types of foods in the daily diet, which are consumed massive, especially in those of artisan production (Ruiz et al, 2017). There are studies in which the presence of bacteria such as Salmonella spp and Escherichia coli and its direct relationship with foodborne illnesses (Caffer, 2016; World Health Organization, 2019; Almenar, 2014). In the case of Arcobacter, members of this genus are not part of the intestinal flora, and humans can become infected due to the presence of this organism in foods of animal origin or in water, among other transmission routes that are not yet well identified (González y Ferrús, 2015).

Currently, many procedures and techniques are known for the control and inhibition of microorganisms, in order to preserve food, one of these is heat treatment; however, there are microorganisms that resist high temperatures. On the other hand, the addition of substances of natural origin provides sensory and microbiological quality to foods, allowing the replacement of chemical additives, which have been classified for several years as the major players in the cause of modern diseases. (Ríos y Recio, 2005). Antibiotics are considered as normally low molecular substances of natural or chemical origin, within the natural order are mainly penicillins, which are a family of widely used β-lactam antibiotics. It can be assured that it is one of the most generous groups of antibiotics, from the point of view of its efficacy and almost zero toxicity, as well as aminoglycosides (AG) such as streptomycin (Paredes, 2009). On the other hand, one of the widely used but chemical antibiotics is ciprofloxacin, which is a second-generation fluoroquinolone, although it has notable potency, its pharmacokinetics are less favorable than in third-generation ones (Sharma et al., 2017). However, nowadays, the cases of resistance of bacteria to these and other antimicrobial agents are on the rise, in such a way that several investigations are recommending the use of other agents of natural order such as extracts and vegetable oils as an alternative to deal with this problem (Bayas-Morejón et al., 2020).

Thus, essential oils are known for their phenolic compounds and are considered as possible antioxidants, antifungal and antibacterial agents (Duke, 2002). The antimicrobial activity of oils is directly related to the presence of phenolic compounds (carvacrol, thymol, eugenol, etc.) that are present in them. The oils with the highest antimicrobial activity are directly proportional to the phenolic compounds they have, although it has been observed that the trace elements are also relevant due to synergy effects to the rest of the components (Palacios y Puente, 2016; Reta, 2013).

With the knowledge of the aforementioned, the objective was to study the antimicrobial capacity of the essential oil of Physalis peruviana (uvilla) on strains of Escherichia coli, Salmonella and Arcobacter, in order to use it as a natural additive that contributes to preservation of health and life of consumers.

2. MATERIALS AND METHODS

Research location.

This research work was developed at the State University of Bolivar, Faculty of Agricultural Sciences, in the facilities of the General Laboratory.

Handling the experiment

The raw material for obtaining the extracts and oil of Physalis peruviana (uvilla) was obtained in the Cochabamba site, La Magdalena parish, canton Chimbo (Ecuador).

Obtaining extracts (stems and leaves)

To obtain the extracts, first the cultivation of uvilla Physalis peruvina seedlings was carried out, as soon as the seedlings were 50 cm high, the stems and leaves were collected and classified. The plant material was already classified, it was transferred to the General Laboratory of the State University of Bolivar, for its subsequent disinfection, then cut into pieces of between 5-10 cm, and left to macerate for 14 days in a dark glass container in a ratio of 220 mL of solvent (ethanol 98 Gl) with 50g of plant material (stems, leaves).

After this time, it was filtered using a previously sterilized funnel. Finally, the extracts were obtained, which were packaged in a glass bottle, storing them in refrigeration for later analysis.

Obtaining oil (berries)

The uvilla fruits previously collected, selected and disinfected were crushed with the help of a mortar and later placed (50 g) in the chambers for the extraction of the oil using the soxhlet method. Then the solvent (hexane) was added through the condenser, the solvent was heated, the vapors rose through the tube condensing in the refrigerant and fell into the container impregnating the solid (berries) found in the sample holder, the container goes slowly filling with liquid until it reaches the top of the tube and discharges into the balloon, this process was repeated until the oil extraction was complete.

Bacterial reactivation

In this investigation we worked with strains characterized in previous works of Arcobacter, Salmonella and E. coli conserved in the Microorganisms Bank of the Molecular Biology Laboratory of the Research and Linkage Department, State University of Bolivar.

- For Arcobacter, 10 strains of the microorganism were selected, and re-cultured on blood agar (Arcobacter broth + bacto agar + 5% sheep's blood); and incubated at 37 °C for 24-48 h under microaerophilic conditions (10% CO_2, 5% O_2, and 85% N_2).

- For Escherichia coli, 6 isolates of the microorganism were selected, cultured on nutrient agar (Nutri Agar) and allowed to incubate at 37 °C for 24 h under aerobic conditions.

- For Salmonella, 4 isolates of the microorganism were selected, cultured on XLD agar (Xylose Lysine Deoxycholate), and incubated at 37 °C for 24h under aerobic conditions.

Inoculum preparation and Antimicrobial Activity using the Kirby Bauer method (disk-plate diffusion).

From the cultures in exponential growth phase, a bacterial suspension of each strain was prepared in 10% saline water to a turbidity of 0,5 on the McFarland scale.

Subsequently, with the use of a sterile swab, the seeding was carried out in Müller Hinton Agar (MH) plates (Pronadisa, 1058.00, USA) in a homogeneous way. After a few minutes at rest, and with the help of a sterile forceps, the discs were placed on the surface of the agar. All the discs were previously immersed in each of the extracts and oil obtained in a ratio of 17 discs for each extract and oil; Likewise, Ciprofloxacin, Streptomycin and Penicillin discs were tested as control. To comply with the experimental design, the tests were carried out in duplicate. Finally, the plates were incubated under conditions of aerophilicity for Salmonella and Escherichia; and microerophilicity for Arcobacter all cases at 37ºC for 24 and 48 hours as appropriate.

After the incubation time, the diameters of the zones of inhibition of the discs were measured. The results were interpreted according to the criteria established by according to the Clinical Laboratory and Standards Institute (CLSI 2010, M45-A2).

3. Study factors

4. Table 1. Study factors for the antimicrobial analysis in Eschericha coli strains

FACTORS	LEVELS
Factor A (Extracts)	a_1: Leaves a_2: Stems a_3: Berries
Factor B (Strains)	b_1: Strain 31 b_2: Strain 34 b_3: Strain 21 b_4: Strain 43 b_5: Strain 50 b_6: Strain 6

5. Table 2. Study factors for the antimicrobial analysis in Salmonella spp.strains

FACTORES	NIVELES
Factor A (Extracts)	a_1 Leaves a_2: Stems a_3: Berries
Factor B (Strains)	b_1: Strain 42

	b_2: Strain 71 b_3: Strain 72 b_4: Strain70

6. Table 3. Study factors for the antimicrobial analysis in Arcobacter spp. strains

FACTORS	LEVELES
Factor A (Extracts)	a_1: Leaves a_2: Stems a_3: Berries
Factor B (Strain)	b_1: C Q6NC2 b_2: C Q3NC2 b_3: C Q8NC4 b_4: C Q64NC4 b_5: C Q24NC1 b_6: C Q18BC1 b_7: C Q89BC2 b_8: C Q94BM b_9: C Q49NC1 b_{10}: C Q18BC2

Nomenclature: Q6NC2 = Cheese 6, “10 de Noviembre” Market, colony 6; Q3NC2 = Cheese 3, “10 de Noviembre” Market, colony 2; Q8NC4 = Cheese 8, “10 de Noviembre” Market, colony 4; Q64NC4 = Cheese 64, “10 de Noviembre” Market, colony 4; Q24NC1 = Cheese 24, “10 de Noviembre” Market, colony 4; Q18BC1 = Cheese 18, “10 de Noviembre” Market, neighborhood 1; Q89BC2 = Cheese 89, Bellavista Market, colony 2; Q94BM = Cheese 49, Bellavista Market; Q49NC1 = Cheese 49, “10 de Noviembre” Market, neighborhood 1; Q18BC2 = Cheese 18, Bellavista Market, colony 2.

In parallel, the antibacterial effect of the extracts and oils was measured with the antibiotics for clinical use: Ciprofloxacin, Streptomycin and Penicillin.

RESULTS AND DISCUSSIONS

7. Analysis for the variable Escherichia coli

Performing the analysis, although there is no statistically significant difference, numerically the best treatment was T8 (Stem-Strain 34) of extracts * strains with 14,67 mm, we can say that the extract of this plant does act as a bactericide in relation to pathogenic microorganisms of Escherichia coli., considering that, Ponce et al. (2008), considers that for there to be such an effect there must be diameters of 9-14 mm

8. Table 4. Functional analysis (Extracts vs Strain) in Escherichia coli

Treatments	Extracts	Strain	Means	Ranks
T8	2,00	2,00	14,67	A
T7	2,00	1,00	14,00	A
T10	2,00	4,00	14,00	A
T1	1,00	1,00	14,00	A
T15	3,00	3,00	13,33	A
T9	2,00	3,00	13,33	A
T13	3,00	1,00	13,33	A
T11	2,00	5,00	13,00	A
T4	1,00	4,00	13,00	A
T17	3,00	5,00	12,67	A
T2	1,00	2,00	12,33	A
T3	1,00	3,00	12,33	A
T16	3,00	4,00	12,00	A
T14	3,00	2,00	12,00	A
T6	1,00	6,00	11,67	A
T12	2,00	6,00	11,67	A
T18	3,00	6,00	11,33	A
T5	1,00	5,00	11,00	A

Means with a common letter do not present significant difference (p >0,05)

When comparing the effects of the extracts with the antibiotics for clinical use, the extracts were slightly below the halos achieved with the antibiotics streptomycin and penicillin whose mean value was 16,75 mm. While Ciprofloxacin showed a better effect with a mean halo value of 22,66 mm, When analyzing the results of antibiotics with the tables of the CLSI (Clinical Laboratory and Standards Institute), the antimicrobial effect was Intermediate resistance to antibiotics (12 -24 mm).

9. Analysis for the variable Salmonella

Performing the analysis of the best treatment T9 (Berries-Strain 42) and T5 (Berries-Strain 42) both with 14 mm in diameter, when comparing with the tables according to Ponce et al. (2008), it would correspond to a degree of susceptibility because the range in the tables is 9-14 mm, therefore, the extract of this plant does act as a bactericide against Salmonella.

10. Table 5. Functional analysis (Extracts vs Strain) in Salmonella.

Treatments	Extracts	Strains	Means	Ranks
T9	3,00	1,00	14,00	A
T5	2,00	1,00	14,00	A
T12	3,00	4,00	13,67	A
T11	3,00	3,00	13,33	A
T10	3,00	2,00	13,00	A
T2	1,00	2,00	13,00	A
T3	1,00	3,00	12,67	A
T8	2,00	4,00	12,67	A
T1	1,00	1,00	12,00	A
T7	2,00	3,00	12,00	A
T4	1,00	4,00	11,67	A
T6	2,00	2,00	11,00	A

Means with a common letter do not present significant difference (p >0,05)

The inhibition results of the extracts and berries against Salmonella are well below the values obtained with antibiotics, where cipropfloxacin had a halo diameter of 31 mm, streptomycin 26,25 mm, and penicillin 25,5 mm. When these antibiotic values are compared with the tables according to the CLSI (Clinical Laboratory and Standards Institute), it corresponds to a degree of susceptibility because the range in the tables is ≥21 mm.

11. Analysis for the variable Arcobacter.

Performing the analysis of the best treatment T7 (Leaves-Strain 7) and T9 (Leaves-Strain 9) both with a value of 3,5 mm, when compared with the values according to Ponce et al (2008), it would correspond to a degree of resistance Because the range in the tables is ≤8mm, considering in this way that the extract of this plant does not act as a bactericide on Arcobacter.

12. Table 6. Functional analysis (Extracts vs Strain) in Arcobacter

Treatments	Extracts	Strains	Means	Ranks
T7	1,00	7,00	3,50	A
T9	1,00	9,00	3,50	A
T5	1,00	5,00	3,00	A B
T12	2,00	2,00	3,00	A B

T1	1,00	1,00	3,00	A B
T23	3,00	3,00	3,00	A B
T19	2,00	9,00	3,00	A B
T17	2,00	7,00	2,50	A B
T11	2,00	1,00	2,50	A B
T2	1,00	2,00	2,50	A B
T27	3,00	7,00	2,00	A B
T29	3,00	9,00	2,00	A B
T4	1,00	4,00	2,00	A B
T18	2,00	8,00	2,00	A B
T24	3,00	4,00	2,00	A B
T22	3,00	2,00	2,00	A B
T6	1,00	6,00	2,00	A B
T13	2,00	3,00	2,00	A B
T10	1,00	10,00	2,00	A B
T16	2,00	6,00	2,00	A B
T28	3,00	8,00	1,50	A B
T25	3,00	5,00	1,50	A B
T8	1,00	8,00	1,50	A B
T14	2,00	4,00	1,50	A B
T21	3,00	1,00	1,50	A B
T30	3,00	10,00	1,50	A B
T3	1,00	3,00	1,50	A B
T15	2,00	5,00	1,00	B
T20	2,00	10,00	1,00	B
T26	3,00	6,00	1,00	B

Means with a common letter do not present significant difference (p >0,05)

The extracts did not show an encouraging antimicrobial effect against Arcobacter, in fact, the results were well below those achieved by antibiotics for clinical use. In this sense, the antibiotic that presented the best inhibitory effect was cycprofloxacin with a mean value of 24,78 mm, followed by streptomycin with 13,15 mm, and penicillin with 4,83 mm. When these values are compared with the tables according to the CLSI (Clinical Laboratory and Standards Institute), it corresponds to a degree of susceptibility because the range in the tables is ≥16.

CONCLUSIONS

A great antimicrobial effect of the leaves, stems and berries of Physalis peruviana (uvilla) was evidenced against Escherichia coli and Salmonella, however, it was discouraging against Arcobacter. This suggests that the berry extracts and oils analyzed are effective against γ proteo-bacteria, but not against ε proteo-bacteria.

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AUTHORS' CONTRIBUTIONS

The author declares that this work was done by the author named in this article.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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