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Original Article

ANTIBACTERIAL ACTIVITY OF GOLDEN BERRY (*PHYSALIS PERUVIANA*) EXTRACT AGAINST *ESCHERICHIA COLI*SPP. ISOLATES FROM MEATS IN ECUADOR

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ABSTRACT

Objective: The resistance of pathogenic bacteria to multiple antimicrobials has become a real global threat in terms of food safety and public health. To Study of antibacterial activity of golden berry extracts against *E. coli* isolated from meats in Ecuador.

Methods: Two solvents (water and ethyl alcohol) were used as well as three parts of the Goldenberry (mature berries, semi-mature berries and leaves) for its transformation into extracts. The extracts obtained were used in tests of anti-*E. coli* activity, contrasting with Ampicillin and gentamicin by means of the disk difusión method.

Results: Extracts in ethyl alcohol from semi-ripe berries and leaves were more effective at inhibiting the development of *E. coli*, with A2B1 and A2B3 treatments being those with the best antibacterial effect with 35.3% and 50% respectively. These results were close to those obtained with the antibiotics under study.

Conclusion: The use of uvilla extracts could be a viable natural alternative to act against pathogens.

Keywords: Antibacterial activity, Goldenberry extracts, *E. coli*, Meats

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INTRODUCTION

An antibacterial agent is a substance that kills or inhibits the growth of bacteria by dividing into two types: bactericidal and bacteriostatic [1]. Antibiotic resistance is currently the biggest challenge for the effective treatment of infections worldwide. The continuous emergence of new strains resistant to antibiotics every day has become an important problem for public health [2, 3]. Bacteria have the genetic ability to transfer and acquire resistance to drugs, which are used as therapeutic agents [4]. Therefore, alternatives to these chemical antibiotics have become necessary. The side effect associated with the available antibiotics is also alarming. Therefore, there is considerable interest in investigating the antimicrobial effects of different types of plant extracts as possible sources of natural antimicrobials against a wide range of microorganisms.

Medicinal plants are important therapeutic agents for medicine because of their alternative to chemical agents, in addition, most plants have valuable bioactive compounds. There are investigations where fruits have been studied for the presence of bioactive compounds such as: tannins, carotenoids, polyphenols and anthocyanins [4]. Similarly, the antimicrobial activity exhibited by plant extracts against pathogenic bacteria isolated from food has been demonstrated [5]. *Physalis peruviana* L. (golden berry) is a member of the Solanaceae family, it has a characteristic golden color (fig. 1).

Fig. 1: Berries and leaves of *Physalis peruviana*

The fruits of the golden berry have a high nutritional value because they have a high content of minerals, antioxidants and vitamins [6]. These plants also have potential medicinal properties such as antibacterial, anti-inflammatory and antioxidant properties [7-9]. In addition, it is known that this plant induces apoptosis in different phases of the formation of cancer cells [9]. Therefore, interest in this vegetable has grown in many regions of the world. *Escherichia coli* is a gram-negative bacillus that does not form spores, may or may not be mobile. The organism is a facultative anaerobic and ferments simple sugars such as glucose to form lactic, acetic and formic acids [10]. *Escherichia coli* is a common inhabitant of the intestinal tract of man and warm-blooded animals. Most strains of *E. coli* are harmless and are part of the normal intestinal microflora [11]. These strains fulfill a useful function in the body by suppressing the growth of harmful bacteria and synthesizing appreciable amounts of vitamins. But some *E. coli* are pathogenic and can contaminate food, water and the environment [12]. In addition, within the species, there are 4 strains or categories that cause diarrheal diseases or diseases. These 4 categories are: enter pathogenic *E. coli*, enteroinvasive *E. coli*, enterotoxigenic *E. coli* and enter hemorrhagic *E. coli* [13]. In addition, they detect the presence of *E. coli* O157: H7 in food samples based on microbiological cultures and molecular methods [14].

It is known that there are no data on the anti-growth activity of *E. coli* from *Physalis peruviana* L. The sensitivity of the microorganism to an antimicrobial agent can be tested using the antimicrobial susceptibility test. The sensibility of the microorganism to natural extracts is related to the size of the microbial growth inhibition zone. According to the diameter of inhibition halo, microorganisms are classified in: resistant or nor sensible (d<8 mm), sensible (9 mm. d<14 mm), very sensible (14 mm. d<19 mm.) and extremely sensible (d>20 mm) [15]. Considering everything previously described, our objective was to study the anti listerial activity of *Physalisperuvina* L. extract on fruits and leaves against *Escherichia coli* spp. Strains isolated from three types of meats.

MATERIALS AND METHODS

Uvilla samples and extraction of extracts

The extracts analyzed in this study were obtained from the mature berries and leaves of *Physalis peruviana* L. purchased of different local retail shops from the city of Guaranda (Ecuador). The berries and healthy leaves were washed and triturated, in parallel also were lyophilized in a Freeze drier (Labconco Free Zone 2.5, USA), for their later processed. Fifteen grams of uvilla (berries and leaves) were put to maceration in 100 ml of 95% ethanol and 100 ml of distiller water at room temperatura for 6 d. According to the method established by Çakir*et al.* [6] for ethyl extract and Areiza *et al.* [16] for aqueous extract with so memodifications. In table 1, the treatments performed in this research are detailed where a DBCA was applied with factorial arrangement AxB: A (the type of Extract) and B (Parts of the vegetable).

Table 1: Factors combination for obtaining extracts

Code	Factors combination	
A1B1	Aqueous extract of mature golden berry fruits	[a]
A1B2	Aqueous extract of semi-mature golden berry fruits	
A1B3	Aqueous extract of golden berry tender leaves	
A2B1	Ethylextract of mature golden berry fruits	
A2B2	Ethylextract of semi-mature golden berry fruits	
A2B3	Ethylextract of golden berry tender leaves	[b]

[a]Original method of Areiza *et al.*, but did not use it to inhibit microorganisms, [b]Original method of OzgurCakir *et al.*, used to inhibit E. Coli.

All extracts obtained were supplied in the antibiogram effect test at a concentration of 100µg/disk. The extract was centrifuged while the residue was further extracted under the same conditions twice. The supernatant collected from separate extractions and were stored at 4 °C in flask samber.

Collection of the inoculum

Thirty four strains of *E. coli* were used, isolated from samples of bovinemeat (9), porcinemeat (14) and chicken (11) acquired from the market place located in the Guaranda-Ecuador city center and preserved in cryovials (Microbank ™ ProlabDiagnostics, USA) at-80 °C in the Molecular Biology Laboratory of Bolivar State University. These strains were reactivated and cultured onto Nutri Agar (Oxoidbrand, UK), incubated overnight at 37 °C. The reference strain was *E. coli* migula (ATCC 1053) was rehydrated and cultured according to the Culture Collections instructions.

Screening of antilisterial activity

The antibacterial activity of the eight extracts against *E. coli* spp. Strains was tested by the paper disk diffusion method applied by Shokeen *et al.* [17]. Colonies of fresh pure culture from each isolate and of the references strains (*E. coli* migula ATCC 1053) were suspended in physiological serum until the turbidity was adjusted to match the McFarland 0.5 standards. Bacteria from each suspensión were inoculated onto Muller Hilton Agar (7101A, Neogen, Michigan-USA) using a sterilecotton-tipped swab and the plates were left standing for 5 min.

Sterile filter paper disks (TSMX 7215, Oxoid, UK) of 6 mm diameter were applied to the agar surface and soaked with 10 ml of each extract. Sterile wáter was used as negative control. The commercially available standard antibiotics, gentamicin and ampicillin (CN C1333; AN C133, Bioanalyse, Turkey) were used as reference antibiotic controls. All assays were performed in duplicate.

Statistical analysis

Statistic alanalyses of the data were undertaken using Statgraphic Centurion VII program. The results were subjected to statistic alanalysis; differences between the eight treatments (extracts) were carried out by Tukey´s test. With a confidence level of 95%.

RESULTS AND DISCUSSION

Screening of antibacterial activity

The P-Values prove the statistical significance of the factor A, Strains and Interaction AxB since they are less than 0.05, these factors have a statistically significant effect on the volumen with a 95% level of confidence (table 2). The type of solvent directly influences the inhibitory effect of *E. coli*. After the comparison analysis of means, the inhibition ranges were of 0.06-7 mm, moreover, the treatment A2B3 (Ethyl extract of golden berry tender leaves) was the one with the best antibacterial effect, followed by the treatment A2B1 (Ethyl extract of mature golden berry fruits), both in the same group of ranges ordered (table 3). It is evident that ethyl alcohol proved to be the best solvent, due to which the bioactive components of physalis peruviana are better extracted with it. Devi *et al.* [18], determined that each solvent has different yield characteristics in the extraction process of vegetables and pointed out said characteristics' dependence on polarity and the nature of the solvents.

Table 2: Analysis of variance (ANOVA) of the obtained inhibition diameter

F. V.	SS	GL	MS	F-value	p-value
A	800.08	1	800.08	141.78	<0.0001
B	31.66	2	15.83	2.80	0.0634
Strains	716.29	33	21.71	3.85	<0.0001
A*B	504.48	2	252.24	44.70	<0.0001
Error	931.12	165	5.64		
Total	2983.63	203			

Table 3: Tukey test to compare inhibition diameter

Treatments	Medias	Ranks
A2B3	7.09	A
A2B1	5.50	A
A1B2	3.09	B
A2B2	2.68	B
A1B3	0.24	C
A1B1	0.06	C

According to Settani *et al.* (2014) [19], the sensitivity to natural agents depends on the type of isolate, this explains the existing variability. In this study extracts A2B1, A2B2 and A2B3 showed anti bacterial activity against the majority of studied strains. Ethanolic extracts showed considerably more activity tan the aqueous extract. Nair *et al.* [20]. Demonstrated that alcoholic plant extracts inhibit a greater number of pathogens than aqueous extracts. This is because alcohol retains the bioactive components of plants better [21]

Table 3: Inhibitory activity of *Physalis peruviana* L. extracts against *E. coli* spp.

		Treatments						Control	
N °	**Strains**	**A1B1**	**A1B2**	**A1B3**	**A2B1**	**A2B2**	**A2B3**	**AN**	**GM**
1	res5c1	0	6	0	10	9	12	14	12
2	res6c1	0	4	0	0	0	0	12	12
3	res10c1	0	6	0	4	0	6	16	14
4	res10c2	0	4	0	4	0	8	14	12
5	res10c3	0	0	0	5	4	4	15	14
6	res15c3	0	4	0	4	0	4	14	12
7	res19c1	0	4	0	11	7	12	18	14
8	res19c2	0	8	0	4	2	6	14	12
9	res19c3	0	4	0	10	10	10	14	12
10	res19c4	0	2	0	13	2	14	12	10
11	res20c1	0	0	0	0	0	2	14	12
12	pol12c1	0	0	2	4	2	4	15	14
13	pol12c2	0	2	0	4	4	8	16	14
14	pol12c3	0	8	0	12	4	14	14	16
15	pol13c3	0	4	0	9	2	12	12	12
16	pol15c2	0	0	0	0	0	4	12	12
17	pol17c2	0	4	0	10	0	12	14	12
18	pol19c2	0	2	0	4	0	8	14	12
19	pol19c3	0	2	0	0	0	4	16	14
20	pol20c1	0	2	0	6	5	5	16	14
21	cer1c1	0	2	0	6	4	10	14	14
22	cer1c5	0	0	0	0	0	8	12	12
23	cer9c1	0	4	0	12	2	4	12	12
24	cer9c2	0	1	2	9	4	14	12	12
25	cer9c2	0	4	0	4	2	2	14	12
26	cer12c1*	0	0	0	0	0	0	4	6
27	cer13c1	0	4	0	10	8	14	14	16
28	cer13c1	2	7	2	8	4	10	14	12
29	cer14c3	0	2	0	2	0	2	12	12
30	cer18c1	0	0	0	2	2	4	12	12
31	cer19c4	0	4	0	2	2	4	14	14
32	cer 22c1	0	2	2	6	4	8	12	12
33	cer34c1	0	4	0	2	0	2	12	14
34	cer56c1	0	4	0	10	8	10	14	14
*E. coli*migula (ATCC 1053)		0	2	0	8	10	10	12	12

Res: code to identify isolated strains of beef; pol: code to identify isolated strains of chicken; cer: code to identify isolated strains of pork, *: resistant strain.

In fact, the A2B1 (12/34) and A2B3 (17/34) extracts showed antimicrobial activity in 35.3% y 50% respectibility of the *E. coli* strains analyzed. ThetreatmentA2B2 (4/34) present inhibition in just 11.7%. This considering according to Ponce *et al.* [15], which establish that a pathogen is sensitive if the diameter of the halo is<8 mm.

These results show that within the best solvent (ethyl alcohol) the most suitable matrix for best results is the semi-mature leaves and fruits of golden berry. Moreover, it was evidenced that a strain from pork (Cer12c1) was the only one that presented resistance to the two antibiotic agents under study, as well as to all the extracts analyzed. The other strains were sensitive to both ampicillin and gentamicin with halo sizes ≥ 10 mm

In studies developed by Goztok and Zengin [8]. and Çakir *et al.* [6], evaluated the antimicrobial activity of *Physalis peruviana* L versus *Bacillusmegaterium* DSM 32, *Proteus vulgaris* FMC 1, *Klebsiellapneumoniae* FMC 66032, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DMS 50071, *Enterobacteraeregenes* CCM 2531, *Staphylococcusaureus* A950277, *Staphylococcusepidermidis* 14990, *Lactococcuslactis* ATCC 11454, *Escherichia coli* DH5-α and *Erwiniaherbicola*. Itisthefirst time in Ecuador that the antimicrobial effect of *Physalis peruviana* L. extracts against *E. coli* spp isolated from meats has been studied.

According to studies of characterization of the uvilla, the phenolic components are them ostabundant in both leaves and berries, them ost outstanding being 1-hexanol, eucalyptol and 4-terpenol [21, 7], as well as organic acids with antioxidant properties. There are studies that have demonstrated that monoterpenes are the components that act in the inhibition of microorganisms [22].

In conclusion, in this study, the ethanolic extracts acted against *E. coli* spp. Of equal effectiveness that the antibiotics of clinical use analyzed. The use of uvilla extracts could be a viable alternative to act against pathogens, and could be part of naturally occurring antimicrobials.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

All author declares no conflict of interest.

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