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Antibacterial Effects of Uvilla (*Physalis peruviana* L.) extracts against *Listeria* spp. Isolated from Meat in Ecuador

Ma. Bernarda Ruilova Cueva[1], Rosa Angelica Tigre León[1], Ma. Magdalena López[1], Angel Yanchaliquín[1], Isidro Favian Bayas Morejón[1]* and Herminia Sanaguano Salguero[2]

[1]Centro de Investigación y Desarrollo Biotecnológico, Departamento de Investigación, Universidad Estatal de Bolívar, EC-020150, Guaranda – Ecuador
[2]Centro de Investigación Ambiental, Departamento de Investigación, Universidad Estatal de Bolívar, EC-020150, Guaranda-Ecuador
**Corresponding author*

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A B S T R A C T

Listeria spp. are opportunist human pathogens that may cause listeriosis, this illness is associated to ingestion of contaminated foods. Nowadays there are various antibacterial to act against *Listeria* spp. However, this bacterium has been demonstrated resistance to several antibiotics. In this regards, the antimicrobial activity of eight *Physalis peruviana* L. extracts obtained from leaves and berries (by aqueous and ethanolic media both lyophilized and fresh state) was evaluated against *Listeria* spp. previously isolates from meats. The ethanolic extracts showed higher antimicrobial activity than the aqueous extracts, and the MFrE extract inhibited the 95% (57/60), followed by the LFrE extracts with 91.7% (55/60), LLyE with 90 % (54/60) and MLyE with 83.3% (50/60) of the analyzed strains. The activity of these extracts was similar to that shown by the antibiotics Gentamicin and Ampicillin. While the aqueous extracts MFrW and MLyW were the only ones that had acceptable antimicrobial activity. Maceration of fresh leaves and berries showed more antimicrobial activity than lyophilized ones, probably in the lyophilization process, part of the bioactive properties of the uvilla is lost.

Introduction

An antibacterial agent is a substance that kills or inhibits the growth of bacteria. Antibacterial substance can be of two types - bactericidal and bacteriostatic (Huang *et al*., 2016). Antibiotic resistance is currently the greatest challenge to effective treatment of infections globally. The continuous emergence of new antibiotic resistant strains day by day has become a major problem for patients (Bayas Morejón *et al*., 2017; Chavan and Bansode, 2015; Thu Vu *et al*., 2016). Bacteria have the genetic ability to transfer and acquire resistance to drugs, which are utilized as therapeutic agents (Khushwaha *et al*., 2012). Therefore, alternatives to these chemical antibiotics have become necessary. The side effect associated with the available antibiotics is alarming too. Hence, with the increase of microbial resistance to antibiotics, there is considerable interest in investigating the antimicrobial effects of different types of plant extracts as potential sources of natural antimicrobials against a wide range of microorganisms.

Medicinal plants are important therapeutic agents for medicine due to their alternatively to chemical products and most of plants have valuable bioactive phytochemicals compounds. Besides plants, fruits also have been studied by the researchers for the presence of bioactive compounds close related to herbs, commonly referred as phytochemicals such as tannins, carotenoids, polyphenols and anthocyanins (Khushwaha *et al*., 2012). Crude extracts from plants with a history of use in folk medicines have been screened *in vitro* for antibacterial activity by many research groups (Suzgec-Selcuk and Birteksoz, 2011).

Physalis peruviana L. (golden berry) is a member of solanaceae family. The fruits of golden berry have high nutritional value because of possessing high minerals, antioxidants and vitamins content (Cakir *et al*., 2014).

These plants have also potential medicinal properties like anti-bacterial, antiinflammatory, and antioxidant properties (Corrales-Bernal *et al*., 2015; Göztok and Zengin, 2013; Yen, *et al*., 2010). Besides this plant is known to induce apoptosis in different phases and has anti-cancer activity (Yen *et al*., 2010). Therefore, the interest in this genus has grown in many regions of the world in recent decade.

Listeria is a widely distributed bacterium in nature and commonly found in soil, sewage, dust, water and causes listeriosis in humans and animals. Listeriosis is a relatively rare food-borne illness, but can be life threatening with high fatality rates. It is mainly associated with the consumption of processed foods that require no further cooking by the consumer (Shantha and Gopal, 2014).

In Ecuador, listerisis have been associated with foods especially of animal origin and water (DNVE-Ecuador, 2016). During 2013, in Ecuador there were 163 deaths from intestinal infectious diseases, according to the Ministry of Health. Of these, 141 were due to diarrhea and gastroenteritis of presumed infectious origin (MSP, 2014).

Is of our known that there are not data about of the antilisterial activity of *Physalis peruviana* L. The sensitivity of amicroorganism towards an antimicrobial agent can be tested using the antimicrobial susceptibility test. Conventionally, antimicrobial test results are reported qualitatively and/or quantitatively. Qualitative results are often reported as susceptible (S), intermediate (I), or resistant (R) (Coyle, 2005).

Our objective was to study the antilisterial activity of *Physalis peruvina* L. extract on fruits and leaves against *Listeria* spp. strains isolated from meat.

Materials and Methods

Listeria spp. strains

Sixty strains of *Listeria* spp. were used in this study. All strains, belonging to the Department of Investigation, Centro de Investigación y Desarrollo Biotecnógico de la Universidad Estatal de Bolívar (Ecuador), were previously isolated from three types of meats (beef, pork and chicken), following the ISO method 11290e1:1996 (ISO 11290e1:1996). Bacterial strains were stored in cryovials (Microbank™ Prolab Diagnostics, USA) at -80 ºC.

The strains were reactivated and sub-cultured onto Chromogenic *Listeria* Agar (CLA, 7502A, Neogen, USA), incubated overnight at 37 ºC. The reference strain (*Listeria innocua* "ATCC 33090") was rehydrated and cultured according to the Culture Collections instructions.

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) and collected during May 2016 to January 2017.

The berries and healthy leaves were washed and triturated, in parallel also were lyophilized in a Freeze drier (Labconco FreeZone 2.5, USA), for their later processed.

Five, teen and five teen grams of uvilla (berries and leaves) were put to maceration in 100 mL of 95% ethanol and 100 mL of distiller water at room temperature for 6 d. according to the method established by Cakir *et al*. (2014) and Areiza *et al*. (2013) with some modifications. In table 1, the treatments performed in this research are detailed.

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 ºC in flasks amber.

Screening of antilisterial activity

The antibacterial activity of the eight extracts against *Listeria* spp. strains was tested by the paper disc diffusion method applied by Shokeen *et al.* (2009).

Colonies of fresh pure culture from each isolate and of the references strains (*Listeria* innocua Seeliger ATCC 33090) were suspended in Saline water 5%, until the turbidity was adjusted to match the McFarland 0.5 standards. Bacteria from each suspension were inoculated onto Muller Hilton Agar (7101A, Neogen, Michigan-USA) using a sterile cotton-tipped swab and the plates were left standing for a few minutes.

Sterile filter paper discs (TSMX 7215, Oxoid, UK) of 6 mm diameter were applied to the agar surface and soaked with 10 mL of each extract (Randazzo *et al*., 2016). Sterile water 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

Statistical analyses of the data were undertaken using Statistix IX (Tallahassee University, Florida, USA). The results were subjected to statistical analysis; differences between the eight treatments (extracts) were carried out by Tukey´s test. A probability value of less than 5% was deemed to be significant.

Results and Discussion

Screening of antibacterial activity

The antibacterial activity of *Physalis peruviana* L. extract of both solvents (ethanolic and aqueous) against *Listeria* isolates by the disk diffusion method are shown in table 2. Most of the extracts resulted statistically different ($P \leq 0.001$ and $P \leq 0.01$) in inhibiting the strains studied, with a range of 2-8 mm. According to Settani *et al*. (2014), the sensitivity to natural agents depends on the type of isolate, this explains the existing variability.

In this study extracts MFrE, LFrE, LlyE and MLyE showed antibacterial activity against the majority of studied strains. As shown in figure 1, ethanolic extracts showed considerably more activity than the aqueous extract. Nair *et al*. (2005) 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 (Yilmaztekin *et al*., 2014).

In fact the MFrE extract showed antimicrobial activity in 95% (57/60) of the *Listeria* strains analyzed, only strains Lspp 7, Lspp 24 and Lspp 47were not inhibited for this extract. Other extracts with a high number of susceptible strains were LFrE with 91.7% (55/60), LLyE with 90% (54/60) and MLyE with 83.3% (50/60).

Of the aqueous extracts, only MFrW extract had a high antibacterial effect with 73.3% (44/60) of the analyzed strains, whereas in the other aqueous extracts, the inhibition was in less than 50% of the strains.

In studies developed by Göztok and Zengin, (2013) and Çakir *et al*. (2014), evaluated the antimicrobial activity of *Physalis peruviana* L versus *Bacillus megaterium* DSM 32, *Proteus vulgaris* FMC 1, *Klebsiella pneumoniae* FMC 66032, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DMS 50071, *Enterobacter aeregenes* CCM 2531, *Staphylococcus aureus* A950277, *Staphylococcus epidermidis* 14990, *Lactococcus lactis* ATCC 11454, *Escherichia coli* DH5-α and *Erwinia herbicola*, But the authors did not evaluate the activity of the extracts against *Listeria* spp. The extracts from berries and leaves lyophilized had a lower antimicrobial effect than those extracts from fresh state berries and leaves (Figure 1). A disadvantage of the lyophilizate is that apart from being expensive, in the freeze-drying process some of the bioactive properties of the plants are lost (Otobone *et al*., 2007). The reference strain *L. innocua* (ATCC 33090) presented the same activity to the obtained extracts as the strains tested.

It is the first time that the antimicrobial effect of *Physalis peruviana* L. extracts against *Listeria* spp. has been studied. Is also the first time that the difference between leaves and berries lyophilized versus non-lyophilized berries and leaves has been studied.

According to studies of characterization of the uvilla, the phenolic components are the most abundant in both leaves and berries, the most outstanding being 1-hexanol, eucalyptol and 4-terpenol (Yilmaztekin *et al*., 2014; Corrales-Bernal *et al*., 2015), as well as organic acids with antioxidant properties (Ramadan *et al*., 2015). There are studies that have demonstrated that monoterpenes are the components that act in the inhibition of microorganisms (Ramadan *et al*., 2015, Randazzo *et al*., 2015).

Table.1 Plant´s parts and treatments of maceration for obtaining extracts

Extracts	Plant´s parts	Treatments
MFrE	Mature berries	15 gr in fresh state, maceration in ethanol
MFrW	Mature berries	15 gr in fresh state, maceration in distilled water
MLyE	Mature berries	5 gr lyophilized, maceration in ethanol
MLyW	Mature berries	5 gr lyophilized, maceration in distilled water
LFrE	Leaves	10 gr in fresh state, maceration in ethanol
LFrW	Leaves	10 gr in fresh state, maceration in distilled water
LLyE	Leaves	5 gr lyophilized, maceration in ethanol
LLyW	Leaves	5 gr lyophilized, maceration in distilled water

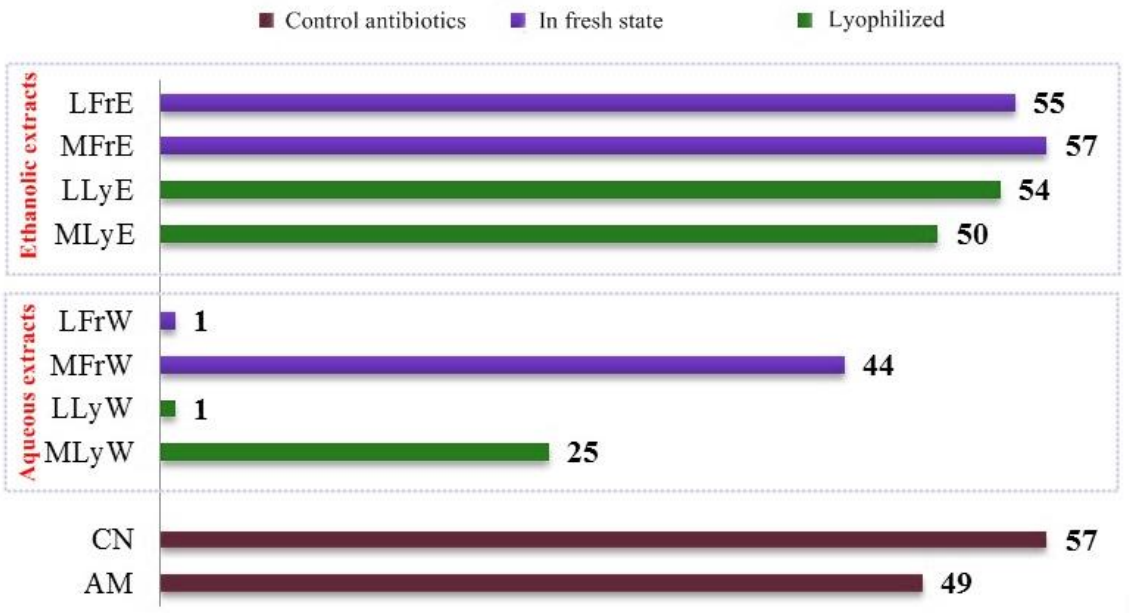
Table.2 Inhibitory activity of *Physalis peruviana* L. extracts against *Listeria* spp. isolated from meat tested by disc diffusion assay

Strain code	Extracts								Statistical significance	AM	CN	Source
	MFrE	MFrW	MLyE	MLyW	LFrE	LFrW	LLyE	LLyW				
Lspp.1	2	2	2	2	6	0	3	0	*	2	2	pork
Lspp.2	2	0	3	2	1	0	5	0	*	2	3	pork
Lspp.3	3	2	2	1	8	0	2	0	**	1	3	chicken
Lspp.4	2	0	4	3	2	0	4	0	**	0	1	chcken
Lspp.5	1	0	5	1	2	0	2	0	***	2	2	pork
Lspp.6	2	1	3	2	2	0	2	0	*	0	1	chicken
Lspp.7	0	0	0	1	5	0	5	0	***	0	2	chicken
Lspp.8	2	0	7	0	0	0	0	0	***	5	2	chicken
Lspp.9	4	0	4	0	0	0	0	0	***	0	1	chicken
Lspp.10	2	0	7	0	3	0	4	0	***	5	4	chicken
Lspp.11	4	0	1	0	0	0	1	0	**	5	5	pork
Lspp.12	5	2	0	0	2	0	2	0	**	3	6	beef
Lspp.13	3	5	0	0	0	0	0	0	***	3	7	beef
Lspp.14	1	2	0	0	0	0	0	0	Ns	2	3	beef
Lspp.15	2	2	4	0	1	0	3	0	**	9	9	pork
Lspp.16	2	2	3	1	3	0	2	0	*	1	9	chicken
Lspp.17	3	1	2	0	5	0	4	0	***	1	9	chicken
Lspp.18	5	2	2	0	4	0	4	0	***	5	12	pork
Lspp.19	2	0	1	0	4	0	2	0	***	3	10	pork
Lspp.20	2	1	1	0	4	0	3	0	**	10	10	beef
Lspp.21	3	2	3	0	2	0	2	0	**	12	12	beef
Lspp.22	2	0	2	0	4	0	2	0	**	14	14	beef
Lspp.23	1	1	2	0	4	0	3	0	**	1	1	pork
Lspp.24	0	0	0	7	2	0	2	0	***	1	2	chicken
Lspp.25	5	0	2	0	4	0	3	0	**	2	10	pork
Lspp.26	4	1	1	0	4	0	7	0	***	15	15	pork
Lspp.27	2	3	3	0	3	0	6	0	***	7	12	pork
Lspp.28	5	2	5	0	2	0	6	0	***	15	15	beef
Lspp.29	3	3	2	0	2	0	4	0	**	12	14,5	beef
Lspp.30	2	2	2	0	2	0	3	0	*	12	14	beef
Lspp.31	2	2	3	3	5	0	3	0	**	5	1	chicken
Lspp.32	3	3	2	3	4	0,5	4	0	*	5	2	chicken
Lspp.33	2	1	3	0	3	0	2	0	**	2	2	pork
Lspp.34	4	2	5	0	4	0	2	0	***	2	2	pork
Lspp.35	3	3	2	0,5	3	0	3	0	**	3	3	beef
Lspp.36	2	0	3	0	2	0	2	0	***	7	12	chicken
Lspp.37	4	2	5	0	4	0	3	0	***	5	2	beef
Lspp.38	2	1	0	3	2	0	3	0	***	0	2	chicken
Lspp.39	1	2	1	1	2	0	1	0	Ns	0	1	chicken
Lspp.40	3	2	3	0	3	0	4	0	**	10	15	beef

Lspp.41	2	3	2	1	2	0	0	0	***	3	10	chicken
Lspp.42	2	3	1	1	2	0	0	0	**	3	9	chicken
Lspp.43	2	3	0,5	0	2	0	2	0	**	4	10	chicken
Lspp.44	2	2	6	0	3	0	3	0	***	5	13	chicken
Lspp.45	2	3	0	0	2	0	2	0	***	3	10	pork
Lspp.46	1	0	0	0	4	0	4	0	***	0	4	pork
Lspp.47	0	0	2	0	3	0	3	0	***	0	0	pork
Lspp.48	1	1	1	0	5	0	4	0	***	0	0	beef
Lspp.49	3	2	0	1	1	0	1	0	**	0	8	chicken
Lspp.50	2	0	1	0	1	0	2	0	Ns	2	2	chicken
Lspp.51	4	1	2	0	3	0	5	0	***	5	10	chicken
Lspp.52	1	1	2	0	3	0	3	0	**	5	2	pork
Lspp.53	4	3	3	1	4	0	4	0	**	2	1	pork
Lspp.54	1	3	0	1	2	0	2	0	*	1	1	pork
Lspp.55	2,5	1	0,5	1	5	0	3	0	***	5	2	chicken
Lspp.56	4	3	1	2	2	0	3	0	**	5	8	chicken
Lspp.57	5	1	4	1	2	0	8	1	***	9	10	chicken
Lspp.58	1	1	2	1	3	0	4	0	***	5	1	chicken
Lspp.59	1	1	1	1	3	0	2	0	**	0	0	chicken
Lspp.60	2	1	3	1	1	0	3	0	**	5	1	chicken
ATCC 33090	3	2	3	1	2	0	3	0	***	5	4	

P value: *, P≤0.05; **, P≤0.01; ***, P≥0.001; ns, not significant. The values are expressed in mm.

Fig.1 Number of inhibited strains according to the analyzed extracts



In addition, all *Listeria* spp. Strains were studied for their susceptibility to Gentamicin (CN) and Ampicillin (AM). CN was effective against 95% of the analyzed strains, whereas AM acted against 81.7% of strains.

On the other hand, the isolated Lspp. 47, Lspp. 48 and Lspp. 59 showed resistance to the two control antibiotics studied, whereas the strains Lspp.4, Lspp. 6, Lspp. 7, Lspp. 9, Lspp. 38, Lspp. 46, Lspp. 48, Lspp. 49 were for AM only.

In conclusion, in this study the ethanolic extracts acted against *Listeria* spp. of equal or superior 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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