ANTIMICROBIAL AND ANTIOXIDANT EFFECT OF NATURAL EXTRACTS FROM LEAVES, ROOT, STEM AND FLOWERS OF BACCHARIS LATIFOLIA FROM ECUADOR

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INTRODUCTION

The continuous increase of microorganisms resistant to different antimicrobial agents has been a major problem for health and food safety, it is also known that with the appearance of antibiotics the lives of millions of human beings have been saved, however, is approaching a reality of dimensions not yet considered.

Among the most used antibiotics, are the antibiotics from the group of Fluoroquinolones [1]. On the other hand, Penicillin G, according to Flores et al [2], is an antibiotic belonging to the group of Beta-lactams, they have a mechanism of action inhibiting the cell wall of the bacteria, especially Gram+ such as Listeria, Salmonella, E. coli [3-5].

The world could be in a serious phase caused by multiple lethal bacteria resistant to antibiotics, this due as the lack of innovation and development of new antibiotics, especially of a natural origin [6]. The antibiotics derived especially from vegetables (medicinal plants), have been proven to be less toxic than synthetic agents [7].

The use of extraction techniques to obtain substances with bioactive principles is of great importance at the time of obtaining the component, even, when orthodox medicines are available, a large percentage of the population still uses herbal remedies together with conventional medicines [8, 9].

These compounds are obtained by contact with solvents through extraction techniques like maceration. The effectiveness of extraction generally depends on the polarity and nature of the solvent used [10].

Antioxidant activity of plants

Oxidative damage caused by free radicals is related to the development of various diseases such as atherosclerosis, cancer, arthritis and other inflammatory diseases [11]. The existence of synthetic substances (Butylated hydroxyanisole (BHA) or Butylated hydroxytoluene (BHT)) that are efficient scavengers of free radicals; but they are being restricted because they can be considered carcinogenic [12]. So, there is a growing interest in the search for antioxidants of natural origin, especially from plants.

In most cases, the antioxidant activity of these plants is mainly due to the presence of phenolic compounds, which are powerful oxygen scavengers and also capable of inhibiting enzymes that produce free radicals [13].

The chilca (Baccharis latifolia) (fig. 1), is one of the 46 species of Baccharis genus that is widely distributed in Ecuador, in provinces such as Pichincha, Imbabura, Cañar, Cotopaxi, Chimborazo, Bolívar, Azuay, Loja, Napo, Sucumbíos and Zamora Chinchipe [14].

**Fig. 1: Baccharis Latifolia plant**

Baccharis latifolia is commonly used in poultices to relieve external inflammations, fractures, dislocations and rheumatic
pains; in infusions, it is used as an antidiarrheal, for asthma, menstrual pains, antidiabetic and insomnia [15]. Also, *Baccharis latifolia* has been used in Latin America for medicinal purposes, such as antidiarrheal, anti-inflammatory, antidiabetic, antidepressant, analgesic and disinfectant of wounds, ulcers and antimicrobial [16].

**Objective**

The objective of the present study was to determine the antimicrobial and antioxidant activity of the extracts of *Baccharis latifolia*.

**MATERIALS AND METHODS**

The leaves, stems and roots of *Baccharis latifolia* (Bl) were collected from young plants during the months of July 2017 to July 2018 on the grounds of the Faculty of Agricultural Sciences of the Universidad Estatal de Bolivar (Ecuador). The selected plants were clean and free of damage.

**Preparation of extracts**

The selected leaves, stems and flowers were exposed to maceration during 6 d in 96% ethyl alcohol in a ratio 50 gr of the vegetal matter: 100 ml of alcohol, after this time, the extracts were obtained by centrifugation. The extracts were used as a sterile filter paper discs (Oxoid, CT0998B, United Kingdom) of 6 mm, sensitive (9 mm d<14 mm), very sensitive (14 mm d<19 mm) and extremely sensitive (d>20 mm) [19].

**Antioxidant capacity of the Baccharislatifolia extracts**

The ability of plant extracts to remove H$_2$O$_2$ can be estimated according to the method of Ruch et al. [20]. In this work a solution of H$_2$O$_2$ (40 mmol) was prepared in Potassium Phosphate Monobasic-Sodium Hydroxide buffer (50 mmol, pH 7.4) (SB108-1, Fisher

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**Table 1: Microorganisms used in the study**

| Type of meat (origin) | Sample number and selected colony | Code | Identified microorganism |
|-----------------------|-----------------------------------|------|-------------------------|
| Beef                  | Sample1-Colony 2                  | B1C2 | Listeria spp            |
| Beef                  | Sample3-Colony 2                  | B3C2 | Listeria spp            |
| Chicken               | Sample3-Colony 1                  | C3C1 | Listeria spp            |
| Chicken               | Sample4-Colony 2                  | C4C2 | Listeria spp            |
| Chicken               | Sample5-Colony 1                  | C5C1 | Listeria spp            |
| Chicken               | Sample6-Colony 1                  | C6C1 | Listeria spp            |
| Chicken               | Sample8-Colony 2                  | C8C2 | Listeria spp            |
| Chicken               | Sample14-Colony 3                 | C14C3| Listeria spp            |
| Chicken               | Sample18-Colony 1                 | C18C1| Listeria spp            |
| Pork                  | Sample19-Colony 1                 | P19C1| Listeria spp            |
| ATCC 33090            | Beef                              | B3C3 | Salmonella spp          |
| Beef                  | Sample5-Colony 1                  | B5C1 | Salmonella spp          |
| Beef                  | Sample15-Colony 3                 | B15C3| Salmonella spp          |
| Beef                  | Sample27-Colony 1                 | B27C1| Salmonella spp          |
| Chicken               | Sample2-Colony 1                  | C2C1 | Salmonella spp          |
| Chicken               | Sample13-Colony 2                 | C13C2| Salmonella spp          |
| Pork                  | Sample1-Colony 3                  | P1C3 | Salmonella spp          |
| Pork                  | Sample1-Colony 5                  | P1C5 | Salmonella spp          |
| Pork                  | Sample5-Colony 3                  | P5C3 | Salmonella spp          |
| Pork                  | Sample14-Colony 3                 | P14C3| Salmonella arizonae     |
| ATCC 13314            | Beef                              | B2C1 | Escherichia coli        |
| Beef                  | Sample2-Colony 1                  | B2C1 | Escherichia coli        |
| Beef                  | Sample3-Colony 1                  | B3C3 | Escherichia coli        |
| Beef                  | Sample3-Colony 3                  | B3C3 | Escherichia coli        |
| Beef                  | Sample5-Colony 1                  | B5C1 | Escherichia coli        |
| Chicken               | Sample2-Colony 3                  | C2C3 | Escherichia coli        |
| Chicken               | Sample3-Colony 2                  | C3C2 | Escherichia coli        |
| Pork                  | Sample2-Colony 1                  | P2C1 | Escherichia coli        |
| Pork                  | Sample4-Colony 1                  | P4C1 | Escherichia coli        |
| Pork                  | Sample6-Colony 1                  | P5C1 | Escherichia coli        |
| Pork                  | Sample14-Colony 3                 | P14C3| Escherichia coli        |
| ATCC 10536            | Beef                              | B2C1 | Escherichia coli        |

The microorganisms used in this study were previously identified by biochemical and molecular methods.

**Antimicrobial analysis**

The antibacterial activity of the four *Baccharis latifolia* extracts (Bl-E) of root, stem, leaves and flowers against *Listeria* spp, *Salmonella* spp and *Escherichia coli* strains were tested by the paper disc diffusion method applied by Shokeen et al. [17].

Colonies of fresh pure culture from each isolate and of the reference strains were suspended in the physiological saline solution until turbidity of 0.5 McFarland standard (equivalent to 1.5x10° UFC/m). Bacteria from each suspension were inoculated onto Muller Hilton Agar (MHA) (Neogen, 7101A, USA) using a sterile cotton-tipped swab and the plates were left standing for 10 min.

The sterile filter paper discs (Oxoid, CT0998B, United Kingdom) of 6 mm diameter were immersed in 10 ml of each extract for 7 min, then applied to the surface of the agar [18]. Sterile water was used as a negative control. The commercially available standard antibiotics, Penicillin G (Oxoid, CT0034B, UK) and Ciprofloxacin (Bioanalyse, 181129B, Turkey) were used as reference antibiotic controls. All assays were performed in duplicate. The sensitivity of microorganisms to natural extracts is related to the size of the microbial grown inhibition zone. According to the diameter of inhibition zone, microorganisms are classified in: resistant (d<8 mm), sensitive (9 mm d<14 mm), very sensitive (14 mm d<19 mm) and extremely sensitive (d>20 mm) [19].
Antimicrobial activity of \( \text{Table 2} \).

[22], the control strain proved susceptible to the quinolone resistance with a zone size discs, 5 isolates (B3C2, C3C1, C4C2, C5C1, C14C3), showed

The control strain was susceptible to the antibacterial with 21 sizes

For penicillin G (gr), an average of 16.9 mm in diameter was obtained, not so far from the average of our best extracts. It should be noted that this antimicrobial is specific to fight infections

The percentage of the sweep of hydrogen peroxide is calculated with the following formula:

Antioxidant capacity (% of H\(_2\)O\(_2\) sequestered) = \([A_i - A_t]/A_i\]x100.

Where: \(A_i\) = absorbance of the reference standard; \(A_t\) = Absorbance of the sample.

RESULTS AND DISCUSSION

Antimicrobial activity of Bl-E against Listeria isolates

Through the inhibition analysis, it was determined that the extract of leaves and flowers presented greater effectiveness against \(L\)isteria isolates, in fact, the size of the inhibition zone presented a mean greater than 15 mm. The third extract that showed effectiveness in the analysis was root, but with smaller zone size, as shown in fig. 2.

The isolates: B1C2, B3C2 and CH5C1 showed resistance to the root extract of Bl isolates: B5C2, CH5C1 and CH14C3 resisted the stem extract; the extracts of leaves and flowers did not show effectiveness against the isolated CH5C1 and CH14C3.

On the other hand, in the control strain (\(L\)isteria innocua, ATCC 33090) presented 23 and 22 mm in diameter of the zone in the extracts of leaves and flowers respectively, however, these two extracts did not inhibit the development of the isolate, as shown in table 2. Resistant isolates were considered to those that presented a zone size ≤8 mm in diameter [19].

![Antimicrobial effect of Bl-E against Listeria, R-E: root extract; S-E: stem extract; L-E: leave extract; F-E: flower extract](image)

**Fig. 2: Antimicrobial effect of Bl-E against Listeria**

Anti-listerial effect of Bl-E and antibiotics for clinical use

For penicillin G (gr), an average of 1.69 mm in diameter was obtained, not so far from the average of our best Bl extracts. It should be noted that this antimicrobial is specific to fight infections caused by \(L\)isteria, taking as reference the parameters established by CLSI2012, [22], it can be said that of the 10 isolates studied, 4 were resistant to this agent (B3C2, C5C1, C6C1 and C18C1) with zone sizes ≤14 mm.

Through the inhibition analysis, it was determined that the extract of leaves and flowers presented greater effectiveness against \(L\)isteria isolates, in fact, the size of the inhibition zone presented a mean greater than 15 mm. The third extract that showed effectiveness in the analysis was root, but with smaller zone size, as shown in fig. 2.

The isolates: B1C2, B3C2 and CH5C1 showed resistance to the root extract of Bl isolates: B5C2, CH5C1 and CH14C3 resisted the stem extract; the extracts of leaves and flowers did not show effectiveness against the isolated CH5C1 and CH14C3.

The control strain was susceptible to the antibacterial with 21 mm diameter. On the other hand, after applying ciprofloxacin discs, 5 isolates (B3C2, C3C1, C4C2, C5C1, C14C3), showed resistance with a zone size ≤15 mm, according to the CLSI 2012 [22], the control strain proved susceptible to the quinolone (table 2).

**Table 2: Antibacterial activity of Bl-E against strains of Listeria spp**

| N°  | Code | R | S | L | F | P | Cp |
|-----|------|---|---|---|---|---|----|
| 1   | B1C2 | 8 | 12| 12| 18| 21| 24 |
| 2   | B3C2 | 4 | 4 | 14| 21| 18| -  |
| 3   | C3C1 | 10| 11| 14| 11| 22| -  |
| 4   | C4C2 | 12| 14| 20| 21| 18| -  |
| 5   | C5C1 | 8 | 2 | - | - | 9 | 14 |
| 6   | C6C1 | 15| 12| 22| 24| - | 24 |
| 7   | C8C2 | 24| 18| 20| 15| 22| 20 |
| 8   | C14C3| 10| 8 | 16| 9 | 4 | 27 |
| 9   | C18C1| 20| 24| 16| 9 | 4 | 27 |
| 10  | P19C1| 12| 11| 18| 18| 18| 18 |
| Mean | 12.3| 11.6| 15.3| 15.1| 16.9| 19.1|
| \(L\)isteria innocua, ATCC 33090 | 20| 18| 23| 22| 21| 14 |

R = root; S = stem; L = leaves; F = flowers; Cp = ciprofloxacin; P = penicillin

Anti-listerial effect of Bl-E and antibiotics for clinical use

On the other hand, in the control strain (\(L\)isteria innocua, ATCC 33090) presented 23 and 22 mm in diameter of the zone in the extracts of leaves and flowers respectively, however, these two extracts did not inhibit the development of the isolate, as shown in table 2. Resistant isolates were considered to those that presented a zone size ≤8 mm in diameter [19].

![Antimicrobial effect of Bl-E against Salmonella](image)

**Fig. 3: Antimicrobial effect of Bl-E against Salmonella**

Antimicrobial activity of Bl-E against Salmonella isolates

After having measured the inhibition diameters, it was determined that all the \(S\)almonella isolates were susceptible to the Bl extracts studied, where the extracts of leaves and flowers presented greater effectiveness, in fact, the size of the zone had a mean greater than 20 mm fig. 3.

On the other hand, in the control strain, the root of Bl extract with a zone of 22 mm acted better, followed by the leaves extract with 21 mm of inhibition zone, as shown in table 3.
Antibiotic activity of penicillins, they are specific for Gram + microorganisms [23], so that it justifies the ineffectiveness of this antibiotic against these isolates. After applying quinolone.

Antimicrobial activity of penicillin G, an average of 4.0 mm in diameter was obtained. Considering the parameters established by the CLSI 2012 [22], all the isolates showed resistance to this antibiotic, the size zone > 14 mm. However, it is important to consider that in a group of penicillins they are specific for Gram + microorganisms [23], so that it justifies the ineffectiveness of the antibiotic against Salmonella.

With ciprofloxacin, all isolates were shown to be susceptible to this antimicrobial agent with a zone diameter size > 14 mm, according to the CLSI, 2012 [22]. The control strain was also susceptible to this quinolone.

Antimicrobial activity of BI-E against Escherichia coli isolates

After finishing each of the zones of inhibition, it was determined that the extract of flowers and stem showed greater effectiveness against isolates of Escherichia coli with a zone size of 7.2 and 7.1 mm respectively. However, according to Ponce et al. [19], it should be considered that the microorganism is susceptible to the natural extract or oil, if the zone size is greater than 9 mm, so that the root extract alone inhibited the P2C1 strain; stem extract, the strains: B3C3 and P4C1; leaves extract, the strains: B2C1 and C3C2 and flowers extract only strain B3C3, as shown in fig. 4.

Anti-Salmonella effect of BI extracts and antibiotics for clinical use

In the clinical antimicrobials, for penicillin G, a mean of 21 mm in diameter was obtained (in strains with inhibitory effect) value not so far from the average of the extracts of BI, in fact, the extracts of Bl leaves acted better than this antibiotic. There were 8 strains of Salmonella that showed total resistance to Penicillin (B5C1, B3C3, C2C1, C13C2, P1C3, P1C5, P5C3, P14C3) zone size < 14 mm as established by the CLSI 2012 [22] (table 2). However, it is important to consider that in the group of penicillins they are specific for Gram + microorganisms [23], so that it justifies the ineffectiveness of the antibiotic against Salmonella.

With ciprofloxacin, all isolates were shown to be susceptible to this antimicrobial agent with a zone diameter size > 14 mm, according to the CLSI, 2012 [22]. The control strain was also susceptible to this quinolone.

Table 3: Antibacterial activity of BI-E against strains of Salmonella spp

Table 4: Antibacterial activity of BI-E against strains of Escherichia coli

Anti-E. coli effect of BI extracts and antibiotics for clinical use

For penicillin G, an average of 4.0 mm in diameter was obtained. Considering the parameters established by the CLSI 2012 [22], all the isolates showed resistance to this antibiotic, the size zone < 12 mm. However, it is important to consider that in a group of penicillins they are specific for Gram + microorganisms [23], so that they justify the ineffectiveness against these isolates. After applying the ciprofloxacin discs, all the isolates sensitivity to this antibiotic, with inhibition zone size > 13 mm.

Antioxidant capacity of BI extracts

After this analysis it was possible to determine that the flower BI extract in 60 mg/ml presents a higher percentage of sequestration of H2O2 with 47.25%, followed by the concentration of 20 mg/ml with...
46.36%. In relation to the extract obtained from the stem of BL, the concentration of 20 mg/ml was enough to be able to sequester H₂O₂ by 4.00%; followed by the 40 mg/ml concentration with 19.23%.

The leaves of BL extract with the greatest effect on the retention of H₂O₂ was that of 20 mg/ml concentration. While the root extract did not show any positive effect for this analysis, as shown in table 5.

| Table 5: Antioxidant capacity test of Baccharislatifolia extracts |
|------------------|------------------|
| Extract          | Concentration (mg/ml) | % of kidnapped peroxide |
| Flower           | 20               | 46.36                   |
|                  | 40               | 41.04                   |
|                  | 60               | 47.25                   |
|                  | 80               | 42.08                   |
| Stem             | 20               | 19.23                   |
|                  | 40               | 18.72                   |
|                  | 60               | 5.62                    |
| Leaves           | 40               | 3.92                    |
|                  | 60               | 0.84                    |
|                  | 80               | 0.00                    |
| Root             | 40               | 0.00                    |
|                  | 60               | 0.00                    |

DISCUSSION

The extracts of medicinal herbs showed inhibitory activity, as determined by a work developed by Yoon and Choi [24], where the extracts of Bogolji and Gosam showed antibacterial capacity with zone diameter>10 mm; also, Eruteya and Badon [25], obtained antilisteria activity of ethanol extracts of Morinda oleifera, with zone of inhibition>11 mm from extract concentrations of 200 mg/ml. Similar results were obtained by Rulova et al. [18], obtained antilisteria effect of ethanolic extracts of Physalis peruviana fruits, but with zone sizes<7 mm. Odedina et al. [26], used Rhodomyrtustomentosa ethanolic leaf extract as biocontrol against Listeria monocytogenes. Also, in the work carried out by Carrizo et al. [27], reported that the essential oil of B. salicifolia inhibits the growth of Listeria monocytogenes CLIP 74904, but was inactive against the Gram-negative organisms analyzed. It should also be noted that there are no studies on the antilisterial activity of Baccharislatifolia, which shows that our research group is the first to work with extracts of this plant against Listeria spp. isolates.

In a study developed by Shan et al. [28], reported that the extracts of 26 medicinal herbs positively inhibited the development of Salmonella anatum (mean = 7.2 mm, 4.7-19.2 mm). On the other hand, the acid environment improved the antibacterial activity of the extract of Filipendulaulmaria when tested against S. enteritidis PT4, whose aqueous methanol extract contains a variety of phenolic compounds [29].

In a study conducted by N’guessan et al. [30], the aqueous extracts of Thonningiasanguinea showed an antimicrobial effect for all Salmonella strains of multiple drug resistance (S. typhi, S. typhimurium and S. hadar). In another study conducted in South Korea, by Lee et al. [31], the aqueous and methanolic extracts of Schizandraefructus showed antibacterial activity against the three Salmonella serotypes (S. typhi ATCC 19943, S. paratyphi A and S. gallinarum ATCC 9184). In addition, the root of Euphorbia balsamifera had shown high activity against S. typhimurium in comparison with the extracts of leaves and stems [32]. As well in the study developed by our research group, the inhibitory effect of extracts of Physalis peruviana L against isolates of Salmonella spp. with inhibition zones between 8 and 10 mm [33].

In the research developed by BachtalRahm and Benali [34] shows that the essential oil of Eucalyptus globulus is effective to inhibit the development of Escherichia coli with zone sizes ranging from 8 to 26 mm in diameter. According to Argote-Vera et al. [35], mentions that the essential oils of eucalyptus and mandarin inhibit in a 13.2 μl/ml and lemon 14.6 μl/ml, demonstrating that the essential oils of eucalyptus, lemon peel and mandarin have the inhibitory capacity to the bacteria Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923). Also, in the research developed by Bastos [36], oregano oil was more effective against Escherichia coli with 0.35% CBM (minimum bactericidal concentration), with an inhibition zone of 29.5±3.4 mm.

Similarly, Sequeda et al. [16], studied extracts of Baccharislatifolia obtained by percolation, maceration and soxhlet, but did not observe an inhibitory effect on E. coli, although it did act positively against other pathogens.

In general, the chemical compound of the plant extract has revealed the presence of several components, most of which have important antimicrobial properties [37]. The properties present in Baccharis species are constituted mainly in flavonoids, monoterpenes, diterpenes, triterpenes, tannins, quinones, saponins, as well as some phenolic compounds, where flavonoids are distinguished by confer protection/resistance against attack of microorganisms [38, 37, 16]. Coumarins and essential oils have also been obtained of Baccharis species [39].

In addition, there are studies that show that monoterpenes are the components that also act in the inhibition of microorganisms, [40]. Other researchers state that the phenolic compound in the plant contributes significantly to its antimicrobial and antioxidant properties [41]. This study is the first to analyze the antibacterial effect of extracts of Baccharislatifolia (root, stem, leaves and flowers) on isolated Listeria, Salmonella and E. coli.

In a study developed by Guerra [42], they analyzed the antioxidant activity of the essential oil of the Baccharislatifolia-β carotene test, with concentrations of 26 and 64 mg/ml of BL oil obtained values of 40.56% and 46.20% respectively. In our study, the extracts of flowers were the only ones that approximate these results. Cucurbita pepo extracts inhibited the peroxidation of linolic acid at 5.1-30.4% after incubation for 96 h [43].

In another study conducted by Hossain et al. [44], obtained a value of 48.6% with lipophilic extracts of mixed Cucurbita in Peru a study by Doroteo et al. [45], determined the antioxidant effect of cat’s claw (Uncaria tomentosa) with an effect of 47%.

So also, Rodriguez et al. [46], determined the effect of an extract of Bocconiafrutencens L with a capture value of 40% with an extract concentration of 25 mg/l.

CONCLUSION

Extracts of leaves and flowers of Baccharislatifolia acted better against Listeria and Salmonella isolates, whereas in E. coli isolates; Flower and stem extracts were the best. In short, these extracts proved to be equal to or better than the antibiotics for clinical use, thus considering the extracts of BL as an alternative natural product to inhibit the development of pathogens.

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CONFLICT OF INTERESTS
All the authors have contributed equally.

AUTHORS CONTRIBUTIONS
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