Bioactive properties of blueberry extracts obtained by different drying techniques against Helicobacter pylori

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INTRODUCTION
Helicobacter pylori (H. pylori) is one of the most successful and prevalent human pathogens that infect more than 50% of the world’s population [1]. Chronic inflammation of the gastric mucosa is one of the main consequences of this infection and is associated with the risk of gastric cancer [2]. Blueberries are rich in different bioactive compounds with antibacterial and anti-inflammatory properties [3] that could contribute to reduce the problems associated with H. pylori infection. However, these properties may vary depending on the blueberry variety. Furthermore, the industrial processing of blueberry extracts involves the use of a number of technical procedures that could affect these bioactive properties [4]. For this reason, the main objective of the present work was to evaluate the antibacterial and anti-inflammatory properties of three blueberry extracts from different varieties and obtained by different drying methods against H. pylori.

MATERIALS & METHODS
- **Blueberry extracts**
  - Bluejay
  - Berkley
  - Bluecrop
cultivars

- **Drying procedures**
  - Drying blueberry extracts by:
    - freeze drying (FD)
    - vacuum drying (VD)
    - spray drying (SD)

- **Bacterial strain, growth media and conditions**
  - Strain: Helicobacter pylori Hp59 from MICROBIO group’s bacterial collection and isolated from gastric biopsy
  - Selective growth media: Brucella broth supplemented with 10% horse serum (BBH) and Mueller-Hinton agar supplemented with 5% sheep blood (MHB).
  - Growth conditions: strains reactivation into MHB and incubation for 72h at 37ºC, in microaerophilic atmosphere in VAIN workstation (85% H2, 10% CO2, 5% O2).

- **Antibacterial activity**
  - Procedure:
    - Bacterial culture mixture:
      - 1 mL of extracts (2 mg/mL) dissolved in BBH (or only BBH for control growth)
      - 4 mL BB supplemented with 10% horse serum
      - 100 μL bacterial inoculum (~1×10^9 CFU/mL)
      - Incubation for 24h at 37ºC, 150 rpm, in microaerophilic atmosphere (VAIN).
      - Serial decimal dilutions of bacterial cultures were plated onto fresh MHB agar.
      - Incubation microaerobically for 72h at 37ºC in microaerophilic atmosphere (VAIN).
      - Antibacterial activity determination by CFU counting.

- **Anti-inflammatory activity on infected-gastric AGS cells**

RESULTS

- **Antibacterial activity**

  Table 1. Antibacterial activity of blueberry extracts (2 mg/mL) on the viable counts of H. pylori Hp59 after 24 hours of treatment. Results are expressed as CFU/mL (mean ± standard deviation) (n=4).

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Bluejay</th>
<th>Berkley</th>
<th>Bluecrop</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD</td>
<td>1.90 ± 0.58x10^9 *</td>
<td>4.00 ± 0.50x10^9 *</td>
<td>&lt;1.00 ± 0.50x10^9 *</td>
</tr>
<tr>
<td>VD05</td>
<td>&lt;1.00x10^9 *</td>
<td>&lt;1.00x10^9 *</td>
<td>&lt;1.00x10^9 *</td>
</tr>
<tr>
<td>VD70</td>
<td>2.25 ± 1.0x10^9 *</td>
<td>&lt;1.00x10^9 *</td>
<td>&lt;1.00x10^9 *</td>
</tr>
<tr>
<td>VD90</td>
<td>2.13 ± 1.25x10^9 *</td>
<td>&lt;1.00x10^9 *</td>
<td>&lt;1.00x10^9 *</td>
</tr>
<tr>
<td>SD</td>
<td>&lt;1.00x10^9 *</td>
<td>3.75 ± 0.35x10^9 *</td>
<td>&lt;1.00x10^9 *</td>
</tr>
</tbody>
</table>

* Different superscript letters denote statistical difference within a row (p<0.05, ANOVA Tukey test).
* Different subscript letters denote statistical difference within a column (p<0.05, ANOVA Tukey test).

- **Anti-inflammatory activity**

  Table 1. Effect of blueberry extracts (2 mg/mL) on pro-inflammatory cytokine IL-8 production in AGS cells infected by H. pylori Hp59. Results are expressed as % production of IL-8 respect to the untreated infected cells (mean ± standard deviation) (n = 3).

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Bluejay</th>
<th>Berkley</th>
<th>Bluecrop</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD</td>
<td>78.2 ± 1.2 x*</td>
<td>82.9 ± 3.4 x*</td>
<td>81.5 ± 4.0 x*</td>
</tr>
<tr>
<td>VD05</td>
<td>76.8 ± 1.0 x*</td>
<td>84.1 ± 2.2 x*</td>
<td>89.9 ± 2.6 x*</td>
</tr>
<tr>
<td>VD70</td>
<td>74.5 ± 0.7 x*</td>
<td>80.4 ± 4.3 x*</td>
<td>67.3 ± 5.0 x*</td>
</tr>
<tr>
<td>VD90</td>
<td>72.9 ± 2.1 x*</td>
<td>77.8 ± 4.3 x*</td>
<td>67.3 ± 0.7 x*</td>
</tr>
<tr>
<td>SD</td>
<td>78.1 ± 4.8 x*</td>
<td>83.0 ± 1.9 x*</td>
<td>85.3 ± 2.3 x*</td>
</tr>
</tbody>
</table>

* Different superscript letters denote statistical difference within a row (p<0.05, ANOVA Tukey test).
* Different subscript letters denote statistical difference within a column (p<0.05, ANOVA Tukey test).
* Asterisk denotes statistical difference in comparison with the control growth (untreated AGS cells) (p<0.05, ANOVA Tukey test).

CONCLUSIONS
- All blueberry extracts showed significant antibacterial activity against H. pylori Hp59 and some of these extracts showed bactericidal effect.
- The rest of blueberry extracts without bactericidal effect were able to reduce H. pylori Hp59 growth by more than 5 log CFU/mL.
- Bluecrop extracts showed the most effective antibacterial activity, because all extracts obtained by the different drying methods resulted bactericidal.
- Vacuum drying (VD) at 50ºC were the most effective drying method since the extracts of the three varieties obtained by this method were bactericidal.
- Regarding anti-inflammatory activity, all blueberry extracts reduced IL-8 production in H. pylori-infected AGS cells.
- Bluecrop extracts obtained by the VD at 70ºC and 90ºC showed the most active anti-inflammatory effect reducing IL-8 production by 32% and 30%, respectively.
- These results suggest that the selection of the blueberry variety and drying method can be an effective tools for modulating the antibacterial and anti-inflammatory properties of blueberry extracts.

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REFERENCES: