ANTIBACTERIAL ACTIVITY OF Beta vulgaris L. POMACE EXTRACT

Aleksandra S. Velićanski*, Dragoljub D. Cvetković, Siniša L. Markov, Jelena J. Vulić and Sonja M. Dilas

University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

Antibacterial activity of Beta vulgaris L. (beetroot) pomace extract (concentration 100 mg/ml) was tested against five Gram positive and seven Gram negative bacterial strains (reference cultures and natural isolates). Disc diffusion method with 15 µl of extract and agar-well diffusion method with 50 and 100 µl were used. Antibiotic (ceftaxime/clavulanic acid) was used as a control sample. The tested extract showed the highest activity against Staphylococcus aureus and Bacillus cereus, where clear zones (without growth) appeared. There was no any activity against other tested Gram-positive bacteria, except for Staphylococcus epidermidis, with a small zone of reduced growth. Growth of all tested Gram-negative bacteria was inhibited usually with 100 µl of extract. The most susceptible were Citrobacter freundii and Salmonella typhymurium. The tested antibiotic gave clear, usually large zones for all tested strains except for Staphylococcus cohnii spp. cohnii, where only a zone of reduced growth appeared.

KEY WORDS: Beta vulgaris L., beet root pomace extract, antibacterial activity, agar-well diffusion method

INTRODUCTION

Beta vulgaris L. (beetroot) is ranked among the ten most powerful vegetables with respect to antioxidant capacity ascribed to a total phenolic content of 50–60 mmol/g dry weight (1, 2, 3). It contains a significant amount of phenolics: catechin hydrate, epicatechin, ferulic, protocatechuic, vanillic, p-coumaric, p-hydroxybenzoic, caffeic and syringic acids (4, 5). Beetroot is a potential source of valuable water-soluble nitrogenous pigments, called betalains, which are composed of two main groups, the red betacyanins and the yellow betaxanthins. In addition to their red color, betalains possess several desirable biological activities, including antioxidant, antiinflammatory, hepatoprotective, and anti-tumor properties (6, 7, 8). Previous investigations of antimicrobial activity of Beta vulgaris L. extracts showed its slight activity against tested strains, while Gram–positive bacteria were more resistant than Gram–negative bacteria and yeasts (9, 10, 11).

* Corresponding author: Aleksandra S. Velićanski, University of Novi Sad, Faculty of Technology, 21000 Novi Sad, Bulevar cara Lazara 1, Serbia, e-mail: sanja@tf.uns.ac.rs
By-products of plant food processing represent a major disposal problem for the industry concerned, but they are also promising sources of compounds which may be used because of their favourable technological or nutritional properties (12). These phytochemicals from waste materials deriving from agro-industrial production may be used as functional food ingredients and as natural antioxidants to replace their synthetic equivalents that have experienced growing rejection.

Taking into account biological activities of beetroot as well as possible potential of plant by-products, in this study, beetroot pomace extract was used to screen antimicrobial activity to twelve bacterial strains. Among the species used, there were wild isolates and reference cultures. According to previous results, where *Beta vulgaris* L. extracts had a low activity, a larger beetroot pomace extract concentration or volume was needed, so different methods were used in this study: disc diffusion method with the limited capacity of discs and agar-well diffusion method with much higher volume of holes.

**EXPERIMENTAL**

**Preparation of beetroot pomace extract**

Beetroot (*Beta vulgaris* L.) was obtained from Zdravo Organic, Selenča, Serbia. Organic beetroot was washed in running water, cut in pieces and the pulp was prepared using a domestic food processor (Neo SK-400). After juice separation, the sample of the obtained beetroot pomace was stored at -20°C until analysis. Sample of beetroot pomace (120 g) was extracted for 30 min on an ultrasonic bath Branson model b-220 SmithKline Co., Shelton, USA (50/60 Hz, 125 W). The extraction was performed with a 50% ethanol aqueous solution containing 0.5% acetic acid (1200 ml). The obtained extract was evaporated to dryness under reduced pressure. The yield, average of triplicate analysis, of the extract was 9.89±0.48 g.

**Sample for the determination of antibacterial activity**

Sample for the determination of antibacterial activity was *Beta vulgaris* L. (beetroot) pomace extract, which was dissolved in distilled water to a concentration of 100 mg/ml.

**Test microorganisms**

Test microorganisms for the determination of antibacterial activity were reference cultures and wild isolates from foodstuffs and water. Gram–negative bacteria were: *Salmonella typhymurium* (ATCC 14028), *Escherichia coli* (ATCC 10536) and wild isolates: *Pseudomonas aeruginosa*, *Citrobacter freundii* and *Enterobacter cloacae*. Gram–positive bacteria were: *Staphylococcus aureus* (ATCC 11632), *Bacillus cereus* (ATCC 10876) and wild isolates: *Bacillus* spp., *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus cohnii* spp. *cohnii* and *Listeria monocytogenes*. Wild isolates were identified using Vitek® 2 Compact System (bioMérieux, France).
Antibacterial activity

Antibacterial activity was determined by disc diffusion and agar–well diffusion method (13). Bacterial strains were grown on Müeller–Hinton agar (Himedia, Mumbai, India) 24 h at 37 °C. Cells were then suspended in a sterile 0.9% NaCl solution. 2 ml of the suspensions for inoculation (1×10^6 cells/ml, estimated by Mc Farland nefelometer) were homogenized with 18 ml of melted (45 °C) Müeller–Hinton agar and poured into Petri dishes.

For disc diffusion method, sterile 6 mm discs (Himedia, Mumbai, India) were placed on the inoculated agar plates and impregnated with 15 µl of extract solution. The antibiotic (cefotaxime 30 µg/clavulanic acid 10 µg discs, Bioanalyse®, Ankara, Turkey) was used as control. For the agar–well diffusion method, wells of 9 mm diameter were made with a sterile metal tube by means of a vacuum pump. The extract solution (50 and 100 µl) was then transferred into the wells of inoculated agar plates. For both methods, the test plates were refrigerated at 8 °C for 1 hour to allow the extract to diffuse into the medium, and then were incubated at 37 °C for 24 hours. After the incubation, the diameters of the inhibition zones were measured and recorded in millimeters (mm). The evaluation of antibacterial activity was carried out in three repetitions.

RESULTS AND DISCUSSION

The results of antibacterial activity of beetroot pomace extract are presented in Table 1.

As it is evident from Table 1, the least activity of beetroot pomace extract for all tested bacterial strains was obtained by disc diffusion method. The zone of reduced growth was found only for Salmonella typhymurium, Staphylococcus aureus and Bacillus cereus (Figure 1, a). This is not unexpected because the volume of the extract solution was 15 µl, unlike 50 or 100 µl which were used by agar–well diffusion method. So, the antibacterial activity increased in parallel with the increase in the extract volume. The tested extract in an amount of 100 µl had an inhibitory activity against all tested Gram–negative bacteria, of which most susceptible strains were Salmonella typhymurium and Citrobacter freundii. Their growth inhibition was caused by 50 µl of the extract, too. The other tested Gram–negative bacteria were more resistant to the beetroot pomace extract, with the inhibition zones of about 13 mm caused by 100 µl of the extract. Growth of two tested Gram–positive bacteria (Listeria monocytogenes and Enterococcus faecalis) was not inhibited by Beta vulgaris L. pomace extract. On the other hand, the tested extract even in an amount of 50 µl exhibited a significant inhibitory activity against Staphylococcus aureus (clear zone around the well), while in the case of Bacillus cereus clear zone appeared using 100 µl of extract (Figure 1,b). So, these bacterial strains were the most susceptible among tested bacteria, with halo zones of about 20 mm, which could indicate the bactericidal activity of Beta vulgaris L. pomace extract. Unlike the reference strains, wild isolates of Bacillus and Staphylococcus were more resistant, so the tested extract did not inhibit growth of Staphylococcus cohnii spp. cohnii and Bacillus spp., while Staphylococcus epidermidis was slightly susceptible.
Table 1. Antibacterial activity of beetroot pomace extract (diameter of the inhibition zone mean (mm) including disc (6 mm) or well (9 mm) ± standard deviation)

<table>
<thead>
<tr>
<th>Group</th>
<th>Tested strains</th>
<th>Method</th>
<th>Control (cefotaxime/clavulanic acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Disc diffusion 15µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>G (-) bacteria</td>
<td><em>Escherichia coli</em></td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td><em>Citrobacter freundii</em></td>
<td>nd</td>
<td>20.33±0.58**</td>
</tr>
<tr>
<td></td>
<td><em>Enterobacter cloacae</em></td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella typhymurium</em></td>
<td>8.0±0.0**</td>
<td>16.0±0.0**</td>
</tr>
<tr>
<td>G (+) bacteria</td>
<td><em>Staphylococcus aureus</em>266</td>
<td>12.5±0.55**</td>
<td>16.0±0.0**</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus epidermidis</em></td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus cohnii spp. cohn</em></td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td><em>Bacillus cereus</em></td>
<td>10.67±1.03**</td>
<td>17.0±1.0**</td>
</tr>
<tr>
<td></td>
<td><em>Bacillus spp.</em></td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td><em>Enterococcus faecalis</em></td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td><em>Listeria monocytogenes</em></td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

nd - non detected inhibition zone  
* - clear zone around the disc/well  
** - zone of reduced growth

Figure 1. Antibacterial activity of beetroot pomace extract against *Bacillus cereus*: a) disc diffusion method, b) agar well diffusion method

The inhibition zones of tested controls (cefotaxime/clavulanic acid discs) were significant, and for all bacteria clear zones around the discs appeared. The antibiotic showed...
the most expressive activity against *Escherichia coli*, *Salmonella typhymurium*, *Staphylococcus aureus* and *Bacillus cereus* (more than 30 mm). The least clear zones (less than 15 mm) appeared for *Listeria monocytogenes* and *Enterococcus faecalis*, while for *Staphylococcus epidermidis* only a zone of reduced growth appeared. Against these strains, the beetroot pomace extract did not show any activity, so, their higher resistance to antimicrobial substances is expected.

However, the absence of antibacterial activity or the presence of slight activity in the case of some bacteria do not indicate the absence of bioactive constituents, nor the plant inactivity. Namely, active components may be present in insufficient quantities to inhibit the growth of the cells. The lack of activity can thus only be proven by using large doses. Also, by applying higher extract quantities, some other constituents may be introduced which exert antagonistic effects or negate the positive effects of the bioactive agents (11).

Koochak et al. (2010) tested the antibacterial activity of *Beta vulgaris* L. ethanolic extracts against ten bacterial clinical isolates by disc diffusion method using 50 µl of extract solution (10). In all tested concentration (50, 100, 200 and 400 mg/ml) the extracts did not show considerable inhibitory activity against Gram–negative bacteria. Among Gram–positive bacteria, the most resistant strains were *Listeria monocytogenes* and *Bacillus cereus*, while significant antimicrobial activity was observed against *Staphylococcus epidermidis*. Rauha et al. (2000) tested the antimicrobial activity of methanolic extracts of *Beta vulgaris* L. by cylinder diffusion method (500 µl of extract, 1 mg/ml) (9). Only slight antibacterial activity was obtained against *Escherichia coli* and *Staphylococcus aureus*, probably because of the use of a low extract concentration (1 mg/ml). In testing of 100 µl of aqueous *Beta vulgaris* L. leaf extract (disc diffusion method), Parekh and Chanda (2008) obtained a slight inhibitory activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*, while ethanolic leaf extract (agar well diffusion method) inhibited growth of *Staphylococcus subfava* (11).

Although the same methods and extract solution volumes were tested in different studies, the beetroot pomace extract tested in this study showed the highest antibacterial activity. The observed differences are probably caused by the different susceptibility of tested natural isolates, as well as the composition and amount of active components extracted from tested materials originated from different geographic areas.

**CONCLUSION**

In the discs diffusion method, *Beta vulgaris* L. (beetroot) pomace extract had a slight antibacterial activity against only three tested strains, because of the least applied extract volume (15 µl). In the agar-well diffusion method, the tested extract inhibited growth of all tested Gram–negative bacteria in a volume of 100 µl. For *Citrobacter freundii* and *Salmonella typhymurium* the inhibition was present when 50 µl was applied. Among all tested bacteria, *Staphylococcus aureus* and *Bacillus cereus* were the most susceptible, because halo zones appeared, which indicate bactericidal activity of tested extract. Against the other tested Gram–positive bacteria, *Beta vulgaris* L. pomace extract in applied quantities did not show any activity.
Acknowledgement

Financial support of the Ministry of Education and Science of the Republic of Serbia (Project TR 31044) is highly acknowledged.

REFERENCES

АНТИБАКТЕРИЈСКА АКТИВНОСТ ЕКСТРАКТА ТРОПА ЦВЕКЛЕ

(Beta vulgaris L.)

Александра С. Велићански, Драгољуб Д. Цветковић, Синиша Л. Марков, Јелена М. Вулић и Соња М. Ђилас

Универзитет у Новом Саду, Технологијски факултет, Булевар цара Лазара 1, 21000 Нови Сад, Србија

Антибактеријска активност екстракта тропа цвекле (Beta vulgaris L.) (концентрације 100 mg/ml) испитана је према пет Грам–позитивних и седам Грам–негативних бактерија (референтних сојева и природних изолата). Коришћене су диск-дифузиона метода (са 15 µl екстракта) и метода бунарчића у подлози) са 50 µl и 100 µl екстракта. Антибиотик (cefotaxime/clavulanic acid) је коришћен као контрола. Испитани екстракт показао је највећу активност према Staphylococcus aureus и Bacillus cereus, код којих се јавила чиста зона (без раста). Није било активности према осталим испитаним Грам–позитивним бактеријама, осим Staphylococcus epidermidis, код којег се јавила мала зона смјешеног раста. Раст свих испитаних Грам–негативних бактерија инхибиран је обично са 100 µl екстракта. Најосетљивије међу њима су Citrobacter freundii и Salmonella typhymurium. Контролни узорак (антибиотик) даје чисту зону за све испитане бактерије осим Staphylococcus cohnii spp. Cohni, код којег се јавља зона редукованог раста.

Кључне речи: Beta vulgaris L., екстракт тропа цвекле, антибактеријска активност, метода „бунарчића“

Received 16 September 2011
Accepted 27 October 2011