Antimicrobial Properties of Daily Consumed Bio Vegetables: Tomato, Chili Pepper, Parsley, Onion and Garlic

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Abstract

Many antimicrobial drugs lost their ability to effectively combat bacteria in recent years. This prompted novel investigations on the antibacterial properties of plants. The aim of this study was to evaluate the antibacterial activity of bio parsley, tomatoes, chili peppers, two types of onions, and garlic against Gram (+) and Gram (-) bacteria. 20 samples derived from 5 plants were assessed using the agar diffusion method against test strains Bacillus subtilis No8751 and Escherichia coli No8752, provided by the National Bank for Microorganisms and Cell Cultures, Bulgaria. Our results demonstrated that the strongest antibacterial power had garlic, followed by orange-skinned onion and chili pepper. Against Gram (+) bacteria inhibitory effect was shown by mature orange and red-skinned onion bulbs and chili pepper tissue. Against Gram (-) most effective were mature garlic bulbs and chili pepper’s tissue. The single vegetable suppressing the growth both of E. coli and B. subtilis was the chili pepper. Obtained results showed that seeds of tomato and chili pepper demonstrated weak effectiveness demonstrating their prophylactic antibacterial role when eaten. In conclusion, single bio vegetables or their combinations are promising sources for novel antibacterial agents.

Keywords: antibacterial activity, tomato, chili pepper, parsley, onion and garlic

Introduction

Due to the multidrug resistance of pathogenic microorganisms, new antimicrobial drugs are now emergently required. The goal of many recently performed investigations was to identify novel bioactive substances from plants. One reason for the antibacterial action of plants is their synthesis of antimicrobial peptides (AMPs). The diversity of AMPs covers thionins, defensins, hevein-like peptides, knottins, a-hairpinins, lipid transfer proteins, and snakins (Tam et al., 2015, Bin Hafeez et al., 2021, Li et al., 2022). AMPs of vegetables cause pore formation in the bacterial cytoplasmic mem-

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brane, agglomeration of the bacterial cells, membrane permeabilization, disruption of the cell membrane, triggering the production of ROS, preventing the formation of bacterial biofilms and degrading the formation of mature biofilms. For example, garlic is rich in antimicrobial peptides Fs-3-a, F3-3-b, F3-3-c, and NpRS (9 Aminoacids), tomatoes produce snakins, onions - peptide Ba49, chili peppers - γ-thionins and defensins. The AMPs’ mode of action is via five mechanisms - barrel, toroidal, carpet, aggregate channel, and flood gate models, shown in Fig. 1.

Another reason for the successful antimicrobial effect of plants is the rich polyphenolic content in their roots, stems, leaves, flowers, and fruits (Abbas et al., 2017; Satchanska, 2022). As described by Satchanska (2022) plant polyphenols consist of more than 9 000 substances containing polyaromatic rings and few hydroxyl groups. They are divided into several main groups: flavonoids, lignans, phenolic acids, and stilbenes. Among them, flavonoids are the most numerous. All polyphenols play an important role in the defense of plants against bacteria, viruses, fungi, insects, and herbivores. Polyphenol synthesis derives from two aromatic amino acids – tyrosine and phenylalanine. As secondary plant metabolites, polyphenol amount is estimated at only 10% of plant metabolites. Usually, the daily polyphenol human intake varies between 20 and 500 mg, taken via onions, tomatoes, red wine, and many other foods and beverages. Gallic acid is a component of apple, ginger, yellow pepper, hazelnuts, and oak bark; salicylic acid was found in cardamom seeds and cereals; hydroxycinnamic acids also known as coumaric acid was found in green pepper, apricot, and blueberry.

Caffeic acid is bountiful in apricots, prunes, salvia, spearmint, thyme, aronia, sunflower seeds, barley, and rye. Ferulic acid was detected in giant fennel, sweet popcorn, and many vegetables’ roots. Warfarin or 4-hydroxycoumarins is concentrated in green leafy vegetables.

Flavone is a valuable component of fruit pomegranate and the herb rosemary, rosemary being the wealthiest in this polyphenol. 3-hydroxyflavone is abundant in brassicas and papaya (Othman et al., 2019; Satchanska, 2022).

According to scientific reports, Petroselinum crispum possesses antibacterial activity against both Gram (+) and Gram (-) bacteria and has a vast application in folk medicine (Bin et al., 2007; El Astal et al., 2005; Peter et al., 2006; Sayyednejad and Damab, 2008). According to the tomatoes, Richard and Pajkovic (2008) stated they are strong antioxidants, scavengers of free radicals, and guard against bacterial pathogens and food spoiling (Wen-

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**Fig. 1.** Barrel, toroidal, carpet, aggregate channel, and flood gate models of action of AMPs inside the bacterial cell. 1-7 represent intracellular mode of action of AMPs inhibiting enzymes responsible for cell wall self-organization (3), DNA replication (4), RNA transcription (5), chaperones synthesis (6), and cell respiration (7), latest damaged by ROS causing leakage of accumulated ATP outside the broken mitochondrial membrane.
Materials and Methods

In our study, 5 different bio-grown vegetable species were used including parsley, two varieties of tomatoes, mature and young orange and red-skinned onion, chili peppers, and mature and young garlic (Fig. 3). Different plant tissues were separated and prepared for analysis as follows: eight tomato samples, three garlic samples, four onion samples, and two parsley samples. The final samples were prepared in a manner most similar to eating. Test bacteria *E. coli* and *B. subtilis* were provided by the National Bank for Microorganisms and Cell Cultures. Analysis was conducted using the agar diffusion method (Balouiri et al., 2016).

Samples of parsley were aseptically divided into stems and leaves. Samples of tomatoes from the D5 (Rose variety) and D6 (Ideal variety) were aseptically prepared as dried tomato pulp, raw tomato pulp, tomato seeds, and tissue slices. The pepper fruits were aseptically opened, and the raw pulp, tissue discs (d=0.5mm), and seeds were excavated. The onion and garlic samples were aseptically separated into mature bulbs, young bulbs, and young leaves.

While the tomato, onion, and garlic samples were homogenized using a sterilized blender, the parsley leaves and stems were homogenized using a sterile mortar and pestle. The dried pulp was obtained by drying raw pulp at 37°C for 24 hours. Chili peppers were manipulated using a sterile scalpel. The test microorganisms (0.1 ml) *E. coli* No 8752 and *B. subtilis* No 8751 were added to Nutrient agar Petri dishes at a concentration of 0.5 McFarland corresponding to $1 \times 10^6$ CFU/ml. Subsequently, the
Nutrient agar was aseptically perforated resulting in 5 wells (d=9mm)/Petri dish to accommodate the samples in three or four repetitions and the Control. The Control well was filled in with sterile water. Instead in the wells, tomato and pepper seeds, tomato tissue slices, and pepper tissue discs were directly placed on the inoculated agar. Dishes were incubated overnight at 37 °C and the inhibitory zones were measured.

Data were handled as an average of three or four replicates (Mishra et al., 2019).

**Results and Discussion**

The resulting data showed that the vegetable samples had varying effects on the test bacteria’s growth depending on the species or the plant organ used. Table 1 presents the summarized results of the antibacterial action of the aforementioned 20 plant samples.

**Antibacterial properties of parsley**

Parsley leaves had shown to be inert against *E. coli* displaying a 2-mm inhibition zone against *B. subtilis*. No antibacterial activity was observed in parsley stems (Table 1). El Astal et al. (2005) examined not raw parsley material but ethanolic parsley extract and stated that Gram (+) bacteria were less impacted by the extract compared to Gram (-).

**Antibacterial properties of tomato**

Raw tomato pulp

Our results shown in Table 1 and Fig. 4 demonstrate that the raw pulp from sample D5 (Rose variety) had no effect against both *B. subtilis* and *E. coli*. The second raw pulp sample D6 (Ideal variety) displayed better results, showing very weak antibacterial activity against both *B. subtilis* (4 mm) and *E. coli* (4.5 mm). The weak zones show the constant and prophylactic effect of raw tomato consumption against bacteria. Wan-Xian (2008) reported an antibacterial effect on *E. coli* by edible tomato films. According to his study, the inhibitory effect is due to the substance carvacrol.

![Fig. 4. Raw tomato pulp antibacterial activity of variety D6 (Ideal) against B. subtilis (4 repetitions, in the center - Control).](image)

**Antibacterial properties of chili peppers**

The results obtained showed that both *B. subtilis* and *E. coli* displayed a 2-mm inhibition zone against *B. subtilis* and *E. coli*. No antibacterial activity was observed in parsley stems (Table 1). El Astal et al. (2005) examined not raw parsley material but ethanolic parsley extract and stated that Gram (+) bacteria were less impacted by the extract compared to Gram (-).

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**Table 1. Antibacterial activity of bio vegetables (inhibitory zones in mm)**

<table>
<thead>
<tr>
<th>Sample/Test strain</th>
<th>Sample</th>
<th>Parsley</th>
<th>Tomato</th>
<th>Chili pepper</th>
<th>Onion</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>D5, D6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. subtilis</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.5</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>E. coli</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.5</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

O* - yellow skinned, R** - red skinned

471
*tilis* and *E. coli* were strongly inhibited by pepper tissue discs and a lower extent — by their seeds. As seen from Table 1 and Fig. 6 pepper discs demonstrated antibacterial activity against *B. subtilis* (24 mm) and *E. coli* (25 mm). Additionally, the chili pepper seeds showed inhibition on both *B. subtilis* (7 mm) and *E. coli* (11 mm), showing considerably higher values for *E. coli*. Chili peppers were found to display an inhibitory effect on both Gram (+) and Gram (-) bacteria. The antibacterial properties of capsicum fruit against both Gram (+) and Gram (-) bacteria have also been reported in studies by Mariângela et al. (2011) and Soediro et al. (1997).

**Fig. 5.** Tomato seed’s antibacterial activity of D6 (Ideal variety) against *B. subtilis* (3 repetitions, upper triangles).

**Fig. 6.** Chili pepper tissue discs and seeds antibacterial activity on *B. subtilis* and *E. coli*; 4 repetitions, in the center - Control.

Hamed et al. (2019) supplied data that ripening and cooking processes lead to an increase in the polyphenol concentration in 16 out of 18 studied cultivars. Chili peppers lead the ranking of antimicrobial activity.

**Antibacterial properties of onions**

According to the results, mature onion bulbs showed antibacterial activity mainly against Gr (+) bacteria (27 and 15 mm for orange and red-skinned ones, respectively) (Table 1). Against Gr (-) bacteria their inhibitory effect was very weak (2x3 mm). Coinciding outcomes were found in the papers of Cammue et al., (1995) and Jonathan et al., (2010) who described *Allium* extracts to be more effective against Gram (+) microorganisms, while Gram (-) bacteria were reported to be less susceptible. Contrary, Freddy et al. (2006) described that even water extracts from yellow onion skin are active against Gram (-) bacteria. Results are shown in Table 1 and Fig. 7. Flavonols as quercetin are responsible for the yellow-brown onion skin. More than 25 different flavonols are currently recovered from the onion. One of them – quercetin was ubiquitous in all onion varieties.

**Fig. 7.** Antibacterial activity of yellow (a.) and red (b.) mature onion bulbs against *B. subtilis* (4 repetitions, in the center - Control).

**Antibacterial properties of garlic**

When it came to mature and young garlic, stronger effect was considerably shown by mature garlic bulb (on *E. coli* - 30 mm/ on *B. subtilis* - 7 mm) (Table 1 and Fig. 8).

**Fig. 8.** Antibacterial activity of mature garlic bulbs against *E. coli* (4 repetitions, in the center - Control).

As seen from the Table 1 fresh garlic leaves are more efficient to both tested bacteria (17 and 13 mm) in comparison to fresh garlic bulbs (2 mm and 0 mm). Comparatively, young onions bulbs and leaves were completely inactive (4x0 mm). Opposite results were discussed by Srinivasan et al. (2009). The authors reported *Allium sativum* to play antibacterial activity against two Gram (-) and two
Gram (+) pathogenic bacteria exhibiting larger inhibition zone on *Bacillus subtilis*. Authors also discussed the antimicrobial effectiveness varies with temperature and time.

A compelling finding is that the synthesis of antibacterial substances in *A. sativum* and *A. cepa* occurs intensively in mature onion and garlic, not in green leafy ones. Moreover, the synthesis of the antibacterial compounds continues when they are stored at room temperature (22°C) but stops when stored in a refrigerator (5–8°C).

**Conclusions**

In conclusion, the strongest antibacterial activity was played by mature garlic bulbs (on Gr (-)), followed by mature yellow and red-skinned onions and chili peppers (on Gr (+)). Seeds are also beneficial for human health due to the antibacterial activity of tomato and chili pepper seeds. Among young onions and garlic, only garlic leaves were potent to inhibit both Gr (+) and Gr (-) bacteria. Bio vegetables and combinations of them are promising sources of novel antibacterial substances.

**Acknowledgments**

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**References**


