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Antibacterial Activity of Organically Grown Vegetables

Alexander Tomov[1*], Ana Doycheva[1], Petra Mladenova[2] and Galina Satchanska[1]

[1]Department Natural Sciences, New Bulgarian University, 21.Montevideo Str. 1618 Sofia
[2]Mladost, 1799, Sofia, Bulgaria

Abstract

In recent years many antimicrobial agents became ineffective against bacteria. This lead to many studies of the antimicrobial effects among plants. Bulgarian vegetables are ingredients in many traditional Bulgarian foods. The aim of the study was to compare the antibacterial activity of organically grown parsley, tomatoes, cayenne peppers, onions and garlic against referent test bacteria - *Bacillus subtilis* No8751 and *Escherichia coli* No8752, supplied by the National Bank for Microorganisms and Cell Cultures. Sixteen vegetable samples were analyzed. Petri dishes were cultivated at 37°C for 24 hours. Our results demonstrated low antibacterial activity of tomato and parsley samples against the Gram(+) *B. subtilis* and from weak to no activity against the Gram (-) *E. coli*. Fresh garlic samples showed low antibacterial activity against Gram (+) bacteria with only fresh garlic leaves displaying wider sterile zones. No antibacterial activity was demonstrated by the fresh onion bulbs and leaves. In contrast, the mature garlic bulbs showed pronounced activity against the Gram (-) *E. coli* (30 mm zone d). Cayenne pepper tissue and seeds possess antibacterial properties against both *Escherichia coli* and *Bacillus subtilis*. Strong antimicrobial activity against *B. subtilis* was shown by the mature onion samples with 25 mm zone diameter for the red onion bulbs and 27 mm zone diameter for the white onion bulbs. Both mature onion samples demonstrated low activity towards *E. coli*.

Резюме

През годините много антимикробни вещества се превърнаха в неефективни. Това доведе до много нови проучвания за антимикробно действие сред растенията. Българските зеленчуци са съставки на много традиционни наши храни. Целта на проучването бе да се сравни антибактериалната активност на органично отглеждани магданоз, домати, лют червен пипер, лук и чесън срещу референтни тестови бактерии - *Bacillus subtilis* No8751 и *Escherichia coli* No8752, предоставени от Националната Банка за Микроорганизми и Клетъчни Култури. В проучването бяха използвани 16 растителни проби. Експериментите бяха проведени по метода дифузия в агар. Петрита бяха култивирани при 37°C в продължение на 24 часа.Нашите резултати показват ниска антибактериална активност на пробите от домати и магданоз срещу Грам (+) *B. subtilis* и от слаба до отсъстваща активност срещу Грам (-) *E. coli*. Пробите пресен чесън показаха ниска антибактериална активност срещу Грам (+) бактерии само листата от пресен чесън, показаха по-широки стерилни зони. Главите и листата от пресен лук не демонстираха антибактериално действие.Зрелите глави чесън, обаче, показват ясно изразена активност срещу Грам (-) *E. coli* (30 мм диаметър на зони). Тъканите и семената от лют червен пипер притежават антибактериални свойства както срещу *E. coli*, така и срещу *B. subtilis*. Силна антимикробна активност срещу *B. subtilis* беше показана от пробите зрял лук с диаметър 25 мм при зоните от червен лук и зони с диаметър от 27 мм за главите бял лук. И двете проби зрял лук показаха ниска активност към *E. coli*.

*Corresponding author: alexanderat@abv.bg, Department Natural Sciences, New Bulgarian University, 21 Montevideo Str., 1618 Sofia

Introduction

Currently there is a need for new antimicrobial agents, because of the multidrug resistance of pathogenic bacteria. Many studies are focused on finding new bio-active compounds in extracts derived from plants. Preparations containing such compounds as the principal physiologically active constituents have been used to treat human diseases. Reports of activity in the field of antibacterial flavonoid research are widely conflicting, probably owing to inter- and intra-assay variation in susceptibility testing (Tim and Lamb, 2006). Yee-Lean *et al.* (2003) reports, that among the vegetable and fruit extracts, all fresh vegetables showed no antibacterial activity, but purple and red vegetable and fruit juices had antibacterial activities.

Petroselinum crispum has been widely used in folk medicine and has been reported to possess antibacterial activity against both Gram (+) and Gram (-) bacteria (Bin *et. al*., 2007; El Astal *et. al*, 2005; Peter *et al*., 2006; Sayyednejad and Damab, 2008).

Tomatoes are potent antioxidants and scavengers of free radicals (Richard and Pajkovic, 2008). Tomato-based bio-films have been reported to protect against bacterial pathogens and spoilage while also enhancing sensory properties of foods (Wen-Xian *et al.,* 2008, 2012).

Capsicum species produce fruits that synthesize and accumulate unique hot compounds known as capsaicinoids in the tissues and seeds (Cesar *et al.,* 2011; Mariângela *et al.,* 2011). Bio-autographic tests have demonstrated that capsaicin is the main antimicrobial component (Soediro *et al.,* 1997). *Capsicum annuum* fruits have also been used in traditional medicine for their antibacterial activity against a broad spectrum of bacterial species (Mariângela *et al.,* 2011; Rose *et al.,* 2012; Soediro *et al.,* 1997). Antimicrobial, antifungal and antioxidant activity has also been reported for *Allium cepa*, showing it as a new potential source of bio-active compounds due to the presence of Flavonoids (Cammue *et al.,*, 1995; Chun-Lin *et al.,* 2013; Freddy *et al.,* 2006; Jonathan *et al.,* 2010).

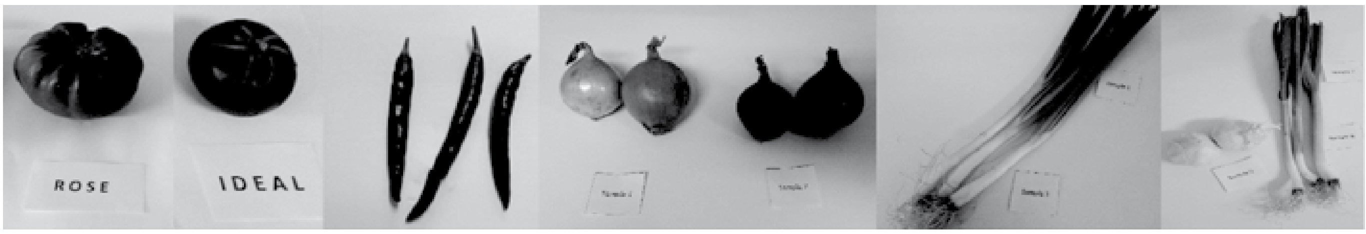
Garlic has been used for its medicinal properties for thousands of years, investigations on its mode of action were recently performed. Recent research shows *Allium sativum* as a promising source of antibacterial agents that can be used in food preservation and pharmaceutics (Srinivasan *et al.,* 2009).

The geographic location of Bulgaria provides one of the best environments for cultivating organic vegetables rich in bio-active substances, without the need for chemically enriched manure or other soil additives. The aim of our study was to compare the antibacterial activity of organically grown Bulgarian parsley, tomatoes, peppers, onions and garlic against referent Gram (+) and Gram (-) test bacteria.

Materials and Methods

Nine different organically grown vegetables were used in our study: parsley, two varieties of tomato, cayenne peppers, white, red and fresh onions, mature and fresh garlic. The vegetable samples were divided in two parsley samples, eight tomato samples, three garlic samples and four onion samples. Samples were prepared in a way closest to human consumption. The samples were analyzed *via*agar diffusion method against Gram (-) *E. coli* and Gram (+) *B. subtilis*, supplied by the National Bank for Microorganisms and Cell Cultures.

- Parsley samples were separated in stems and leaves.
- Tomato samples from two varieties were used: D5 - "Rose" and D6 - "Ideal". The samples were prepared as dried tomato puree, raw tomato puree, tomato seeds and tissue slices in order to note the antibacterial properties of each part of the tomato fruit for both varieties.
- The pepper fruits were cut open with a sterile scalpel and were separated in 3 samples - raw tissues, tissue disks (d = 0.5mm) and seeds.
- The onion samples were divided in white and



a b c d e f g

Samples: tomatoes (a), cayenne peppers (b), white onion (c), red onion (d), fresh onion (e), mature garlic (f) and fresh garlic (g)

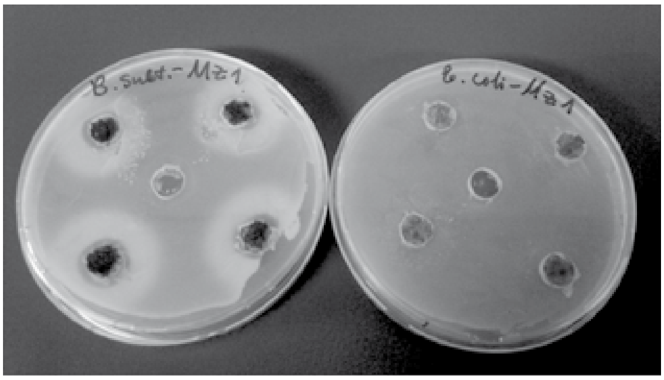


Fig. 1. Antibacterial activity of parsley leaves against *B. subtilis* N°8752 and *E. coli* No8751

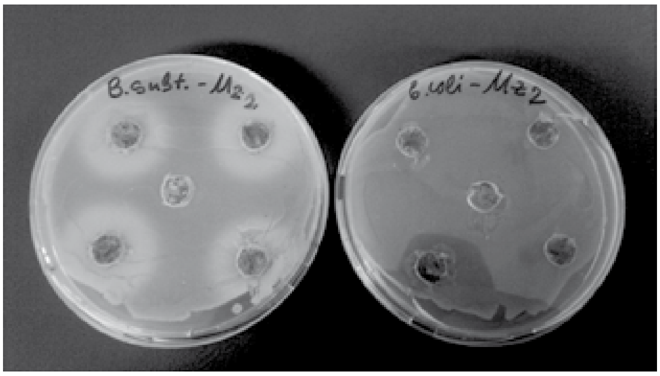


Fig. 2. Antibacterial activity of parsley stems against *B. subtilis* No8752 and *E. coli* No8751

red onion bulbs, fresh onion bulbs and fresh onion leaves.

- Mature garlic bulbs, fresh garlic bulbs and fresh garlic leaves were used for the garlic samples.

The parsley leafs and stems were homogenized using a mortar and pestle, while the tomato, onion and garlic samples were separately homogenized using a sterilized blender. The dried tomato puree was prepared by drying part of the raw puree at 37oC for 24 hours. The cayenne pepper tissues were scraped away with a sterile scalpel.

Nutrient agar petri dishes were inoculated with 0.1ml $1x10^9$ CFU/ml of the test microorganisms the Gram (-) *E. coli* No8752 and Gram(+) *B. subtilis* No8751 (supplied by the National Bank for Microorganisms and Cell Cultures). Wells (d = 9mm) were then aseptically perforated in the Nutrient agar to hold the homogenized samples and the raw cayenne pepper tissues.

The tomato and pepper seeds, tomato tissue slices and the pepper tissue disks were placed on the surface of the inoculated agar.

Petri dishes were cultivated at 37°C for 24 hours. Sterile zones around the wells, seeds and slices were then measured.

Results and Discussion

The obtained results demonstrateddiverse effects of the vegetable samples on the test bacteria's growth ranging from no to strong antibacterial activity as follows:

Parsley Antibacterial Activity

Parsley leafs inhibit the growth of *B. subtilis* (2 mm inhibition zone) and are not active against *E. coli*. Similar results were obtained for parsley stems: no antibacterial activity was registered (Fig. 1, Fig. 2). At the same time, El Astal *et al.* (2005) reports that *E. coli* was more affected by the ethanolic parsley extracts than Gram (+) bacteria (6).

Raw Tomato Puree Antibacterial Activity

Wan-Xian D.'s research (2008) shows that edible films made from tomatoes containing carvacrol possess antimicrobial effects against *E. coli*. Our investigation demonstrated that the raw puree, from sample D5 ("Rose") exhibited no antibacterial activity against *B. subtilis* and *E. coli*. Sample D6 ("Ideal") displayed antibacterial activity against both *B. subtilis* and *E. coli* with zone diameter of 4 mm against *B. subtilis* and about 4.5 mm against *E. coli.* (Fig. 3)

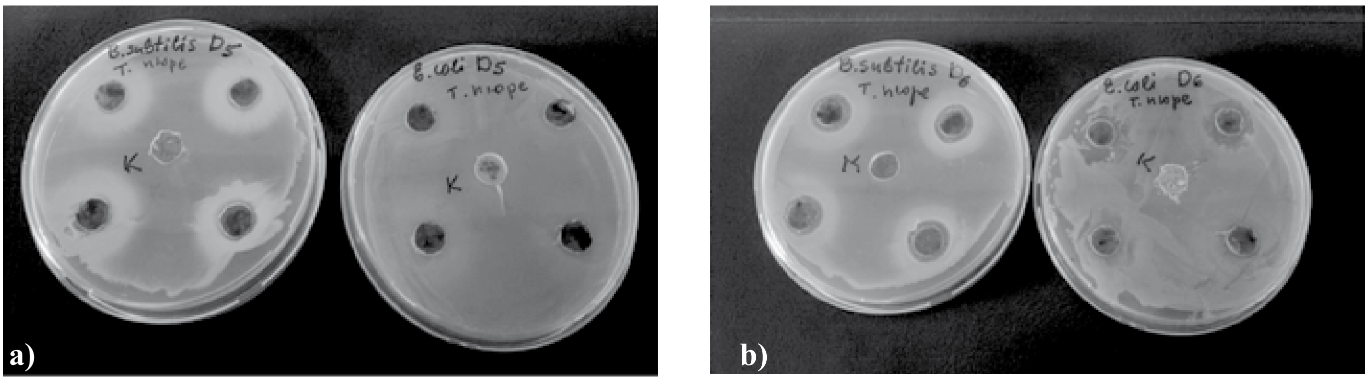
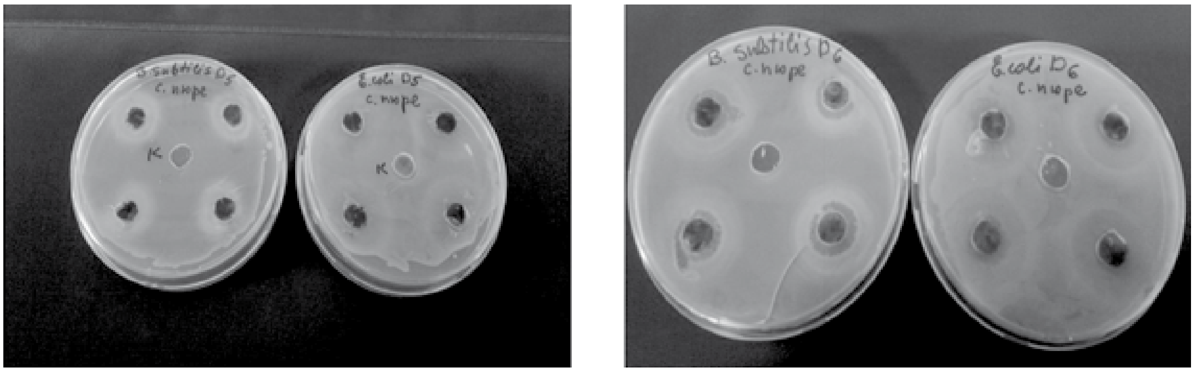
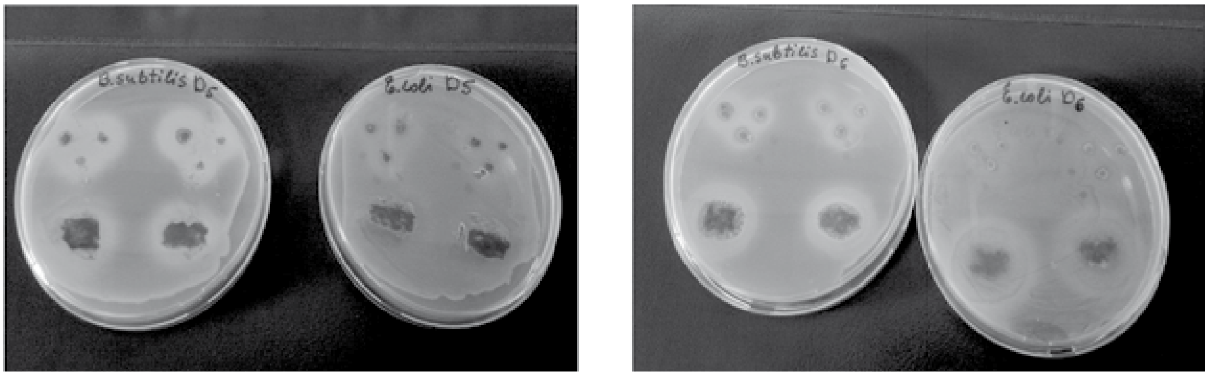


Fig. 3. Antibacterial activity of raw tomato puree,of variety D5 (a.) and D6 (b.), against *B. subtilis* N°8752 and *E. coli* N°8751.



a) b)

Fig. 4. Antibacterial activity of dried tomato puree, of variety D5 (a.) and D6 (b.), against *B. subtilis* N°8752 and *E. coli* N°8751.



a) b)

Fig. 5. Antibacterial activity of tomato seeds and tissue slices, of variety D5 (a.) and D6 (b.), against *B. subtilis* N°8752 and *E. coli* N°8751.

The D5 dried puree samples also displayed no antibacterial activity against *B. subtilis* and *E. coli*. The D6 dried puree samples also displayed-larger sterile zonesthan the D5 dried puree samples against bothbacteria -with zone diameter of 5 mm against *B. subtilis* and about 3.5 mm against *E. coli* (Fig. 4).

Tomato tissue slices from samples D5 and D6 display no antibacterial properties against *B. subtilis*, or against *E. coli*. (Fig. 5)

Tomato seeds of samples D5 (Rose) and D6 (Ideal) inhibit the growth of *B. subtilis*, but display no antibacterial activity against *E. coli*, for both samples D5 and D6. The D5 ("Rose" variety) sample exhibited sterile zones with range between 4 mm. and 7 mm, while the D6 ("Ideal" variety) zones had a range of about 5 mm. A more recent research made by Naseer *et al.,* (2012) showed results similar to ours as it reports low antibacterial activity in tomato fruits against *E. coli* (Fig. 5).

Cayenne Pepper Antibacterial Activity

Cayenne pepper fruits and seeds inhibit the growth of both *E. coli* and *B. subtilis*. Pepper tissues displayed no antibacterial activity. Cayenne pepper discs showed antibacterial activity against both *E. coli* (25 mm inhibition zone diameter) and *B. subtilis* (24 mm). The seeds also exhibited inhibition zones of 11 mm against *E. coli* and 7 mm against *B. subtilis*.The results for the pepper tissues are not shown (Fig. 6 and Fig. 7). Other recent and previous studies, like the ones conducted by Mariângela *et al.* (2011) and Soediro *et al*. (1997), also report the Capsicum's fruit antibacterial activity against both Gram (+) and Gram (-) bacteria.

Onion Antibacterial Activity

The authors Cammue *et al.,* (1995) and Jonathan *et al.,* (2010) describe *Allium* extracts to be more effective against Gram (+) microorganisms, while Gram (-) bacteria were reported to be less susceptible. Freddy *et al*. (2006) described water extracts from yellow onion skin to be active against Gram (-) bacteria. All of our onion samples showed antibacterial activity against both Gr (+) and Gr (-) bacteria except the fresh onion bulbs. White and red onion bulbs displayed inhibition zones of 27 mm and 25 mm against *B. subtilis* and 3 mm against *E. coli*. (Fig. 8). Fresh onion bulbs and leaves showed no antibacterial activity against both bacteria strains (Fig. 9).

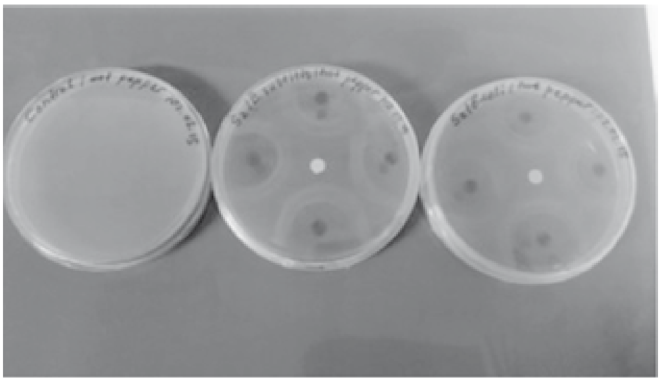


Fig. 6. Antibacterial activity of Cayenne pepper tissue discsagainst *B. subtilis* N°8752 and *E. coli* N°8751.

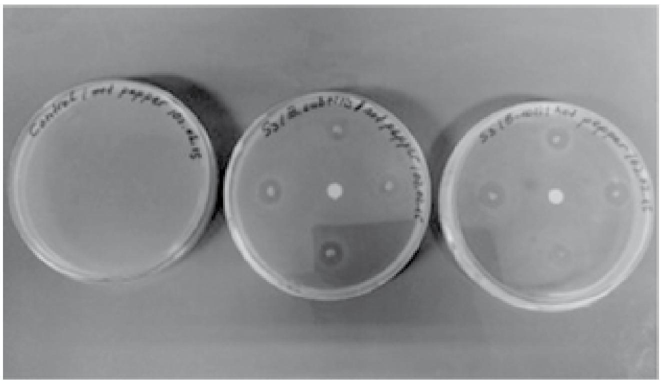
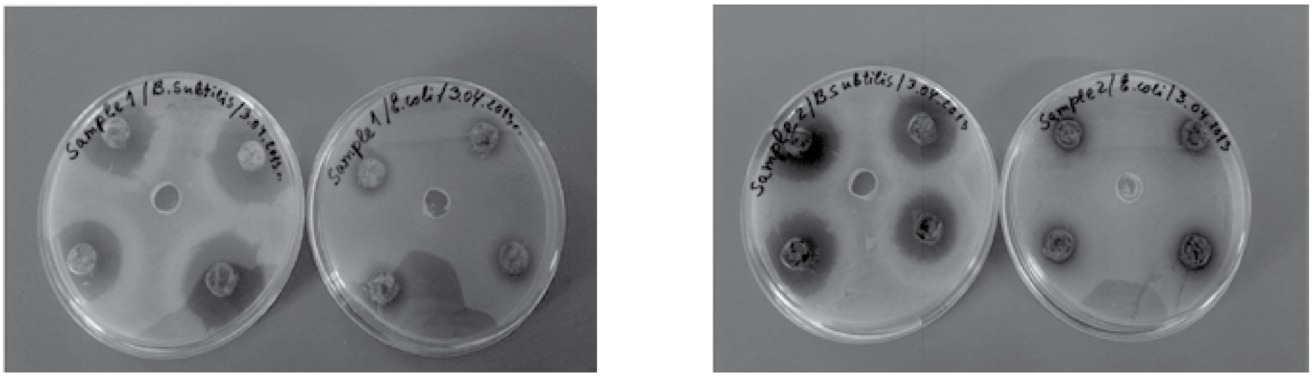


Fig. 7. Antibacterial activity of Cayenne pepper seedsagainst *B. subtilis* N°8752 and *E. coli* N°8751.



a) b)

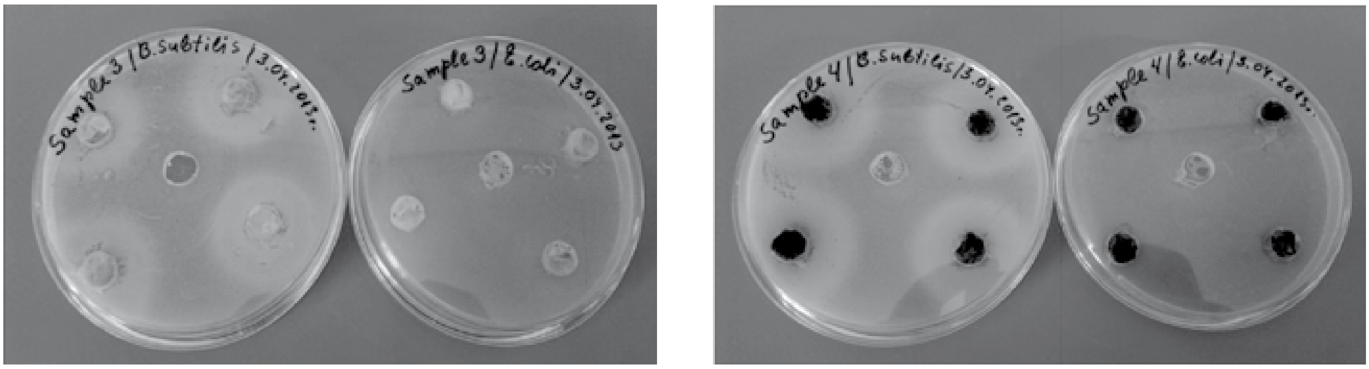
Fig. 8. Antibacterial activity of white (a.) and red (b.) onion bulbsagainst *B. subtilis* N°8752 and *E. coli* N°8751.

Garlic Antibacterial Activity

Mature and fresh garlic bulbs demonstrated low antibacterial activity against *B. subtilis* (7 mm for mature garlic bulbs and 2 mm for fresh garlic bulbs). Fresh garlic bulbs displayed no activity against *E. coli*, but mature garlic bulbs showed inhibition zones on 30 mm against *E. coli*. (Fig. 10) The fresh garlic leafs displayed antibacterial activity against both *E. coli* (13 mm) and *B. subtilis* (17 mm). Srinivasan *et al.,* (2009) reports antibacterial activity of *Allium sativum* against both Gram (+) and two Gram (-) pathogenic bacteria, with the maximum zone of inhibition observed against *Bacillus subtilis*. His analysis revealed that the antimicrobial effectiveness is time- and temperature-dependent (Fig. 11).

Conclusions

In conclusion, cayenne peppers showed antibacterial activity against both *B. subtilis* and



a) b)

Fig. 9. Antibacterial activity of fresh onion bulbs and leavesagainst *B. subtilis* N°8752 and *E.coli* N°8751.

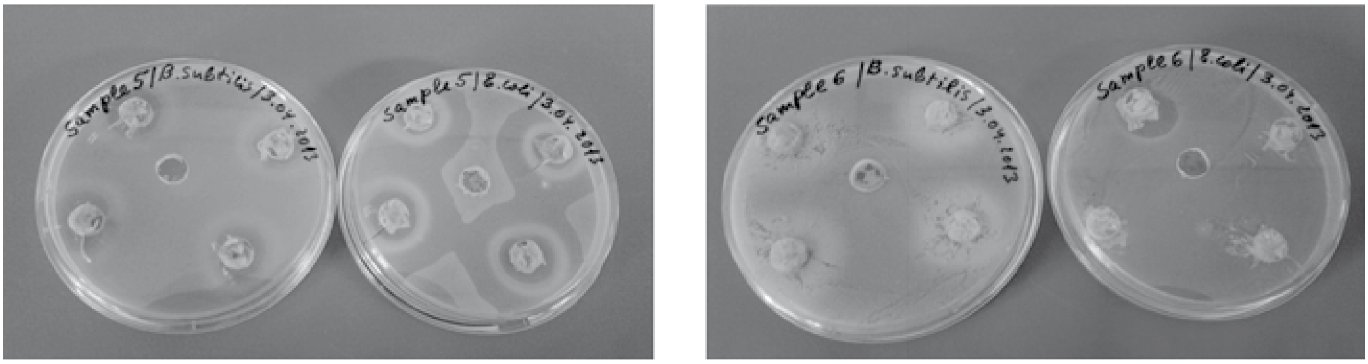


Fig. 10. Antibacterial activity of mature (a.) and fresh (b.) garlic bulbsagainst *B. subtilis* N°8752 and *E. coli* N°8751.

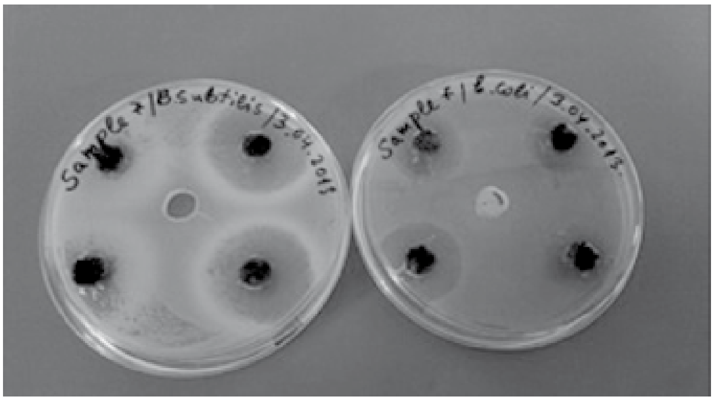


Fig. 11. Antibacterial activity of fresh garlic leavesagainst *B. subtilis* N°8752 and *E. coli* N°8751.

E. coli. Mature onion bulbs were active against only *B. subtilis*. Greatest antibacterial activity was demonstrated by mature garlic bulbs. Moderate antibacterial activity was observed by fresh garlic leaves. Our results demonstrated low antibacterial activity of tomato and parsley samples against the *B. subtilis* and from weak to no activity against the *E. coli*.

Acknowledgements

The study was conducted at the Bio Laboratory of New Bulgarian University and was supported by the Faculty of Basic Education of New Bulgarian University.

Table 1. Antibacterial activity of organically grown vegetables

	Parsley		Tomato								Cayenne Pepper			Onion				Garlic		
Sample	Parsley stems	Parsley leaves	Raw puree		Dried puree		Seeds		Tissue Slices		Raw tissue	Tissue discs	Seeds	White Bulbs	Red Bulbs	Fresh bulbs	Fresh leaves	Mature bulbs	Fresh bulbs	Fresh leaves
			D5	D6	D5	D6	D5	D6	D5	D6										
B. subtilis	0	2	0	4	0	5	6	5	0	0	n.a.	24	7	27	25	0	0	7	2	17
E. coli	0	0	0	4.5	0	3.5	0	0	0	0	n.a.	25	11	3	3	0	0	30	0	13

All figures are in mm

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