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ANTIBACTERIAL ACTIVITY OF MATURE AND GREEN *ALLIUM CEPA*

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Abstract: Antibacterial *in vitro* activity of white (bulb), red (bulb) and spring (bulb and leafs) onion towards the Gram-negative *Escherichia coli* 8752 and the Gram-positive *Bacillus subtilis* 8751 was investigated. Mature onion samples were purchased from a big supermarket while the green onion was organically grown. Experiments were performed *via* agar well diffusion method. Samples were separately homogenized with a blender until reaching fine paste. 0.1 mL of the test bacteria at concentration $1x10^9$ CFU/mL were inoculated on the nutrient agar. The onion paste (0.25 g) was loaded in aseptically perforated in the agar wells with a diameter 9 mm. The petries were cultivated at 37^o C for 24 hours and further the sterile zones around the wells were measured. Our results revealed that the white and red onion bulbs were active against Gram–positive bacteria showing 27 mm and 25 mm inhibition zone and displayed weak activity towards Gram-negative bacteria. Green onion bulbs and leafs did not inhibited the growth of *B. subtilis* and *E. coli*. The highest inhibition grade was registered for the white variety of *Allium cepa.*

Key words: antibacterial activity, medicinal plants, *Allium cepa*; mature onion, green onion

INTRODUCTION

Onion belongs to genus *Allium*, fam. Amarillidaceae. Onion species are cultivated at different altitudes in Europe, Asia, North America and Africa [26,14]. The plant is used not only in culinary but also as a medicinal plant and is selectively cultivated over 5 000 years. In last decade the world production of onions increased with 25 % reaching 45 million tons and became the second most important crop product after tomatoes. Increased interest to onion is due to the heavy promotion of its beneficial health effect [14]. Onion is rich in biologically active substances as flavonoids, sulphur and seleno compounds but the biochemical mechanisms of their action are still not clear [5, 1, 23, 9, 10]. Anthocianins, responsible for the colour of red onion and quecertins which give the yellow and brown colour of onion skin also belong to flavonoids [3].

Onion compounds possess anticarcinogenic, antidiabetic, antithrombotic, antiasthmatic, antioxidant, antimicrobial and antiaging properties [25, 14, 21, 22, 4]. WHO report describes hypoglycaemic and platelet antiaggregation effects of onion bulbs *in vivo.*

Crash of the onion bulb leads to release of trans-(+)-S-(1-propenil)-L-cysteine sulphoxide which is hydrolyzed by the enzyme aliinase stored in the cell vacuoles. Product of the enzymatic reaction is (Z)-propanethial-S-oxide which cause tears [2]. Alkylthiosulphonates are main aroma compounds and release only in fresh chopped onion while the sulphides accumulate only in stored extracts. Propyl- and propenyl disulphides and trisulphides are responsible for the aroma of cooked onion. The aroma of fried onion is due to dimethylthiofenes. Precursors of onion aroma are S-methyl and S-propyl-L-cysteine sulfoxide which are biosynthesized from valine and cysteine [2].

Over 90 % of soluble organic-bound sulfur in onion is found in γ-glutamilcysteine peptides not hydrolyzed by alliinase. γ-glutamilcysteine peptide serve as storage reserve and are important for seeds germination [25]. Sulfur-rich onion compounds metabolized in the human liver microsomes are being substrates for FMO and CYPs [24].

In recent years, large number of pathogenic bacteria showed multidrug resistance (MDR) which force to investigate novel antimicrobial agents, especially from the medicinal plants. Medicinal plants are abundant source of antimicrobial substances and alternative to the toxic synthetic chemicals [12, 16, 20].

Investigations on the antibacterial activity of crude onion juice and onion extracts started in 80^{-es} [11, 13]. According to authors the crude juice is active against Gram-positive bacteria and ineffective upon Gram-negative bacteria. Studying the antibacterial activity of onion extracts on the mouth microflora Elnima et al. [13] and Kim [18] reported that washing the mouth with onion extract leads to moderate bacteriostatic or bactericidal effect.

Antimicrobial activity of *Allium cepa* was reported by Benmalek et al. [4] and of *Allium flavum* - by Curkich [9]. Antibacterial action of onion is due to the peptide compounds, polyphenols and sulfur-rich compounds. One of the organosulphur compounds is the cyclic zwibelane (cis-2,3-dimethyl-5,6-dithiabicyclo[2.1.1] hexane 5-oxide) which enhance the antifungal activity of Polymixin B disrupting vacuole of *Saccharomyces cerevisiae* [6]. The yeast vacuole is a main target for the antifungal agents. According to Kim et al. [19] onion essential oil disposes antiyeast activity towards *Zygosaccharomyces rouxii* preventing its growth for 30 days. The protein Ace-AMP1, unusually rich in arginine and isolated from onion seeds [7] showed activity towards 12 fungal pathogenic strains. The protein inhibited Gram-positive but not the Gram-negative bacteria. According the antibacterial activity of heat treated onion important results were reported by Chen et al. [8]. The authors showed that heat treatment in boiling water for 20 minutes of green onion bulb and leafs lead to destruction of the antibacterial components. Recently, antituberculosis activity of onion was reported by Gupta et al. [15]. Authors examined five plants, including *A. cepa*. Even with moderate activity, onion demonstrated inhibition of *Mycobacterium tuberculosis*. Except its antituberculosis effect onion was reported to inhibits also the growth of clinical isolates of *Vibrio cholerae* [17]. Arnault and Auger [1] described the anticancer activity of onion.

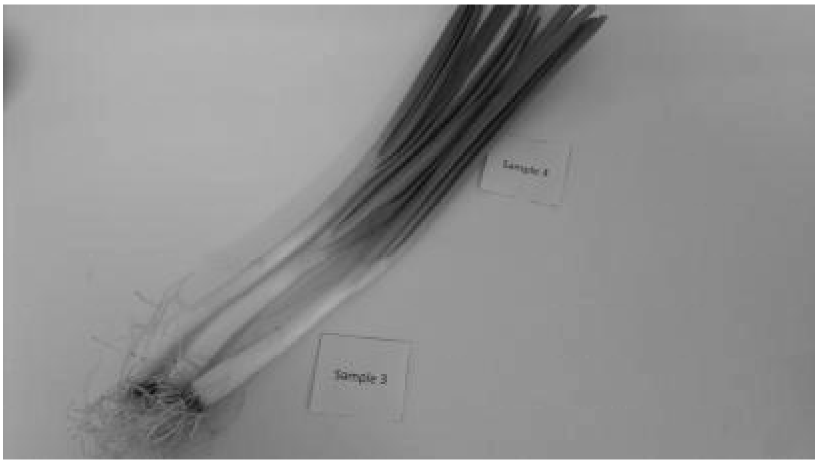
Up to now in the literature were reported data about antibacterial action only of the mature onion bulbs but not for the green onion bulbs or leafs.

Aim of this study was to investigate the antibacterial activity of white and red onion mature bulbs as well of green onion bulbs and leafs. This is the first report describing antibacterial activity of green onion. Investigation is conducted using onion paste – in a way closest to the human consumption.

MATERIALS AND METHODS

Samples 1 (bulb of white onion) and 2 (bulb of red onion) were supplied by a big supermarket. Green onion - sample 3 (bulb) and sample 4 (leafs) was organically grown (Fig.1).

a)



b)

Fig. 1. Studied samples

All samples were separately homogenized with a blender. Test bacteria *Bacillus subtilis* No 8751 and *Escherichia coli* No 8752 were supplied by the National Microbial and Cell Culture Collection, Bulgaria. Antibacterial activity was assessed *via* the agar well diffusion method. Nutrient broth was supplied by HiMedia Laboratories (India). Agar was delivered by Merck (Germany). Further 0.1 mL of the test bacteria ($1x10^9$ CFU/mL) were spread on nutrient agar. Bacterial concentration was assessed using spectrophotometer (Yenway, UK) at 520 nm. The absorption for *E. coli* was 0.740 and 0.360 - for *B. subtilis*. Samples of onion paste (0.25 g) were loaded in aseptically perforated in the inoculated agar wells with diameter 9 mm. In the middle of each petri was left an empty well as a control. Petri dishes were cultivated at 37 ^{o}C for 24 h and sterile zones around the wells were measured. Experiments were performed in four replicas.

RESULTS AND DISCUSION

Newer antimicrobial agents are currently required because of multidrug resistance of the pathogenic bacteria. In this study different mature and green onion samples were examined for their antibacterial activity. Onion samples were prepared in a closest to the human consumption way (Fig. 2).

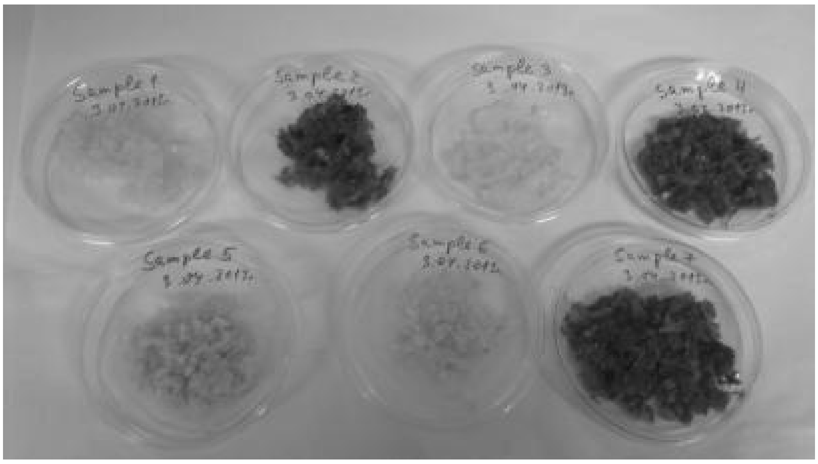


Fig. 2. Homogenized samples (see samples 1-4)

Antibacterial activity of white onion bulbs

As shown on Fig. 3 the bulbs of white variety of *Allium cepa* demonstrated pronounced antibacterial activity against *Bacillus subtilis* (27 mm) and weaker activity against *Escherichia coli* (13 mm). This finding coincide the results reported by Dankert et al. [11]. The authors reported that onion possess low antibacterial activity towards Gram-negative bacteria.

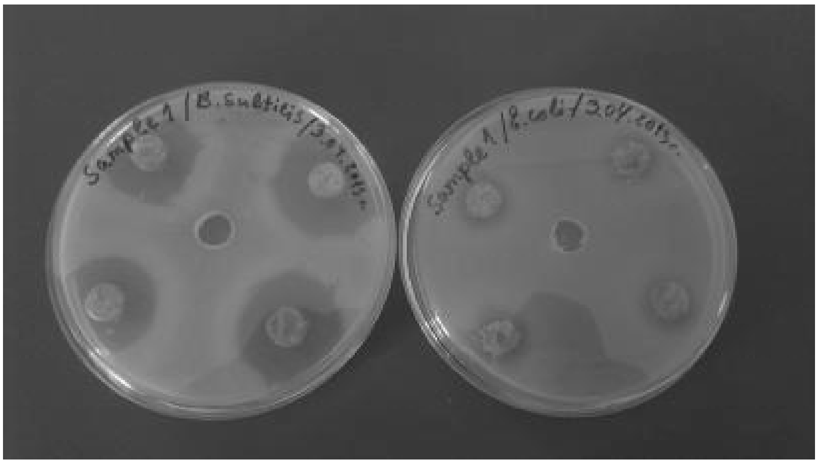


Fig. 3. Antibacterial activity of white onion bulb against *B. subtilis* and *E. coli*

Antibacterial activity of red onion bulbs

Results obtained for red onion bulb are similar to those of the white onion bulb. Sample of red onion bulb inhibited significantly the growth of *Bacillus subtilis* (25 mm) and was less effective to *E. coli* (12 mm) (Fig. 4).

Fig. 4**.** Antibacterial activity of red onion bulb against *B. subtilis* and *E. coli*

Antibacterial activity of green onion bulb

Green onion bulb showed no antibacterial activity against both *Bacillus subtilis* (9 mm) and *E. coli* (9 mm) (Fig. 5). Our report describe for the first time the activity of onion leafs. Results about the antibacterial activity of heat treated green onion were discussed by Chen et al. [8] showing no activity.

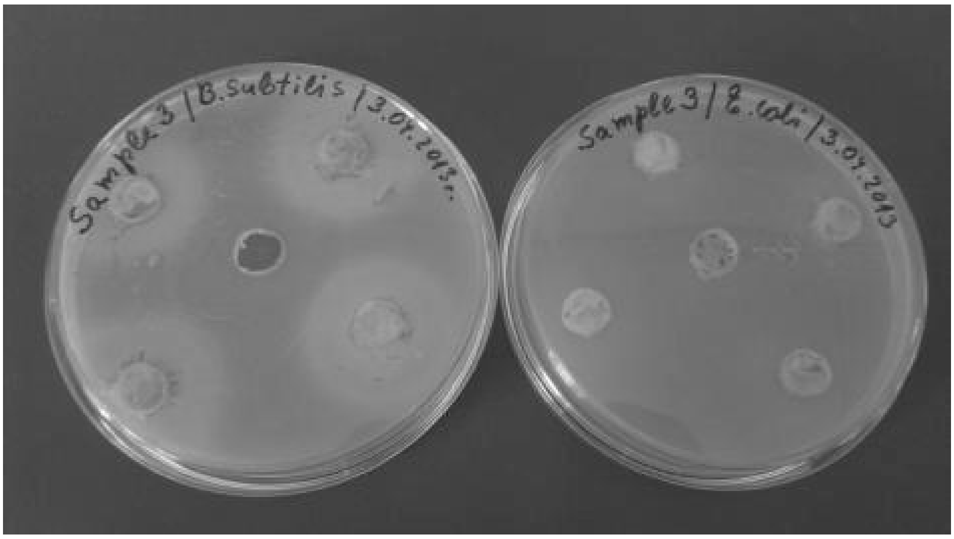


Fig. 5. Antibacterial activity of green onion bulb against *B. subtilis* and *E. coli*

Antibacterial activity of green onion leafs

Leafs of green onion also showed similar negative effect – the sample was not effective against both *B. subtilis* (9 mm) and *E. coli* (9 mm). Data are shown on Fig. 6.

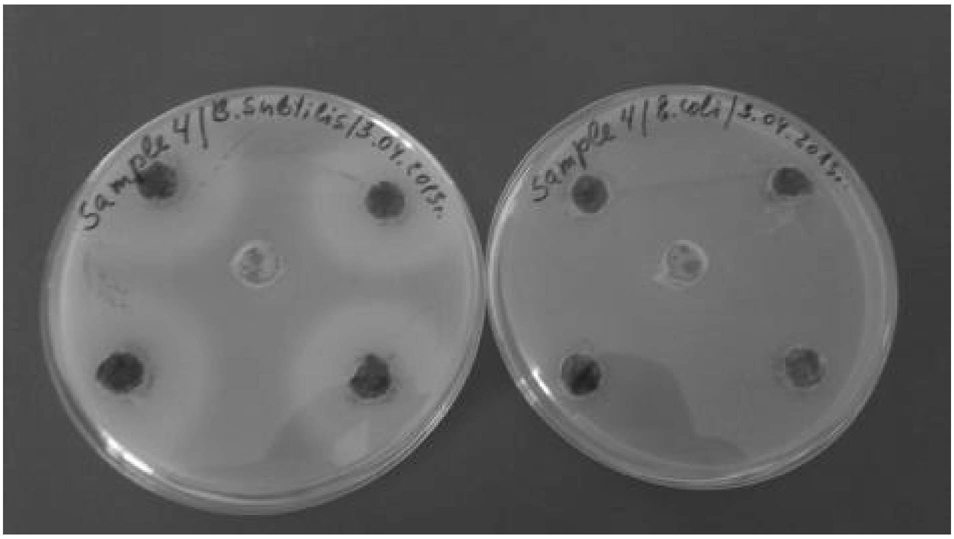


Fig. 6. Antibacterial activity of green onion leafs against *B. subtilis* and *E. coli*

We suppose that the weak antibacterial effect of green onion is due to high water content of the plant and less concentration of sulfur compounds which accumulate during the onion maturation.

CONCLUSIONS

Our investigation demonstrate that white and red onion bulbs possess the highest antibacterial activity. The white onion was active against Gram-positive bacteria - 27 mm sterile zone. The red onion showed 25 mm zone against Gram-positive bacteria. Both samples showed weaker activity towards Gram-negative bacteria – 13 mm and 12 mm, respectively. Green onion bulbs and leafs demonstrated no antibacterial activity against *B. subtilis* and *E. coli.*

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АНТИБАКТЕРИАЛНАТА АКТИВНОСТ НА ЗРЯЛ И ЗЕЛЕН ЛУК

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Резюме: В изследването е проучена антибактериалната активност на бял (луковици), червен салатен (луковици) и пролетен зелен лук (луковица и листа) спрямо Грам-отрицателни - *Escherichia coli* 8752 и Грам-положителни бактерии - *Bacillus subtilis* 8751. Луковиците от зрелия лук бяха закупени от голяма верига супермаркети, докато пролетния зелен лук беше органично отгледан. Експериментите бяха осъществени по метода на дифузия в агар. Пробите бяха хомогенизирани чрез пасатор до получаването на фина маса. 0.1 mL от култура на тестовите бактерии с концентрация $1x10^9$ CFU/mL бяха инокулирани върху месо-пептонен агар. Пробите от лук (0.25 g всяка) бяха поставени в стерилно пробити в агара отвори с диаметър 9 mm. Петритата бяха инкубирани за 24 часа на 37^o C, след което бяха измерени стерилните зони около ямките. Резултатите показаха, че белия (кромид) и червения салатен лук са активни срещу Грам-положителни бактерии, образувайки стерилни зони съответно от 27 mm и 25 mm; двете проби показаха слаба активност срещу Грам-отрицателни бактерии. Пробите от луковица и листа на зеления пролетен лук не подтискаха растежа нито на *B. subtilis*, нито на *E. coli*. В заключение, най-силно подтискане на бактериалния растеж беше установено при белия (кромид) лук.

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