DOI: 10.32999/ksu2524-0838/2022-33-6
УДК 615.28:615

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EVALUATION OF ANTIBACTERIAL ACTIVITY OF COMMERCIAL GREEN TEA

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This study aimed to the impact of infusion derived from commercial green leaf tea (Distributer: Auchan Polska Sp. Z o.o.; Country of origin: China; Netto weight 100 g) on the growth of some Gram-positive and Gram-negative strains was studied. In the current study, Gram-negative strains such as Escherichia coli (Migula) Castellani and Chalmers (ATCC®25922™), Escherichia coli (Migula) Castellani and Chalmers (ATCC®35218™), Pseudomonas aeruginosa (Schroeter) Migula (ATCC®27853™) and Gram-positive strains such as Staphylococcus aureus subsp. aureus Rosenbach (ATCC®25923™), Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) (resistant to vancomycin; sensitive to teicoplanin) and Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™) were used. The testing of the antibacterial activity of green tea was carried out in vitro by the Kirby-Bauer disc diffusion technique. Results of the current study demonstrated that the most sensitive to infusion derived from commercial green tea was the Gram-negative bacterial strain Escherichia coli (Migula) Castellani and Chalmers (ATCC®35218™), where there was the greatest increase in the zone of growth inhibition compared to 96% ethanol (by 98%, $p < 0.05$). According to the Gram-positive bacterial strains, only the Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) strain showed sensitivity to infusion derived from commercial green tea, with a statistically significant increase in the zone of growth inhibition compared to 96% ethanol (by 57.4%, $p < 0.05$). Staphylococcus aureus subsp. aureus Rosenbach (ATCC®25923™) strain was also sensitive to infusion derived from commercial green tea. The zone of growth inhibition was increased by 37% compared to 96% ethanol ($p < 0.05$). These results demonstrate that the use of green tea can be applied to a variety of bacterial infections caused by both Gram-positive and Gram-negative bacterial strains. Further studies are needed to analyze the antimicrobial properties of the compounds in this plant.

Keywords: *green tea, antibacterial activity, Kirby-Bauer disc diffusion technique, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis.*

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ОЦІНКА АНТИБАКТЕРІАЛЬНОЇ АКТИВНОСТІ КОМЕРЦІЙНОГО ЗЕЛЕНОГО ЧАЮ

Це дослідження було спрямоване на вивчення впливу настою, отриманого з комерційного зеленого листового чаю (Дистриб'ютор: Auchan Polska Sp. Z o.o.; Країна походження: Китай; вага нетто 100 г) на ріст деяких грампозитивних і грамнегативних штамів. У поточному дослідженні було використано грамнегативні штами, такі як Escherichia coli (Migula) Castellani and Chalmers (ATCC®25922™), Escherichia coli (Migula) Castellani and Chalmers (ATCC®35218™), Pseudomonas aeruginosa (Schroeter) Migula (ATCC®27853™) і грампозитивні штами, такі як Staphylococcus aureus subsp. aureus Rosenbach (ATCC®25923™), Enterococcus faecalis (Andrewes and Horder) Schleifer і Kilpper-Balz (ATCC®51299™) (стійкі до ванкоміцину; чутливі до тейкопланіну) і Enterococcus

faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™). Тестування антибактеріальної активності зеленого чаю проводили in vitro методом дискової дифузії Кірбі-Бауера. Результати дослідження показали, що найбільш чутливим до настою, отриманого з комерційного зеленого чаю, був штам грамнегативних бактерій Escherichia coli (Migula) Castellani and Chalmers (ATCC®35218™), де спостерігалося найбільше збільшення зони росту, інгібування порівняно з 96% етанолом (на 98%, p < 0,05). Згідно зі штамами грампозитивних бактерій, лише штам Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) виявив чутливість до настою, отриманого з комерційного зеленого чаю, зі статистично значущим збільшенням зони росту інгібування порівняно з 96% етанолом (на 57,4%, p < 0,05). Staphylococcus aureus subsp. aureus Rosenbach (ATCC®25923™) також був чутливий до настою, отриманого з комерційного зеленого чаю. Зона пригнічення росту була збільшена на 37 % порівняно з 96 % етанолом (p < 0,05). Ці результати демонструють, що використання зеленого чаю може бути застосоване до різноманітних бактеріальних інфекцій, викликаних як грампозитивними, так і грамнегативними штамами бактерій. Необхідні подальші дослідження для аналізу антимікробних властивостей сполук цієї рослини.

Ключові слова: *зелений чай, антибактеріальна активність, диско- дифузійний метод Кірбі-Бауера, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis.*

INTRODUCTION

Tea, leaf, or bud from the plant *Camellia sinensis* (L.) Kuntze, make up some of the beverages popularly consumed in different parts of the world as green tea (unfermented), black tea (fully fermented), and oolong (semifermented) [14, 38]. More particularly, as a non-fermented tea, green tea has gained more renown because of the significant health benefits assigned to its rich content in polyphenols [38]. The consumption of green tea has been shown to have many physiological and pharmacological health benefits documenting antioxidant [15], anti-inflammation [24], anticancer [13], antimicrobial [23, 29], antihyperglycemic [37], and antiobesity properties [16, 34]. Recent reports demonstrate that green tea may exert a positive effect on the reduction of medical chronic conditions such as cardiovascular disease [11], cancer [7, 32], Alzheimer's disease [26], Parkinson's disease [21], arthritis [1], stroke [9], genital warts [22], skin wound [39], and diabetes [6]. Nowadays, multiple pharmacologically active components have been isolated and identified from green tea, including tea polyphenols, alkaloids, amino acids, polysaccharides, and volatile components [43].

Many studies have shown that green tea exhibits antibacterial effects against a variety of bacteria. Yee and Koo [41] (2000) first reported that green tea had the ability to inhibit *Helicobacter pylori* activity. Epigallocatechin gallate (EGCG) is probably the active ingredient responsible for most of the anti-*H. pylori* activity of Chinese tea [41]. Anand and co-workers [3] proposed that epigallocatechin-3-gallate may be of importance in the prevention of tuberculosis infection. Green tea extract could effectively inhibit the growth of major foodborne pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Listeria monocytogenes* [10]. The study of Si and co-workers [33] demonstrated a direct link between the antimicrobial activity of tea and its specific polyphenolic compositions. The activity of tea polyphenols, particularly EGCG on antibiotics-resistant strains of *S. aureus*, suggests that these compounds are potential natural alternatives for the control of bovine mastitis and food poisoning caused by *S. aureus* [33]. Sharma and co-workers [31] revealed that green tea extracts showed significant antibacterial activity against skin pathogens *in vitro*, and this mechanism was mainly related to preventing bacterial adhesion. The study of Fournier-Larente and co-workers [8] explored the preventive and therapeutic potential of green tea catechins against periodontal disease. In addition, to inhibit growth and adherence of *Porphyromonas gingivalis*, a green tea extract, and its main constituent EGCG was found to decrease the expression of genes coding for the major virulence factors [8]. Catechins in the tea are potentially anti-cariogenic agents which can reduce

bacterial presence in the oral cavity and have the potential to be further used for the preparation of dentifrice and mouthwash [12]. Green tea mouthwash had an antibacterial effect on saliva and bacterial plaque, suggesting that it could be a beneficial addition to standard oral hygiene measures [30].

Renzetti and co-workers [28] presented a variety of mechanisms for the antibacterial activity of green tea catechins. These mechanisms can be broadly classified into the following groups: (i) inhibition of virulence factors (toxins and extracellular matrix); (ii) cell wall and cell membrane disruption; (iii) inhibition of intracellular enzymes; (iv) oxidative stress; (v) DNA damage; and (vi) iron chelation [28]. Taylor [35] also noted that green tea-derived galloylated catechins have weak direct antibacterial activity against both Gram-positive and Gram-negative bacterial pathogens and are able to phenotypically transform, at moderate concentrations, methicillin-resistant *Staphylococcus aureus* (MRSA) clonal pathogens from full β-lactam resistance to complete susceptibility.

In the current study, the impact of infusion derived from commercial green leaf tea (Distributer: Auchan Polska Sp. Z o.o.; Country of origin: China; Netto weight 100 g) on the growth of some Gram-positive and Gram-negative strains was studied.

MATERIALS AND METHODOLOGY

Green leaf tea. The commercial green leaf tea (Distributer: Auchan Polska Sp. Z o.o.; Country of origin: China; Netto weight 100 g) was used in the current study. Brewing method: Dried leaves were weighed (1 g) and poured with water (10 mL) at a temperature of 80 ^{o}C. The brewing time was 30 min. Green tea infusion was then used for the antibacterial assay.

Determination of the antibacterial activity of plant extracts by the disk diffusion method. The testing of the antibacterial activity of commercial green leaf tea was carried out *in vitro* by the Kirby-Bauer disc diffusion technique [4]. In the current study, Gram-negative strains such as *Escherichia coli* (Migula) Castellani and Chalmers (ATCC®25922™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC®35218™), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC®27853™) and Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC®25923™), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™) were used.

The strains were inoculated onto Mueller-Hinton (MH) agar dishes. Sterile filter paper discs impregnated with green tea infusion were applied over each of the culture dishes. Isolates of bacteria with green tea infusion were then incubated at 37 °C for 24 h. The Petri dishes were then observed for the zone of inhibition produced by the antibacterial activity of green tea. A control disc impregnated with 96% ethanol was used in each experiment. At the end of the 24-h period, the inhibition zones formed were measured in millimetres using the vernier. For each strain, eight replicates were assayed (n = 8). The Petri dishes were observed and photographs were taken. The susceptibility of the test organisms to the green tea was indicated by a clear zone of inhibition around the discs containing the green tea infusion and the diameter of the clear zone was taken as an indicator of susceptibility. Zone diameters were determined and averaged. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤ 10 mm [25, 36].

Statistical analysis. Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean ± standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of the green tea tested. All statistical calculation was performed on separate data from each strain. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., Krakow, Poland) [42].

RESULTS AND DISCUSSION

Figure 1 is presented the results obtained by the mean diameters of the inhibition zone around the growth of some Gram-positive and Gram-negative strains induced by infusion derived from commercial green tea.

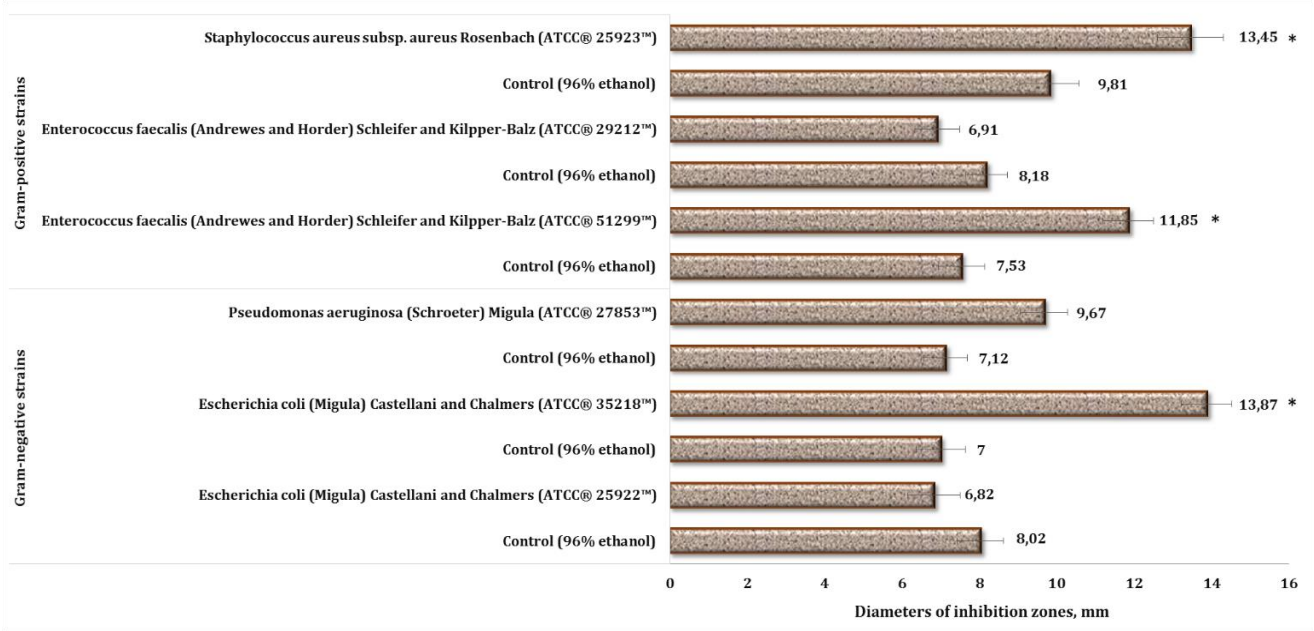

Fig. 1. The mean values of inhibition zone diameters around the growth of some Gram-positive and Gram-negative strains induced by infusion derived from commercial green tea (M ± m, n = 8),
The data were presented as the mean ± the standard error of the mean (S.E.M.).
* denote significant differences between the control (96% ethanol) and infusion derived from commercial green tea ($p < 0.05$).

A statistically non-significant decrease in the growth of inhibition zones of *E. coli* (Migula) Castellani and Chalmers (ATCC®25922™) after the application of infusion derived from commercial green tea by 15% ($p > 0.05$) compared to the 96% ethanol samples (6.82 ± 0.68 mm *vs.* 8.02 ± 0.61 mm) was observed. A similar statistically non-significant change after *in vitro* application of infusion derived from commercial green tea against the *E. faecalis* (Andrewes and Horder) strain Schleifer and Kilpper-Balz (ATCC®29212™) strain was noted, where we observed a decrease in the zone of inhibition by 15.5% ($p > 0.05$) compared to the 96% ethanol samples (6.91 ± 0.57 mm *vs.* 8.18 ± 0.55 mm) (Fig. 1).

A different trend was observed after the application of infusion derived from commercial green tea against the *P. aeruginosa* (Schroeter) Migula (ATCC®27853™) strain, where there was a statistically non-significant increase in the zone of bacterial inhibition by 35.8% ($p > 0.05$) compared to 96% ethanol samples (9.67 ± 0.61 mm *vs.* 7.12 ± 0.56 mm). A similar but statistically significant increase in the zone of growth inhibition by 57.4% ($p < 0.05$) was observed after the application of infusion derived from commercial green tea against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) strain compared to 96% ethanol samples (11.85 ± 0.65 mm *vs.* 7.53 ± 0.6 mm) (Fig. 1).

Similarly, when infusion derived from commercial green tea was applied *in vitro* to the *E. coli* (Migula) Castellani and Chalmers (ATCC®35218™), we noted a statistically significant increase in the zone of growth inhibition of this bacterial strain (by 98%, $p < 0.05$) compared to controls (13.87 ± 0.66 mm *vs.* 7.0 ± 0.64 mm). In a like manner, a statistically significant increase in the zone of growth inhibition was obtained after the application of infusion derived from commercial green tea

against the *S. aureus* subsp. *aureus* Rosenbach (ATCC®25923™) strain, where there was a statistically significant increase in the zone of growth inhibition by 37% ($p < 0.05$) compared to 96% ethanol (13.45 ± 0.85 mm *vs*. 9.81 ± 0.77 mm) (Fig. 1).

Our study is in line with studies conducted by other researchers exhibiting the antibacterial potential of *Camellia sinensis* (green tea) against Gram-positive and Gram-negative bacteria. For instance, Akroum [2] studied the antifungal activity of *Camellia sinensis* crude extracts against four species of *Candida* and *Microsporum persicolor*. The results showed that the acetone crude extract had the most important *in vitro* activity against the growth of *Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei,* and *Microsporum persicolor*. But *in vivo*, it was only the most active against *Candida albicans, Candida glabrata, Candida tropicalis,* and *Microsporum persicolor*. *Candida krusei* was more sensitive to the aqueous crude extract [2].

The antibacterial and antifungal potential of the aqueous extract of *C. sinensis* was investigated by a group of Khan and co-workers [19]. Antibacterial activity was determined by disc and well diffusion assay. MIC and MBC were calculated by broth dilution method. Miles and Misra's technique was used to finding out colony forming unit per/ml. All the test organisms revealed a diverse range of vulnerabilities against aqueous extract. Among Gram-positive, MRSA showed to be the most sensitive with the least MIC and MBC while Gram-negative *P. aeruginosa* exhibited the highest sensitivity. In Miles and Misra, a progressive decline in the log of CFU/ml was observed. In the time-kill assay, a decline was noted in the viable count of *S. aureus* after exposure to an 18% aqueous extract of *C. sinensis*. In the study of Khan and co-workers [19], an aqueous extract of *C. sinensis* was found to be effective against Gram-positive, Gram-negative, and fungi. The most important finding of this study is its aqueous extract inhibitory effect against drug-resistant microorganisms, e.g. MRSA, *P. aeruginosa*, and *C. albicans* [19].

Also, Radji and co-workers [27] have investigated the antimicrobial activity of green tea extract against isolates of methicillin-resistant *S. aureus* and multi-drug-resistant *P. aeruginosa*. The results showed that the inhibition zone diameter of green tea extracts for *S. aureus* ATCC 25923 and MRSA were (18.970 ± 0.287) mm, and (19.130 ± 0.250) mm, respectively, while the inhibition zone diameter for *P. aeruginosa* ATCC 27853 and MDR-*P. aeruginosa* were (17.550 ± 0.393) mm and (17.670 ± 0.398) mm, respectively. The MIC of green tea extracts against *S. aureus* ATCC 25923 and MRSA were 400 μg/mL and 400 μg/mL, respectively, whereas the MIC for *P. aeruginosa* ATCC 27853 and MDR-*P. aeruginosa* were 800 μg/mL, and 800 μg/mL, respectively. Thus, *C. sinensis* leaves extract could be useful in combating emerging drug resistance caused by MRSA and *P. aeruginosa* [27].

Moreover, green tea catechins showed antibacterial activity on *Streptococcus mutans*. S. *mutans*, the primary etiologic agent of dental caries, possesses a series of virulence factors associated with its cariogenicity [40]. Hattarki and co-workers [12] have evaluated the antibacterial effect of green tea catechins namely EGCG on *S. mutans* with two different methods at different concentrations. The results of the agar well diffusion method showed that the EGCG extract has shown zones of inhibition against *S. mutans* at concentrations of 100 μg/mL (28.67 mm), 75 μg/mL (15.33 mm), 50 μg/mL (10.33 mm) while that of MIC test of EGCG extract of concentrations ranging from 0.2 to 100 μg/mL against *S. mutans* shows that the mean MIC value was 1.07. Thus, catechins in the tea are potentially anti-cariogenic agents which can reduce bacterial presence in the oral cavity and have the potential to be further used for the preparation of dentifrice [12].

In the study of Xu and co-workers [39], researchers have investigated the biological effect of epigallocatechin gallate (EGCg) on the virulence factors of *S. mutans* associated with its acidogenicity and acidity. The antimicrobial effects of EGCg on *S. mutans* biofilm grown in a chemically defined medium were also examined. EGCg inhibited the growth of *S. mutans* planktonic cells at a MIC of 31.25 μg/ml and a minimal bactericidal concentration (MBC) of 62.5 μg/ml. EGCg also inhibited *S. mutans* biofilm formation at 15.6 μg/ml (minimum concentration that showed at least 90% inhibition of biofilm formation) and reduced viability of the preformed biofilm at 625 μg/ml (sessile MIC_{80}). EGCg at sub-MIC levels inhibited acidogenicity and acidity of *S.*

mutans cells. Also, EGCg significantly suppressed the ldh, eno, atpD, and aguD genes of *S. mutans* UA159. Inhibition of the enzymatic activity of F_1F_0-ATPase and lactate dehydrogenase was also noted (50% inhibitory concentration between 15.6 and 31.25 μg/ml). These findings suggest that EGCg is a natural anticariogenic agent in that it exhibits antimicrobial activity against *S. mutans* and suppresses the specific virulence factors associated with its cariogenicity [39].

Later, the antimicrobial properties of EGCG were evaluated by Khan and co-workers [19]. These researchers have examined its bactericidal activity, its inhibitory effects against bacterial growth, acid production, acidic end-product formation, and sugar uptake (phosphoenolpyruvate-dependent phosphotransferase system, phosphoenolpyruvate (PEP): carbohydrate phosphotransferase system (PTS) – PEP-PTS activity), and its effects on bacterial aggregation, using monoculture planktonic cells of *S. mutans* and non-mutants streptococci. Co-incubating *S. mutans* with EGCG (1 mg/mL) for 4 h had no bactericidal effects, while it decreased the growth and acid production of *S. mutans* by inhibiting the activity of the PEP-PTS. EGCG (2 mg/mL) caused rapid bacterial cell aggregation and reduced the optical density of *S. mutans* cell suspension by 86.7% at pH 7.0 and 90.7% at pH 5.5 after 2 h. EGCG also reduced the acid production of non-mutant streptococci, including *S. sanguinis*, *S. gordonii*, and *S. salivarius*, and promoted the aggregation of these non-mutants streptococci. Furthermore, these antimicrobial effects of short-term EGCG treatment persisted in the presence of saliva. These results suggest that EGCG might have short-term antibacterial effects on caries-associated streptococci in the oral cavity [19].

The antibacterial effects of water-soluble green tea extracts on multi-antibiotic-resistant isolates of *P. aeruginosa* were evaluated by Jazani and co-workers [17]. Results obtained by these researchers revealed that green tea has significant activity with bactericidal action on multi-drug resistant strains of *P. aeruginosa*. Moreover, 35.6% of isolated strains showed resistance to 5 antibiotics or more and 55.8% of all strains were Multi-Drug Resistant (MDR) strains. The average MICs and MBCs of the extract against all strains of *P. auroginosa* were 2.06 ± 1.76 and 2.54 ± 2.22 $mg{\cdot}mL^{-1}$ respectively [17]. These researchers [18] also evaluated the antibacterial effects of water-soluble green tea extracts on multi-antibiotic-resistant isolates of *Acinetobacter* sp. Seventy-five percent of isolated strains showed resistance to at least 12 antibiotics or more and all the strains were Multi-drug Resistant (MDR) strains. The average MBCs of the extract against all strains of *Acinetobacter* were 387.5 ± 127.6 $\mu g{\cdot}mL^{-1}$. The study of Jazani and co-workers [17] suggests that green tea has significant bactericidal action on multi-drug resistant strains of *Acinetobacter* [18].

In our previous studies, we also investigated the *in vitro* antimicrobial activity of ethanolic extracts derived from leaves of other species belonging to the *Camellia* genus [20]. For example, we assessed the *in vitro* antimicrobial activity of ethanolic extracts of leaves derived from *Camellia japonica* 'Kramer's Supreme', 'C.M. Wilson', 'La Pace', 'Mrs. Lyman Clarke', 'Benikarako', 'Fanny Bolis' against clinical cefuroxime-resistant *Enterobacter cloacae* strain [20]. Ethanolic extracts were prepared by freshly crushed leaves and evaluated for their antimicrobial activity against *E. cloacae* strain locally isolated using disc diffusion assay. Among the six plant extracts, *C. japonica* 'Mrs. Lyman Clarke' exhibited the highest inhibitory zones against the tested strain (the mean of the zone of inhibitions was 12.5 ± 0.7 mm). The least activity was attributed to the *C. japonica* 'La Pace' extract. It can be concluded that *C. japonica* and its cultivars possess a mild antibacterial efficacy. It may also be concluded that the antimicrobial potential of various samples of these plants might be due to a wide variety of compounds present in these plants [20]. In other our study [5], we aimed to determine the antibacterial activity of these six plants, i.e. *C. japonica* and its cultivars against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC®25922™) strain. The increase of the mean of the diameters of the inhibition zone was 58.4% for *C. japonica* 'Kramer's Supreme', 29.2% for *C. japonica* 'La Pace', and *C. japonica* 'Mrs. Lyman Clarke', 22.5% for *C. japonica* 'Fanny Bolis', 19.1% for *C. japonica* 'Benikarako', and 18% for *C. japonica* 'C.M. Wilson' compared to the control samples (96% ethanol). Among the six plant extracts, *C. japonica* 'Kramer's Supreme' exhibited the highest inhibitory zones against the tested strain (the mean of the zone of inhibitions was 14.1 ± 1.1 mm). The intermediate activity was presented by other cultivars

studied [5]. These results could provide a theoretical basis for making full use of *Camellia* species. Moreover, their antibacterial activities can play an important role in medicine, veterinary, food preservation, and other aspects. Mechanisms of antibacterial activities remain to be studied.

CONCLUSIONS

Results of the current study demonstrated that the most sensitive to infusion derived from commercial green tea was the Gram-negative bacterial strain *Escherichia coli* (Migula) Castellani and Chalmers (ATCC®35218™), where there was the greatest increase in the zone of growth inhibition compared to 96% ethanol (by 98%, $p < 0.05$). According to the Gram-positive bacterial strains, only the *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) strain showed sensitivity to infusion derived from commercial green tea, with a statistically significant increase in the zone of growth inhibition compared to 96% ethanol (by 57.4%, $p < 0.05$). *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC®25923™) strain was also sensitive to infusion derived from commercial green tea. The zone of growth inhibition was increased by 37% compared to 96% ethanol ($p < 0.05$). These results demonstrate that the use of green tea can be applied to a variety of bacterial infections caused by both Gram-positive and Gram-negative bacterial strains. Further studies are needed to analyze the antimicrobial properties of the compounds in this plant.

Acknowledgments

This work was supported by The International Visegrad Fund, and the authors are cordially grateful for this.

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Стаття надійшла до редакції / The article was received 08.11.2022