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Research Article OPEN ACCESS

Antimicrobial efficacy of ethanolic extracts obtained from leaves of *Camellia japonica* L. cultivars against *Escherichia coli* strain

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The present study was aimed to determine the antibacterial activity of six plants, i.e. *Camellia japonica* L. (cultivars Kramer's Supreme, C.M. Wilson, La Pace, Mrs. Lyman Clarke, Benikarako, Fanny Bolis) against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain. Ethanolic extracts were prepared by freshly crushed leaves and evaluated for their antimicrobial activity against *Escherichia coli* ATCC® 25922™ strain using disc diffusion assay. The increase of the mean of the diameters of the inhibition zone was 58.4 % for cv. Kramer's Supreme, 29.2 % for cv. La Pace and cv. Mrs. Lyman Clarke, 22.5 % for cv. Fanny Bolis, 19.1% for cv. Benikarako, and 18 % for cv. 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. The findings reported herein give scientific credence to the traditional uses of these plants and suggest that extracts derived from the leaves of *Camellia japonica* and its cultivars merit further chemical study as natural antibiotics to identify the secondary metabolites. These results could provide a theoretical basis for making full use of *Camellia japonica* and its cultivars. 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.

Keywords: *Camellia japonica*, cultivars, leaves, *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), antibacterial efficacy, disc diffusion technique, ethanolic extracts

Introduction

The emergence of new infectious diseases and the development of drug resistance in pathogenic microorganisms prompts scientists to discover novel plant-derived bioactive compounds (Jeyaseelan and Jashothan, 2012). Therefore, medicinal plants are nowadays widely screened to determine their bioactivity and to isolate novel bioactive compounds (Khan et al., 2011). Antimicrobial properties of medicinal herbs are being increasingly reported from different parts of the world.

Camellia japonica L. is one of the best-known species of the genus *Camellia* that belongs to the Theaceae family and is widely grown in Korea and Japan (Lee et al., 2017; Jeon et al., 2018). As the ornamental plant, *Camellia japonica* is widely distributed worldwide. Previous studies have demonstrated that *Camellia japonica* has antioxidant activity. For example, Piao et al. (2011) investigating the antioxidant properties of the ethanol extract of the flower of *C. japonica* (*Camellia* extract), revealed that *Camellia* extract exhibits antioxidant properties by scavenging ROS and enhancing antioxidant enzymes. *Camellia* extract

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contained quercetin, quercetin-3-O-glucoside, quercetin, and kaempferol, which are antioxidant compounds. *Camellia* extract exhibited 1,1-diphenyl-2-picrylhydrazyl radical and intracellular reactive oxygen species (ROS) scavenging activity in human HaCaT keratinocytes. Also, *Camellia* extracts scavenged superoxide anion generated by xanthine/xanthine oxidase and hydroxyl radical generated by the Fenton reaction in a cell-free system. Furthermore, *Camellia* extract increased the protein expressions and activity of cellular antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase (Piao et al., 2011). Results of Kim et al. (2012) indicate that *C. japonica* oil exerts anti-inflammatory effects by downregulating the expression of the inducible isoform of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) genes through inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activator protein 1 (AP-1) signaling. A daily oral administration of camellia oil distillate fraction effectively inhibited spontaneous lung metastasis of BL6 cells (Miura et al., 2007).

Previous studies reported the antimicrobial activity of green tea leaves to Gram-positive organisms (Hamilton-Miller, 1995; Hamilton-Miller and Shah, 2000); however, discrepancies were found regarding its activity concerning Gram-negative rods (Shetty et al., 1994; Gordon and Wareham, 2010). The role of non-polymeric phenolic and polymeric tannin constituents in the antioxidant and antibacterial properties of six brands of green, black, and herbal teas of *Camellia sinensis* were investigated by Chan et al. (2011). Minimum inhibitory dose against Gram-positive *Micrococcus luteus*, *Staphylococcus aureus*, and *Bacillus cereus*, and Gram-negative *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* were evaluated using the disc diffusion method. The susceptibility of the same strains to plant extracts was assessed using the disc diffusion method. Extracts and fractions of all six teas showed no activity against the three Gram-negative bacteria. Green tea inhibited all three Gram-positive bacteria with *S. aureus* being the least susceptible. Black and herbal teas inhibited the growth of *M. luteus* and *B. cereus*, but not *S. aureus* (Chan et al., 2011). Antibacterial activity of tea (*C. sinensis*) and coffee (*Coffee arabica*) with special reference to *Salmonella typhimurium* was assessed by Shetty et al. (1994). Extracts of Black tea, Japanese green tea, China tea, or Coffee inhibited the growth of various bacteria causing diarrhoeal diseases. Tea or coffee also showed bactericidal activity against *Vibrio cholerae*, *S. typhimurium*, and *S. typhi* (Shetty et al., 1994). Antimicrobial activity of the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) against clinical isolates of *Stenotrophomonas maltophilia* was studied by Gordon and Wareham (2010). EGCG has promising *in vitro* antimicrobial activity against *S. maltophilia*.

The results of Lee et al. (2008) strongly suggest that the anti-allergic activity of leaf extract of *C. japonica* is mediated through inhibiting degranulation and allergic cytokine secretion in mast cells. Moreover, an aqueous extract derived from petals of *C. japonica* at a concentration of 100 mg/ml was bacteriostatic against all the foodborne pathogens, i.e. *Salmonella typhimurium* DT104, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* (Kim et al., 2001). The findings of Park et al. (2015) indicated that *C. japonica* fruit extract could be a valuable candidate for herbal medicine for cardiovascular diseases associated with endothelial dysfunction and atherosclerosis. The extracts of *C. japonica* fruits exhibit a strong cardiovascular protective effect, inducing endothelium-dependent nitric oxide (NO)-mediated relaxation *via* the redox-sensitive PI_3-kinase pathway (Park et al., 2015). *C. japonica* fruits could be a potent herbal therapeutic option and source of functional food for the prevention and treatment of atherosclerosis and other diseases associated with hypercholesterolemia (Lee et al., 2016). Anti-inflammatory and gastroprotective mechanisms of *C. japonica* fruits are mediated by modulation of oxidative stress, inflammatory cytokines, and enzymes via suppression of mitogen-activated protein kinases (MAPK)/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways (Akanda and Park, 2017). *C. japonica* oil may be considered as possible wrinkle-reducing candidates for topical applications (Jung et al., 2007) and exerts anti-inflammatory effects (Kim et al., 2012).

Synergistic antimicrobial activity of *C. sinensis* and *Juglans regia* against multidrug-resistant bacteria (350 Gram-positive and Gram-negative strains belonging to 10 different bacterial species) was investigated by Farooqui et al. (2015). *C. sinensis* showed higher antibacterial activity against MDR *S. typhi* than to other Gram-negative isolates (Farooqui et al., 2015).

The domestication of double flower in *Camellia japonica* and other related species has resulted in different types of double flower patterns (Vainstein, 2002). The typical *Camellia japonica* flower was defined as a single flower, with one row of overlapping petals (usually less than 8), and a columnar stamen cluster, and one normal pistil in the center. In general, within cultivated

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Camellia five major types of the double flower were identified by morphological characterizations of flower organ number, organ shape, and compositions (Gao, 2005), suggesting various diversifications of molecular mechanisms underlying the control of double flower development (Li et al., 2017).

In our previous study, the *in vitro* antimicrobial activity of ethanolic extracts of leaves derived from *Camellia japonica* (cultivars Kramer's Supreme, C.M. Wilson, La Pace, Mrs. Lyman Clarke, Benikarako, Fanny Bolis) against clinical cefuroxime-resistant *Enterobacter cloacae* strain was evaluated (Kharchenko et al., 2019). It was revealed that *C. japonica* and its cultivars possess a mild antibacterial efficacy. The current study is a continuous line of our investigations directed towards the assessment of antibacterial potentials of *Camellia* plants.

Escherichia coli is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, diarrhea, urinary tract infection, and other clinical infections such as neonatal meningitis and pneumonia (Orskov and Orskov, 1985; Krogfelt, 1991). Although most genetic subtypes of *E. coli* can be harmless residents of the gastrointestinal tract, it also has the pathogenic capacity to cause severe diarrheal and extraintestinal diseases (Croxen et al., 2013). Moreover, *E. coli* is regarded among clinically important bacteria, which are indicator organisms commonly used in various projects to monitor antibiotic resistance (Boss et al., 2016). *E. coli* is a Gram-negative, oxidase-negative, rod-shaped bacterium from the family Enterobacteriaceae. It can grow both aerobically and anaerobically, preferably at 37 °C, and can either be nonmotile or motile, with peritrichous flagella. Pathogenic variants of *E. coli* (pathovars or pathotypes) cause much morbidity and mortality worldwide (Croxen et al., 2013). Also, the development of bacterial resistance to presently available antibiotics has necessitated the search for new antimicrobial agents.

Thus, the present study was aimed to determine the antibacterial activity of six plant *Camellia japonica* cultivars against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain.

Material and methodology

Collection of plant material

The leaves of *Camellia japonica* (cultivars Kramer's Supreme, C.M. Wilson, La Pace, Mrs. Lyman Clarke, Benikarako, Fanny Bolis) plants cultivated at glasshouses under natural light, were sampled at M.M. Gryshko National Botanical Garden (Kyiv, Ukraine). The leaves were sampled in September 2018.

Figure 1 *Camellia japonica* L. cultivars with various double flower types maintained at M.M. Gryshko National Botanic Garden, NAS of Ukraine

The *Camellia japonica* cultivars included in this study represent four various double flowers types, i.e. "paeony" ('Kramer's Supreme' and 'Benikarako'), "rose" ('C.M. Wilson' and 'La Pace'), "semi-double" ('Mrs. Lyman Clarke'), and "formal double" ('Fanny Bolis') (Figure 1).

Preparation of plant extracts

The collected leaves were brought into the laboratory for antimicrobial studies. Freshly washed leaves were crushed, weighed, and homogenized in 96% ethanol at room temperature to obtain the final concentration of extract 50 mg per 1 mL. The extracts were then filtered and investigated for their antimicrobial activity. The storage of extracts was in dark glass firmly sealed bottles at temperature +4 °C.

The disk diffusion method for evaluation of antibacterial activity of plant extracts

The *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain was used in the current study. Strain tested was plated on TSA medium (Tryptone Soy Agar) and incubated for 24 hr at 37 °C. Then the suspension of microorganisms was suspended in sterile PBS and the turbidity adjusted equivalent to that of a 0.5 McFarland standard. The antimicrobial susceptibility testing was done on Muller-Hinton agar by disc diffusion method (Kirby-Bauer disk diffusion susceptibility test protocol) (Bauer et al., 1966). Muller-Hinton agar plates were inoculated with 200 µl of standardized inoculum (10^8 CFU/mL) of the bacterium and spread with sterile swabs.

Sterile filter paper discs impregnated by extract were applied over each of the culture plates, 15 min after bacteria suspension was placed. A negative control disc impregnated by sterile 96 % ethanol was used in each experiment. After culturing bacteria on Mueller-Hinton agar, the disks were placed on the same plates and incubated for 24 hr at 37 °C. The assessment of antimicrobial activity was based on the measurement of the diameter of the inhibition zone formed around the disks. The diameters of the inhibition zones were measured in millimeters and compared with those of the control and standard susceptibility disks. The activity was evidenced by the presence of a zone of inhibition surrounding the well (CLSI, 2014). The results of the disk diffusion test are "qualitative," in that a category of susceptibility (i.e., susceptible, intermediate, or resistant) is derived from the test rather than a MIC (Jorgensen and Ferraro, 2009).

Statistical analysis

Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean. All variables were randomized according to the antibacterial activity of tested extracts. All statistical calculation was performed on separate data from each extract. The data were analyzed using one-way analysis of variance (ANOVA) using Statistica software, version 8.0 (StatSoft, Poland) (Zar, 1999). 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 (Okoth et al., 2013).

Results and discussion

The study was conducted to evaluate the *in vitro* antimicrobial activity of leaf extracts of 6 *C. japonica* cultivars. The data on zones of inhibition of bacterial growth of plant extracts against the *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain is demonstrated in Figure 2 and 3.

The crude extracts were analyzed for their antibacterial effect by the determination of their inhibitory zones against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain. Among the six plant extracts screened, '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 cultivars La Pace and Mrs. Lyman Clarke (11.5 ±0.9 mm and 11.5 ±1.1 mm), cv. Fanny Bolis (10.9 ±1.2 mm), cv. Benikarako (10.6 ±0.9 mm), and cv. C.M. Wilson (10.5 ±1.0 mm) (Figure 2 and 3). The antibacterial effect of positive control was also recorded (the mean value of the inhibition zone was 9.1 ±0.5 mm). The increase of the mean of the diameters of the inhibition zone was 58.4 % for cv. Kramer's 29.2 % for cv. La Pace and cv. Mrs. Lyman Clarke, 22.5 % for cv. Fanny Bolis, 19.1% for cv. Benikarako, and 18 % for cv. C.M. Wilson compared to the control samples (96 % ethanol).

In this study, we investigated the antimicrobial activity of plant extracts by agar well diffusion. In the current study, cultivars Kramer's Supreme, C.M. Wilson, La Pace, Mrs. Lyman Clarke, Benikarako, Fanny Bolis were less potent against the test bacterium due to the observed zone of growth inhibitions.

It is noteworthy to mention that this slight effect on the *E. coli* growth, Gram-negative organisms, is most likely due to the protective nature of the outer membrane of their cell walls. The comparison of our data, with those

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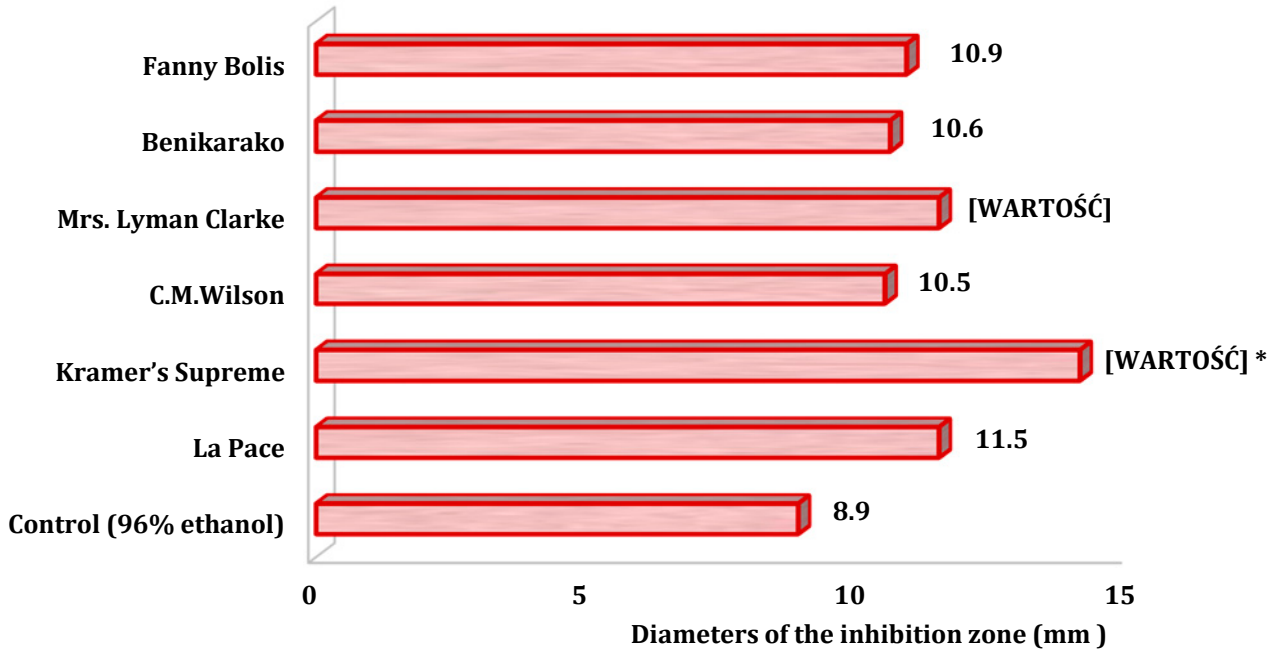

Figure 2 Zone of growth inhibitions of *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain induced by the extracts obtained from leaves of various *Camellia japonica* L. cultivars in millimeter (n = 8)
* changes are statistically significant (p <0.05) compared to 96 % ethanol (control sample)

published by other authors, reveals the findings of other researchers. Greater resistance of Gram-negative bacteria to plant extracts has been reported (Joshi et al., 2009; Koohsari et al., 2015) and this could be attributed to the differences in their cell wall structure (Ikigai et al., 1993).

Hence, these extracts would not be good candidates as drugs lead to an antibacterial agent because of these high values of diameters of inhibition zone due to the importance of the potency of the antibacterial agent in drug development, amongst other factors.

The results obtained in the current study are in line with early reports. The potential presence of naturally occurring antimicrobials in petals of *C. japonica* active against foodborne pathogens in microbiological media and food was studied by Kim et al. (2001). Petals of the *Camellia* flower were extracted with methanol and fractionated into basic, acidic, and neutral fractions. The acidic fraction produced an inhibitory zone of 14 to 19 mm (diameter) in a disk assay against the pathogens *Salmonella typhimurium* DT104, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* on agar plates. Similarly, an aqueous extract from the petals of *C. japonica* had an inhibitory effect on the growth of all pathogens at 37 °C in microbiological media by increasing the lag phase. None of the microorganisms was inhibited completely. Milk was used as a model food system. Aqueous extract at a concentration of 100 mg/ml was bacteriostatic against all the foodborne pathogens in the milk stored at 25 °C for up to 4 days (Kim et al., 2001).

Similar results were obtained also for other species of *Camellia* plants. For example, Zihadi et al. (2019) have investigated the antibacterial potential of ethanolic extract of Green tea (*Camellia sinensis*) and Neem (*Azadirachta indica*) leaves on methicillin-resistant *Staphylococcus aureus* (MRSA) and Shiga-toxigenic *Escherichia coli* (STEC). Results obtained by Zihadi et al. (2019) revealed that the maximum zone diameter of inhibition value was observed for green tea against MRSA (7.5 mm) and minimum for neem (4.9 mm). Moreover, the highest zone diameter of inhibition against STEC was also for green tea and the combination of green tea and neem (4.5 mm). The minimum inhibitory concentration (MIC) values of green tea extract were 15.625 and 31.25 mg/ml against MRSA and STEC, respectively. The combination had a similar MIC (46.87 mg/ml) against both organisms. Green tea showed the lowest minimum bactericidal concentration (MBC) values, 31.25 and 62.5 mg/ml, against MRSA and STEC, respectively. Thus, green tea and neem leaves showed good antimicrobial effects and can be used to explore novel antimicrobial compounds against MRSA and STEC (Zihadi et al., 2019).

Also, Hafiz et al. (2018) have characterized the *in vitro* antibacterial and antioxidant potential of winged

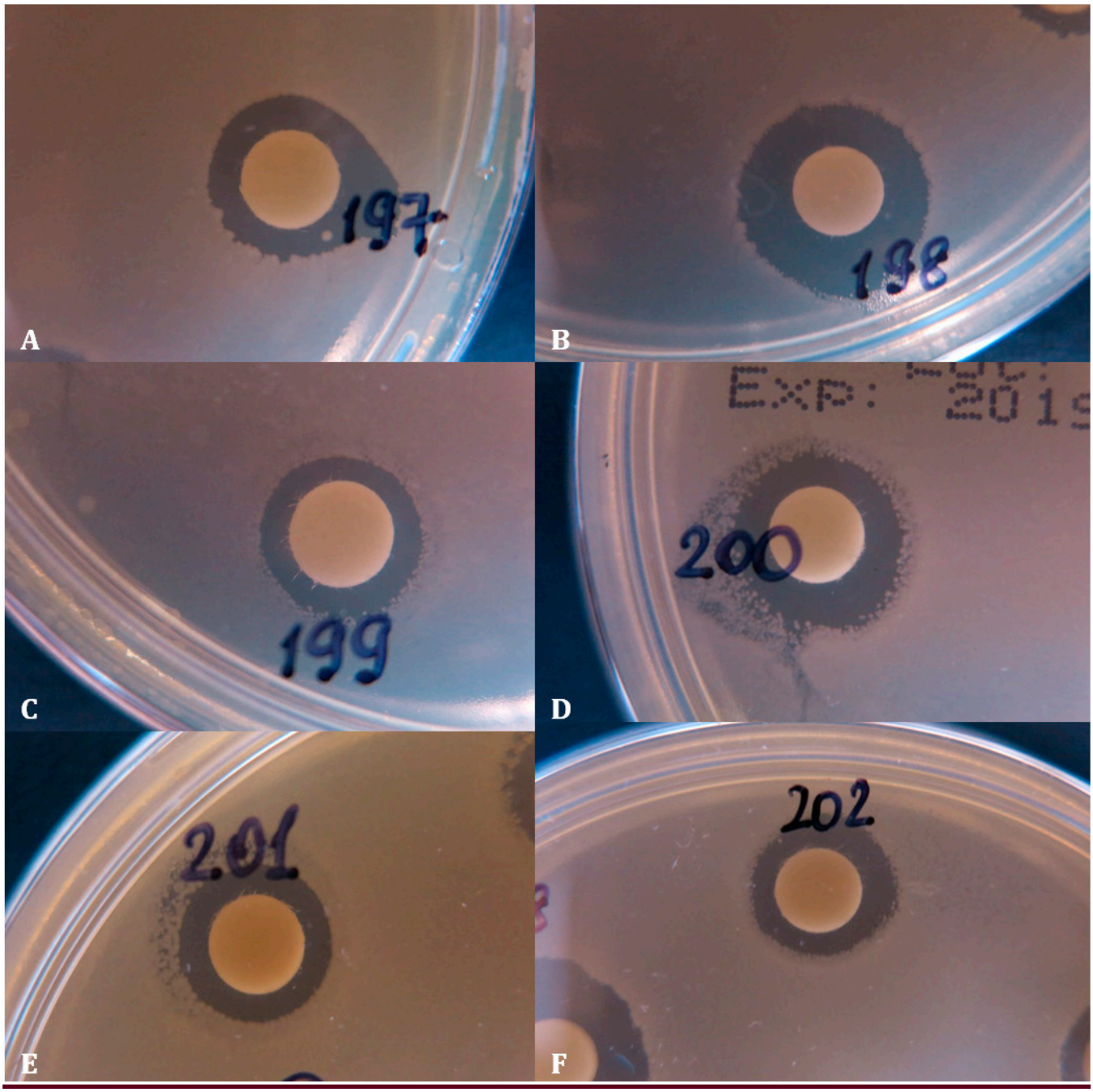

Figure 3 Antimicrobial activity of ethanolic extracts of various *Camellia japonica* L. cultivars analyzed by Kirby-Bauer disc-diffusion assay:
La Pace (A), Kramer's Supreme (B), C.M. Wilson (C), Mrs. Lyman Clarke (D), Benikarako (E), and Fanny Bolis (F)

prickly ash, green tea, and thyme. The antibacterial potential of extracts showed a significant extent of the activity against *Bacillus subtilis* and *E. coli*. Antioxidant potential exhibited the highest phenolic and flavonoid content in *C. sinensis*. The total phenolic content was significantly higher (1456.26 ±12.05 mg gallic acid) in an 80 % ethanolic fraction of *C. sinensis*. The flavonoid content in different plant extracts ranged from 8.17 ±2.02 to 376.29 ±7.11 mg/g. The radical scavenging DPPH assay also showed the significant antioxidant capacity of selected plants with the methanolic (50 %) extract of *C. sinensis* found to be the most potent (78.95 ±7.12 %) (Hafiz et al., 2018).

Extracts of green tea strongly inhibited *Escherichia coli*, *Streptococcus salivarius*, and *Streptococcus mutans*. The antibacterial effect of green and black tea extracts was compared with those of amoxicillin, cephradine, and eugenol (Rasheed and Haide, 1998). Anita et

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al. (2014) have evaluated the *in vitro* antimicrobial activity of *C. sinensis* extract on *Streptococcus mutans* and *Lactobacillus acidophilus*, the representative microbes of dental caries. MIC of green tea extract on *S. mutans* and *L. acidophilus* was found to be 0.2 and 0.3 % respectively, MBC was found to be 0.8 and 0.9 %, respectively. The mean zone of inhibition for 30 µl containing 300 µg of ethanolic extract of green tea and control against *S. mutans* was 18.33 mm and 14.67 mm, respectively. The mean zone of inhibition for 30 µl containing 300 µg of ethanolic extract of green tea and control against *L. acidophilus* was 12.67 mm and 7.33 mm, respectively. Thus, green tea has antibacterial activity against predominant cariogenic bacteria namely *S. mutans* and *L. acidophilus* (Anita et al., 2014).

On the other hand, an aqueous extract of *C. sinensis* was found to be effective against Gram-positive, Gram-negative, and fungi, as well as against drug-resistant microorganisms e.g. MRSA and *P. aeruginosa* and *Candida albicans*. Khan et al. (2019) have investigated the antibacterial and antifungal potential of aqueous extract of *C. sinensis*. Antibacterial activity was determined by disc and well diffusion assay. MIC and MBC were calculated by the broth dilution method. Miles and Misra technique was used to find out the colony-forming unit per/ml. All the test organisms revealed a diverse range of vulnerability against aqueous extract. Among Gram-positive, MRSA showed to be the most sensitive with the least MIC and MBC while Gram-negative *Pseudomonas 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 18 % aqueous extract of *C. sinensis* (Khan et al., 2019).

From a survey of the literature on this subject, it was noticed that *Camellia* species have been a subject of intense phytochemical investigation. In particular, it was shown that *C. sinensis* is the potential source of bioactive phenolic compounds with high antimicrobial and antioxidant properties. The phytochemical screening, antimicrobial, antioxidant, and cytotoxic properties of *C. sinensis* were evaluated in the study of Shah et al. (2018). The phytochemical screening revealed the presence of an applicable amount of lycopene, β-carotenes, flavonoids, and tannins in *C. sinensis*. Among the phytochemicals, tannin was found to be significantly higher in the tea plants. The results showed that the stem part of *C. sinensis* presented greater antimicrobial potential than the leaf and root. Antioxidant activity (assessed through % inhibition of linoleic acid peroxidation test) was the highest (89.22 %) in n-hexane extract of root part as compared to other extracts. Finally, the cytotoxicity analysis (hemolytic activity against human erythrocytes) of plant extract showed the negligible (%) lysis of RBCs ranging from 1.73 to 4.01 % (Shah et al., 2018). Camargo et al. (2016) have investigated the antioxidant and anticandidal activities of leaves obtained from *C. sinensis* by non-fermentation (green and white teas), semi-fermentation (red tea), and fermentation method (black tea). The results showed that non-fermented teas have a higher concentration of phenolic compounds, and then presented the best inhibitory activity of hemolysis, the best inhibition of conjugated diene formation, and more pronounced antioxidant activity in all tests. The highest anticandidal activity was obtained from fermented tea, followed by non-fermented tea (Camargo et al., 2016). Xiang et al. (2018) have determined the disinfectant efficacy of ozonated camellia oil on *Staphylococcus aureus*. According to the plate count method and turbidimetry, the bacterial concentration in the ozonated camellia oil group was lower than that in the negative control group and base oil (camellia oil) group (Xiang et al., 2018).

The crude extracts of six different plants of green tea *C. assamica* and *C. sinensis* were tested by Bashir et al. (2014) against three Gram-positive and four Gram-negative bacteria using the agar disk diffusion method at 50 mg/ml concentration. The maximum inhibition of *Staphylococcus aureus* was recorded by dimethyl sulphoxide extracts of green tea varieties. Maximum scavenging potential activity was found with ethanol, methanol, and dimethyl sulphoxide extracts. Spot screening indicated that the presence of active biological compounds such as flavonoids, proteins, phenols, alkaloids, and glycosides also exhibited strong activity against tested bacterial strains (Bashir et al., 2014).

In a separate study, we also tested these extracts for toxic effects on human erythrocytes. *C. japonica* and its cultivars were found to be non-toxic on the concentrations tested for antimicrobial activity (data not shown). Results obtained in our previous study showed that there is a possibility of using extracts derived from leaves of various *C. japonica* cultivars in intensive aquaculture farms. The results of the study suggested the high antioxidant capacity of *Camellia* cultivars screened give reason to believe that application of these plant extracts signifies a rational curative strategy to prevent and cure various fish diseases involving oxidative stress by increasing the ability of a fish organism to adapt (Kharchenko et al., 2017a,b; 2018).

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Many of the direct effects of tea catechins are a result of the catechins binding to the bacterial lipid bilayer cell membrane which then causes damage to the membrane (Sirk et al., 2008, 2009; Reygaert, 2014). Epigallocatechin-gallate (EGCG) showed the strongest interaction with the lipid bilayer based on the number of hydrogen bonds formed with lipid headgroups (Sirk et al., 2008). The molecular structure and aggregated condition of the catechins significantly influence their absorption, as well as their ability to form hydrogen bonds with the lipid headgroups (Sirk et al., 2009). This damage can then lead to a variety of related antimicrobial effects.

Cho et al. (2007) have found that when exposed to Korean green tea (*Camellia sinensis*) polyphenols, the bacterial response of *Escherichia coli* was changed the regulation of 17 individual genes. One of the major outcomes of this change in regulation was damage to the bacterial cell membrane (Cho et al., 2007). The catechins primarily act on and damage bacterial membranes. The observation that Gram-negative bacteria are more resistant to bactericidal catechins than Gram-positive bacteria can be explained to some extent by the presence of negatively charged lipopolysaccharide (Ikigai et al., 1993). Bacterial cell membrane damage inhibits the ability of the bacteria to bind to host cells (Sharma et al., 2012), and inhibits the ability of the bacteria to bind to each other to form biofilms, which are significant in pathogenesis (Blanco et al., 2005).

Zhang and Rock (2004) have found that green tea components (especially EGCG) inhibit specific reductases (FabG, FabI) in bacterial type II fatty acid synthesis. The presence of the galloyl moiety was essential for activity, and EGCG was a competitive inhibitor of FabI and a mixed type inhibitor of FabG demonstrating that EGCG interfered with cofactor binding in both enzymes. EGCG inhibited acetate incorporation into fatty acids *in vivo*, although it was much less potent than thiolactomycin, a validated fatty acid synthesis inhibitor. Inhibition of fatty acid synthesis by green tea has also been found to inhibit bacterial production of toxic metabolites. The inhibitory effect on the production of toxic end metabolites of bacteria can be attributed to the presence of the galloyl moiety, which is ester-linked with the 3-OH of the catechin moiety in the polyphenolic compounds (Sakanaka and Okada, 2004). Okamoto et al. (2003, 2004) also found that green tea catechins have an inhibitory effect on protein tyrosine phosphatase and cysteine proteinases in certain anaerobic oral bacteria (*Prevotella intermedia*, *Porphyromonas gingivalis*). The inhibitory effect observed is due to the presence of galloyl moiety in the structure (Okamoto et al., 2003, 2004).

The catechins inhibit bacterial DNA gyrase by binding to the ATP binding site of the gyrase B subunit. In the group of four tested catechins, epigallocatechin gallate (EGCG) had the highest activity, followed by epicatechin gallate (ECG) and epigallocatechin (EGC) (Gradisar et al., 2007). The green tea polyphenols can inhibit the enzyme dihydrofolate reductase in bacteria and yeast, effectively blocking the ability of the microorganisms to synthesize folate. In elucidating its mechanism of action, Navarro-Martínez et al. (2005) have shown that epigallocatechin gallate is an efficient inhibitor of *Stenotrophomonas maltophilia* dihydrofolate reductase (Navarro-Martínez et al., 2005). EGCG also acts as an antifolate compound on *Candida albicans*, disturbing its folic acid metabolism (Navarro-Martínez et al., 2006). The bioflavonoids obtained from green tea could also inhibit the activity of bacterial ATP synthase, reducing the ability of the microorganisms to produce enough energy (Chinnam et al., 2010).

Conclusions

The alcoholic extracts of *Camellia japonica* and its cultivars revealed mild antibacterial activity against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain. The antimicrobial ability of various samples of these plants might be due to a wide variety of compounds. The findings reported herein give scientific credence to the traditional uses of these plants and suggest that extracts derived from the leaves of *C. japonica* and its cultivars merit further chemical study as natural antibiotics to identify the secondary metabolites. These results could provide a theoretical basis for making full use of *C. japonica* and its cultivars. 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.

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