



ANTIBACTERIAL ACTIVITIES OF ETHANOLIC EXTRACT OBTAINED FROM *RHODODENDRON MYRTIFOLIUM* SCHOTT & KOTSCHY LEAVES AGAINST CLINICALLY ISOLATED BACTERIAL STRAINS

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The plants belonging to the genus *Rhododendron* L. and isolated compounds demonstrate diverse biological activities including anti-inflammatory, analgesic, anti-microbial, anti-diabetic, insecticidal and cytotoxic activity, as well as antibacterial activity. Our study was designed the effects of *Rhododendron myrtifolium* Schott & Kotschy leaf extract against bacterial strains were monitored *in vitro* by the disk diffusion method. The aim of this study was to assess possible antibacterial effects of an ethanolic extract derived from *Rhododendron myrtifolium* leaves against *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Escherichia coli* strain locally isolated from human biological fluids. Thus, the study contributes to on-going investigations on the bioactivity potential of plant species such as the *Rhododendron*. Leaves of *Rhododendron myrtifolium* were harvested on the side of the road between the Menchul valley and Rogneska valley (Kvasy village, Rakhiv district, Zakarpattia region, Ukraine). The results revealed that extract exerts antibacterial activity against *Citrobacter freundii*. However, the *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Escherichia coli* were resistant to *R. myrtifolium* leaf extract. Maximum *in vitro* inhibition was scored against *Citrobacter freundii*, followed by *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*, which presented inhibition zones of (12.1 ± 0.9) mm, (9.1 ± 0.5) mm, (7.5 ± 0.6) mm, and (7.2 ± 0.5) mm, respectively. In the case of the positive controls, 96% ethanol possesses a mild antibacterial effect, which presented inhibition zones of (6.5 ± 0.7) mm. The results from the screening study performed by the disc diffusion method revealed that *R. myrtifolium* possesses a mild antibacterial activity against *C. freundii*. However, further investigation is needed to determine the bioavailability of the active compounds and to determine the dose and toxicity before it can be used as therapeutic agents.

Keywords: *Rhododendron*, leaf extracts, agar disk diffusion assay, antibacterial activity, inhibition zone diameter

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Introduction

Antimicrobial resistance has become a pre-eminent concern in medicine and public health. This problem is widespread, and the causative factors are uncontrolled (Mah and Memish, 2000). Control of infections caused by multi-drug resistant Gram-positive and Gram-negative bacteria has become a major problem in various countries in the prevention of infectious diseases. Currently, the spread of multi-drug resistant bacteria is not only through nosocomial infections, but also occurs in the community (Radji et al., 2013). Several multi-drug resistant bacteria that are most commonly found, especially through nosocomial infections, are *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. (Rice, 2008). In the past few decades, these strains become increasingly important pathogens in hospitals and play an important role in the colonization and infection of hospitalized patients by a variety of nosocomial infections including bacteremia, urinary tract infections, and nosocomial pneumonia (Radji et al., 2013).

Treatment of these infections is often very difficult due to the cross-resistance of these bacteria with a large group of antibiotics. Therefore, it seems reasonable to explore alternative antimicrobial agents for control multi-drug resistant bacteria. Recently, there has been growing interests to find antimicrobial compounds from medicinal plant extracts as an alternative approach to discover new antimicrobial compounds (Ríos and Recio, 2005; Radji et al., 2013). *Rhododendron* species have been proved to possess medicinal and health promotion properties, including the ability to inhibit the growth of some types of pathogenic bacteria (Innocenti et al., 2010; Silici et al., 2010; Popescu and Kopp, 2013; Rezk et al., 2015; Wang et al., 2015; Li et al., 2016; Hakeem Said et al., 2017; Shrestha et al., 2017).

The genus *Rhododendron* L. (Ericaceae) is one of the most species-rich among angiosperms, comprising over 1000 species spreading across the northern hemisphere and with the center of diversity in southeastern Asia (Irving and Hebda, 1993). These plants are morphologically diverse, including evergreen and deciduous shrubs, subshrubs, and trees, mostly terrestrial but sometimes chasmophytic or epiphytic, with greyish-brown striate bark, spirally arranged branches, and leaves, often in pseudo-whorls, and umbellate to pyramidal racemes of 1–30 conspicuous fragrant flowers. Because of its foliage and flowers, the genus is well-known in ornamental cultivation and gardening, with numerous varieties artificially bred thanks to rhododendrons' natural ability to interspecific hybridization (Cullen, 2005). A noteworthy European member of the genus is *R. myrtifolium* Schott & Kotschy, an evergreen clump-forming dwarf shrub up to 50 cm in height, occurring in high-mountain habitats of the eastern and southern Carpathian Mountains and northern Balkans, largely within altitudes of 1,400–2,500 m. The species is featured in small narrowly elliptic to obovate coriaceous leaves abaxially covered with glandular scales containing essential oils, terminal inflorescences of tubular-campanulate pinkish flowers, and long-pedunculate dry multilocular capsules containing numerous diminutive seeds (Cullen, 1980; Mircea, 2005; Boratyński et al., 2006; Voloschuk and Prokopiv, 2011). Although endangered in countries of its distribution, *R. myrtifolium* has been used in folk medicine for the preparation of herbal teas (Dihoru and Boruz, 2014; Nedelcheva and Draganov, 2014) and presents a major touristic attraction during

its mass flowering period in mountains (Rivers, 2017). Its evolutionary closest relatives, *R. ferrugineum* L. and *R. hirsutum* L. (e.g., Sosnovsky et al., 2017) have been shown to possess cytotoxic, antibacterial, and antiviral effects of their extracts (Louis et al., 2010; Gescher et al., 2011; Seephonkai et al., 2011; Rezk et al., 2015b), while the biochemical features and bioactive potentials of *R. myrtifolium* remain unexplored.

Rhododendron species have been traditionally used in China, Nepal, Russia, and North America against inflammation, pain, skin ailments, common cold, and gastrointestinal disorders and for treating human diseases like asthma and skin diseases. These species are known to be a good source of polyphenolic plant secondary plant metabolites (Shrestha et al., 2017). The plant extracts belonging to the genus *Rhododendron* and isolated compounds demonstrated diverse biological activities including anti-inflammatory, analgesic, anti-microbial, anti-diabetic, insecticidal and cytotoxic activity (Popescu and Kopp, 2013), as well as antibacterial activity (Innocenti et al., 2010; Silici et al., 2010; Popescu and Kopp, 2013; Rezk et al., 2015; Wang et al., 2015; Li et al., 2016; Hakeem Said et al., 2017; Shrestha et al., 2017).

The aim of this study was to assess possible antibacterial effects of an ethanolic extract derived from *Rhododendron myrtifolium* leaves against *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Escherichia coli* strain locally isolated from human biological fluids. Thus, the study contributes to on-going investigations on the bioactivity potential of plant species such as the *Rhododendron*. Hence, the effects of *Rhododendron myrtifolium* leaf extract against bacterial strains were monitored *in vitro* by the disk diffusion method.

Materials and methodology

Collection of Plant Materials

Leaves of *Rhododendron myrtifolium* were harvested on the side of the road between the Menchul valley and Rogneska valley (Kvasy village, Rakhiv district, Zakarpattia region, Ukraine; N 48° 09' 28.4", E 24° 20' 05.6", 1,485 m a.s.l.) (Figure 1). Plant samples were thoroughly washed to remove all the attached materials and used to prepare the ethanolic extract.

Preparation of Plant Extracts

Freshly leaves were washed, weighed, crushed, and homogenized in 96% ethanol (in proportion 1 : 19, w/w) at room temperature. The extract was then filtered and investigated for antimicrobial activity.

Antimicrobial susceptibility testing

Non-repetitive clinical strains of isolated from biological materials infected patients were collected from Koszalin Hospital during March-April, 2019. The purity, as well as the identity of isolate, was confirmed in the laboratory conditions by standard microbiological methods and were interpreted according to the guidelines of the Clinical Laboratory Standards Institute (2014).



Figure 1 *Rhododendron myrtifolium* plant (Photo: Viktor Nachychko, Yevhen Sosnovsky)

First, the samples were examined by a microscopic Gram stain examination. Samples with Gram-negative results were inoculated on plates of nutrient agar, Clede agar, MacConkey's, and blood agar (Merck, Germany) and then incubated at 37 °C for 24 hours. The colony that showed fermenting of lactose on MacConkey agar and Cled agar media were purified and identified according to their morphology as circular, rose-pink to red colonies on MacConkey agar medium and yellow colonies on Cled agar. The isolates were identified by some biochemical reactions (e.g. catalase enzyme, potassium hydroxide test, Indole and methyl red test, Voges Proskauer reaction, urease and citrate, H₂S and oxidase test).

Susceptibility testing of the isolates was performed by disk diffusion according to the Guidelines of Clinical and Laboratory Standard Institute (CLSI, 2014). The following antimicrobial agents were used (µg): amikacin (30), ampicillin (10), aztreonam (30), ceftazidime (30), ciprofloxacin (5), cephalexin (30), gentamicin (10), erythromycin (15), imipenem (10), meropenem (10), clindamycin (2), aztreonam (30), norfloxacin (10), oxytetracycline (30), oxacillin (1), enrofloxacin (5), tetracycline (30), amoxicillin (25), piperacillin (100), piperacillin-tazobactam (100/10), tobramycin (10), ceftazidime (30), chloramphenicol (30), doxycycline (30), cefadroxil (30), pefloxacin (5), and cefepime (30). MIC was determined by E-test strips (according to manufacturer's instruction). The resistance breakpoints were the same as the ones defined by the National Committee for Clinical Laboratory Standards (NCCLS, 2000).

Phenotypic Disc Confirmatory Test was performed as recommended by the CLSI (Clinical and Laboratory Standards Institute, 2014). The antibacterial susceptibility profile of the isolates revealed that many isolated strains were classified as multi-drug resistant (MDR) bacteria.

For the current study, four bacterial strains were used for the study of susceptibility or resistance of bacteria to the phytochemicals:

- ▶ Isolate 1 – *Citrobacter freundii* strain was susceptible to all antibiotics used;
- ▶ Isolate 2 – *Enterobacter cloacae* strain was resistant to the amoxicillin, cefuroxime, trimethoprim-sulphamethoxazole, and cefotaxime;
- ▶ Isolate 3 – *Klebsiella pneumoniae* was resistant to piperacillin-tazobactam (100/10 µg), gentamicin (10 µg), tobramycin (10 µg), and ciprofloxacin (5 µg);
- ▶ Isolate 4 – *Escherichia coli*, not β -lactamase (ESBL)-producing strain, was a sensitive strain to antibiotics tested.

Bacterial Growth Inhibition Test by the Disk Diffusion Method

Strains tested were plated on TSA medium (Tryptone Soy Agar) and incubated for 24 hr at 25 °C. Then the suspension of microorganisms was suspended in sterile PBS and the turbidity adjusted equivalent to that of a 0.5 McFarland standard. 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 sample were applied over each of the culture plates, 15 min after bacteria suspension was placed. The antimicrobial susceptibility testing was done on Muller-Hinton agar by the disc diffusion method (Kirby-Bauer disk diffusion susceptibility test protocol) (Bauer et al., 1966). A negative control disc impregnated by sterile 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. Each test was repeated six times.

Statistical analysis

Statistical analysis of the data obtained was performed by employing the mean \pm standard error of the mean (S.E.M.). All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p > 0.05$). In order to find significant differences (significance level, $p < 0.05$) between groups, the Kruskal-Wallis test by ranks was applied to the data (Zar, 1999). All statistical analyses were performed using Statistica8.0 software (StatSoft, Poland). The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (*S*) ≥ 15 mm, Intermediate (*I*) = 11–14 mm, and Resistant (*R*) ≤ 10 mm (Okoth et al., 2013).

Results and discussion

The ability of the ethanolic extract obtained from leaves of *R. myrtifolium* to inhibit *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Escherichia coli* growth was determined in this study. The results revealed that extract exerts antibacterial activity against *Citrobacter freundii*. However, the *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Escherichia coli* were resistant to *R. myrtifolium* leaf extract. Maximum *in vitro* inhibition was scored against *Citrobacter freundii*, followed by *Escherichia coli*, *Klebsiella*

pneumoniae, and *Enterobacter cloacae*, which presented inhibition zones of (12.1 ±0.9) mm, (9.1 ±0.5) mm, (7.5 ±0.6) mm, and (7.2 ±0.5) mm, respectively. In the case of the positive controls, 96% ethanol possesses a mild antibacterial effect, which presented inhibition zones of (6.5 ±0.7) mm (Figure 2).

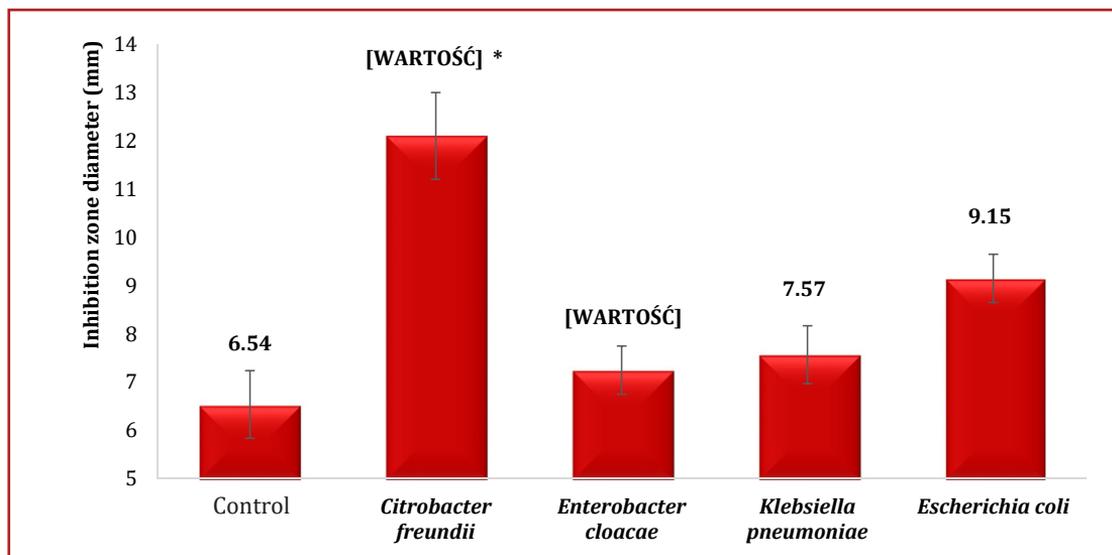


Figure 2 The mean of inhibition zone diameters of the ethanolic extract obtained from leaves of *Rhododendron myrtifolium* against *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Escherichia coli* strains locally isolated ($M \pm m$, $n = 6$)
* – the changes are statistically significant ($p < 0.05$) compared to the control group (96% ethanol)

Detailed data regarding the zones of inhibition by the ethanolic extract obtained from leaves of *R. myrtifolium* against *Citrobacter freundii* and *Enterobacter cloacae* were recorded and presented in Figure 2.

In line with the growing interest in the antibacterial potential of different plants, we examined the antibacterial properties of the ethanolic extract obtained from *R. myrtifolium* against *Rhododendron myrtifolium* leaves against *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Escherichia coli* strains. The results from the screening study performed by the disc diffusion method revealed that *R. myrtifolium* possesses a mild antibacterial activity against *C. freundii* (Figure 2, 3A).

The results of this study are consistent with other studies that have previously been reported that extracts of *Rhododendron* leaves have antibacterial activity against resistant bacteria strains. Crude extracts of *Rhododendron* leaves were tested for their antibacterial activity using agar diffusion and minimum inhibitory concentration assays and results were presented in the study of Hakeem Said et al. (2017). The antibacterial activity of *R. colletianum* Aitch. & Hemsl. was compared to a series of inactive extracts. Three metabolites were found to distinguish *R. colletianum* from other species indicating the ability to suggest potential bioactive substances (Hakeem Said et al., 2017).



Figure 3 Antimicrobial activity of ethanolic extract obtained from leaves of *R. myrtifolium* against *Citrobacter freundii* (A) and *Enterobacter cloacae* (B) measured as inhibition zone diameter

The polyphenolic profile of fruits, flowers, and leaves of different ages of *Rhododendron ambiguum* Hemsl. and *Rhododendron cinnabarinum* Hook. f. was studied by Shrestha et al. (2017). Fifty-nine different polyphenols including isomers were identified in these species. The leaves and fruits contained more polyphenols than the flowers. Also, the antibacterial activity of these parts (leaves, fruits, and flowers) against gram-positive bacteria was studied. There was no bioactivity observed for crude extracts of all samples against *Escherichia coli*. Against Gram-positive bacteria, the bioactivity of *R. ambiguum* ranged between 0.5 and 0.7 cm, while for *R. cinnabarinum* between 0.5 and 0.8 cm. Antibacterial effects of fruit and leaf extracts were in the same order of magnitude. However, there was a reduced antibacterial activity observed for the flowers of *R. cinnabarinum* and *R. ambiguum*. This could be due to the evolutionary aspect as flowers have a short blooming period in a year compared to the leaves and fruits. In general, *B. thioparus* was the most sensitive bacteria species towards the plant parts for both *Rhododendron* species (Shrestha et al., 2017).

A higher antibacterial effect of *Rhododendron* species against Gram-positive and higher effect for *R. cinnabarinum* was demonstrated in the antibacterial screening by Rezk et al. (2015a). *Erwinia amylovora* 1189 (wild type), *Escherichia coli* TG1 (wild type), *Pseudomonas syringae* DC3000 (wild type) as well as the respective mutants with deletions in *acrAB*, *tolC*, or *mexAB*. Additionally, knockout mutants of *E. amylovora* and *E. coli* with deletions in both, *acrAB* and *tolC* were subjected to the antimicrobial analysis. The leaf extracts of 17 *Rhododendron* species exhibited significant growth-inhibiting activities against Gram-positive bacteria. In contrast, only very few of the leaf extracts affected the growth of Gram-negative bacteria. Five out of the 120 *Rhododendron* leaf extracts showed no growth inhibitory activity against any of the Gram-positive tester organisms: *R. elliottii* Watt ex Brandis, *R. hylaeum* Balfour & Farrer, *R. ponticum* L., *R. keiskei* Miquel, and *R. eriocarpum* (Hayata) Nakai. However, crude extracts

obtained from 38 other *Rhododendron* species exhibited moderate antimicrobial activity against 12 out of the 26 tested bacterial species. The crude extracts derived from the remaining 77 *Rhododendron* species showed significant bioactivities against at least one of the 26 tester organisms. The spectrum of microbial susceptibilities towards *Rhododendron* extracts varied widely and showed dramatic differences, i.e. some of the tester organisms were susceptible to leaf extracts from most of the *Rhododendron* species while other bacterial tester organisms were susceptible towards very few *Rhododendron* leaf extracts. *Bacillus thioparus* was the most sensitive tester species susceptible to all 77 potentially bioactive *Rhododendron* leaf extracts. The other tested Gram-positive bacteria showed similar susceptibility only towards leaf extracts derived from the 17 most bioactive *Rhododendron* species. All leaf extracts with antimicrobial bioactivity were extracted from representatives of the subgenus *Rhododendron*, with 15 from the sub-section *Rhododendron* and two belonging to the section *Pogonanthum*. The use of bacterial multidrug efflux pump mutants revealed remarkable differences in the susceptibility towards *Rhododendron* leaf extract treatment. In contrast, susceptibilities of the tested Gram-negative bacterial strains were classified into either low or moderate extent. Only one Gram-negative species, *Sinorhizobium meliloti*, belonging to the order of α -proteobacteria exhibited susceptibility to most of the bioactive *Rhododendron* extracts and was therefore similar in its susceptibility to the majority of Gram-positive bacteria. The results of Rezk et al. (2015a) suggested that common genetic traits are responsible for the production of bioactive secondary metabolite(s) which act primarily on Gram-positive organisms, and which may affect Gram-negative bacteria independence of the activity of multidrug efflux pumps in their cell envelope. In other studies, Rezk et al. (2015b) have assessed the cytotoxicity exerted by leaf extracts from plants of the genus *Rhododendron* towards epidermal keratinocytes and intestine epithelial cells. Different concentrations of DMSO-dissolved remnants of crude methanol *Rhododendron* leaf extracts were incubated for 24 h with cultured epidermal keratinocytes (human HaCaT cell line) and epithelial cells of the intestinal mucosa (rat IEC6 cell line) and tested for their cytotoxic potential. In particular, the cytotoxic potencies of the compounds contained in antimicrobial *Rhododendron* leaf extracts were assessed by quantifying their effects on plasma membrane integrity, cell viability and proliferation rates, cellular metabolism, cytoskeletal architecture, and determining initiation of cell death pathways by morphological and biochemical means. Extracts of almost all *Rhododendron* species, when applied at 500 $\mu\text{g}/\text{mL}$, were potent in negatively affecting both keratinocytes and intestine epithelial cells, except material from *R. hippophaeoides* var. *hippophaeoides*. Extracts of *R. minus* and *R. racemosum* were non-toxic towards both mammalian cell types when used at 50 $\mu\text{g}/\text{mL}$, which was equivalent to their minimal inhibitory concentration against bacteria. At this concentration, leaf extracts from three other highly potent antimicrobial *Rhododendron* species proved non-cytotoxic against one or the other mammalian cell type: Extracts of *R. ferrugineum* were non-toxic towards IEC6 cells, and extracts of *R. rubiginosum*, as well as *R. concinnum*, did not affect HaCaT cells. In general, keratinocytes proved more resistant than intestine epithelial cells against the treatment with compounds contained in *Rhododendron* leaf extracts (Rezk et al., 2015b).

The essential oil of *Rhododendron anthopogon* was investigated by Innocenti et al. (2010). The anti-microbial activity of *R. anthopogon* essential oil was evaluated by reference microdilution assays against a series of Gram-positive and Gram-negative reference strains. Gram-positive

and Gram-negative bacteria: *Staphylococcus aureus* (ATCC 20202), *Enterococcus faecalis* (ATCC 29216), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27753) were evaluated to measure the anti-bacterial activity; a total of six strains of *Candida albicans* and nine strains of *Candida* spp., all clinical isolates, were selected to test the antifungal activity. Furthermore, *Mycobacterium tuberculosis* reference strain H37Rv was employed to determine the anti-tubercular activity. Although topical anti-inflammatory activity was obtained only at very high concentrations, a remarkable anti-microbial activity was detected against *B. subtilis* and *M. tuberculosis*. Moreover, the *R. anthopogon* essential oil inhibited most clinical strains of *Candida* spp. at doses comparable with reference antifungal drugs and the strongest activity was found against a clinical isolate of *C. pseudotropicalis*. Moreover, the oil was able to reduce cancer cell growth independently of the cell line and the treatment protocols used (Innocenti et al., 2010).

The properties of *Rhododendron* species which inhibit bacterial growth are mainly related to their chemical components including phenolic compounds, especially flavonoids, essential oils, procyanidins, chromones, terpenoids, and steroids against various Gram-positive and Gram-negative bacteria. It has many biological properties such as antioxidant, anti-inflammatory, antiviral, antibacterial, anticancer, antidiabetic, immunomodulatory, cardioprotective and hepatoprotective among others due to their polyphenolic constituents (Demir et al., 2016).

The biological activities of major procyanidins isolated from the leaf extract of *R. formosanum* Hemsl. were investigated by Wang et al. (2015). Four compounds, including two procyanidin dimers, procyanidin A1 (1) and B3 (2), and two procyanidin trimmers, procyanidin C4 (4) and cinnamtannin D1 (5), were isolated and identified on the basis of spectroscopic data. The structure of a new procyanidin dimer, rhodonidin A (3), was elucidated by 2D-NMR, CD spectrum, and MS. The procyanidin trimmers and rhodonidin A are reported for the first time in Ericaceae. The biological activities of these procyanidins were evaluated using anti-bacterial and anti-oxidative assays. Only the new compound 3 demonstrated strong antibacterial activity against *Staphylococcus aureus* at a MIC value of 4 µg/mL. All compounds showed pronounced antioxidant activities and the activities are enhanced as the amount of OH groups in procyanidins increased (Wang et al., 2015).

The proanthocyanidins of the leaves of 16 taxa of the *Rhododendron* genus [*Rhododendron catawbiense* Michx. cv. *Grandiflorum*, *Rhododendron* 'Cunningham's White' (syn. *Rhododendron caucasicum* Pall. × *Rhododendron ponticum* L.), *Rhododendron smirnowii* Trautv., *Rhododendron calophytum* Franch., *Rhododendron dichroanthum* ssp. *scyphocalyx* (Balf. f. & Forrest) Cowan, *Rhododendron micranthum* Turcz., *Rhododendron praeevernum* Hutch., *Rhododendron ungeronii* Trautv., *Rhododendron kaempferi* Planch., *Rhododendron degronianum* ssp. *heptamerum* var. *hondoense* (Nakai) H. Hara, *Rhododendron fortunei* Lindl., *Rhododendron ponticum* L., *Rhododendron galactinum* Balf. f. ex Tagg., *Rhododendron oreotrephes* W. W. Sm., *Rhododendron brachycarpum* ssp. *brachycarpum* D. Don ex G. Don, and *Rhododendron insigne* Hemsl. & E. H. Wilson] were investigated qualitatively by liquid chromatography-mass spectrometry by Jaiswal et al. (2012). Four caffeoylquinic acids, six p-coumaroylquinic acids, epigallocatechin, galocatechin, catechin, epicatechin, epigallocatechin gallate, catechin gallate, epicatechin gallate, galocatechin gallate, two quercetin-O-hexosides, quercetin-O-galloyl-hexoside, quercetin-O-pentoside,

quercetin-O-rhamnoside, quercetin-O-pentoside-O-hexoside, quercetin-O-rhamnoside-O-hexoside, quercetin-O-feruloyl-hexoside, quercetin-O-(p-hydroxy)benzoyl-hexoside, taxifolin-O-pentoside, myricetin-O-rhamnoside, two myricetin-O-pentosides, three myricetin-O-hexosides, and two myricetin-O-galloyl-hexosides were detected (Jaiswal et al., 2012).

The seven flavanones were isolated from the aerial parts of *Rhododendron hainanense* Merr. and were tested for their antimicrobial activities against six bacteria and six plant pathogenic fungi by Li et al. (2016). Within the series of flavanones tested, farrerol (1) displayed moderate antibacterial activities against *Bacillus cereus*, *B. subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Erwinia carotovora*, with MICs ranging from 15.6 to 125 µg/mL. Furthermore, farrerol (1) exhibited excellent inhibitory activities against six plant pathogenic fungi: *Fusarium oxysporum* sp. *niveum*, *Colletotrichum gloeosporioides*, *Penicillium italicum*, *Rhizoctonia solani*, *Fusarium oxysporum* sp. *cubense* and *Phytophthora melonis*, with EC50 values of 9, 18, 35, 39, 46 and 66 µg/mL, respectively (Li et al., 2016).

The antioxidant properties and cytotoxic activity of species belonging to the *Rhododendron* genus were also studied. For example, Demir et al. (2016) have evaluated the antioxidant properties and cytotoxic activity of the extract of flowers of *Rhododendron luteum* Sweet on three cancers (human breast, colon, and liver carcinoma) and human foreskin fibroblast cells for the first time. *R. luteum* extract exhibited selective cytotoxicity against colon and liver cancer cells compared to normal fibroblast cells, while this selective cytotoxicity was not observed in breast cancer cells (Demir et al., 2016). The essential oil of *Rhododendron anthopogon* D. Don. showed marginal antibacterial and cytotoxic activities against MCF-7, MDA-MB-231, and 5637, but no antifungal effects (Dosoky et al., 2016).

Fifty *Rhododendron* honey samples obtained from the Black Sea Region of Turkey were also screened for total phenolic content and for potential antioxidant activity by Silici et al. (2010). The antimicrobial activity was studied by using eleven bacteria and two yeasts. The 13 microorganisms containing eleven bacteria and two yeasts were used as test organisms: *Aeromonas hydrophila* ATCC 7965, *Bacillus cereus* FMC 19, *Bacillus subtilis* ATCC 6630, *Escherichia coli* O157:H7 RS_932, *Listeria monocytogenes* 1/2B, *Mycobacterium smegmatis* RUT, *P. mirabilis* BC_3624, *P. aeruginosa* ATCC 27853, *Salmonella typhimurium* NRRLE_4463, *Staphylococcus aureus* ATCC 28213, *Candida albicans* ATCC 1223, *Saccharomyces cerevisiae* BC 5461, and *Yersinia enterocolitica* ATCC 1501. The honey samples showed the highest antimicrobial activity against *P. aeruginosa* and *P. mirabilis*. In addition, *S. aureus*, *A. hydrophila*, *L. monocytogenes*, *B. subtilis*, *M. smegmatis*, and *S. typhimurium* were moderately sensitive to the antimicrobial activity of *Rhododendron* honey. However *B. cereus*, *E. coli* O157:H7, and *Y. enterocolitica* were the most resistant microorganisms. In addition, honey samples also had no inhibitory effects on two yeasts; *C. albicans* and *S. cerevisiae* (Silici et al., 2010).

Conclusions

The results revealed that extract exerts antibacterial activity against *Citrobacter freundii*. However, the *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Escherichia coli* were resistant to *R. myrtifolium* leaf extract. Maximum *in vitro* inhibition was scored against *Citrobacter freundii*, followed by *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*. It can be

concluded that *Rhododendron myrtifolium* leaves extract can be as complementary medicine in treating diseases caused by multidrug-resistant strains of *Citrobacter freundii*. However, further investigation is needed to determine the bioavailability of the active compounds and to determine the dose and toxicity before it can be used as therapeutic agents.

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