In vitro activity of Turkish plant extracts against biofilm-producing food-related bacteria

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Abstract

The identification of effective antimicrobial agents also active on biofilms is a topic of crucial importance in food and industrial environment. For that purpose methanol extracts of Turkish plants, *Ficus carica* L., *Juglans regia* L., *Olea europaea* L., *Punica granatum* L. and *Rhus coriaria* L., were investigated. Among the extracts, *P. granatum* L. and *R. coriaria* L. showed the best antibacterial activity with minimum inhibitory concentrations of 78-625 µg/ml for *Listeria monocytogenes* and *Staphylococcus aureus* and 312-1250 µg/ml for *Escherichia coli* and *Pseudomonas aeruginosa*. SubMICs produced a significant biofilm inhibition equal to 80-60 % for *L. monocytogenes* and 90-80 % for *S. aureus*. The extracts showed also the highest polyphenols content and the strongest antioxidant activity. Bioassay-guided and HPLC procedures demonstrated the presence of apigenin 4′-O-β-glucoside in *P. granatum* L. and myricetrin and quercitrin in *R. coriaria* L. Antigenotoxicity of plant extracts was also observed. The present findings promote the value-adding of *P. granatum* L. and *R. coriaria* L. leaves as natural antimicrobial/antioxidant agents for control of food-related bacterial biofilms.

1.Introduction

Most of the pathogens involved in food-borne diseases are capable to adhere to and form biofilms on different surfaces (Le Magrex-Debar et al., 2000). The biofilm development on the food-product or food-contact surfaces is a potential source of contamination that may compromise food quality or cause pathogen transmission with health hazards (Bridier et al., 2015; Gibson et al., 1995). Once firmly established, a biofilm can be very difficult to eradicate because the bacteria embedded in a self-produced polymeric substance exhibiting poor susceptibility to conventional antimicrobial agents (Olsen, 2015). Alternative strategies or more effective agents are needed. An interesting approach to limit the formation of bacterial biofilms could involve the use of natural products (Buommino et al., 2014; Nostro et al., 2007). The acceptance of traditional medicine as an alternative form of health care has led to an increased use of medicinal plants. The World Health Organization estimates that plant extracts or their active constituents are utilized in traditional medicine in ~ 80% of the world's population (Anonymous, 1993). In this context, it is worth emphasizing the importance of scientific research in the identification of new natural compounds with a proven antimicrobial-antibiofilm activity. Literature already reports experimental evidences of the use of secondary plant metabolites, such as alkaloids, flavonoids, tannins, terpenes and terpenoids, for their potential antimicrobial role in biomedical, industrial and food fields (Cowan, 1999).

Turkey is known as one of the richest country of plants in the world. The number of flora species is estimated to be around 3.000-5.000 in the 1960s and 8.500-9.000 today (Guner et al., 2000; Turker and Koyluoglu, 2012). Around 3.500 of these species are endemic plants (Yeşilada et al., 1993; Yeşilada et al., 1995). Owing to their biological properties, several plants belonging to the flora of Turkey are used in the traditional medicine. However, only a small proportion of these plant species have been thoroughly studied and investigated for their antimicrobial activity and very few studies on biofilm production have been published (Marino et al., 2010).

The first aim of this study was to study the polyphenols content and the biological properties such as antibacterial**,** antibiofilm and antioxidant activities of methanolic extracts derived from Turkish *Ficus carica* L., *Juglans regia* L., *Olea europaea* L., *Punica granatum* L. and *Rhus coriaria* L. The most active extracts were successively tested by antibacterial and antioxidant (HP)-TLC bioautographic assay in order to determine the active fractions and characterize the compounds. In addition the antigenotoxic potential was also assessed.

2. Materials and methods

2.1. Plant materials and extraction procedures

The selected drugs *Ficus carica* L. leaf, *Juglans regia* L. leaf and fruit peel, *Olea europaea* L. leaf, *Punica granatum* L. leaf and *Rhus coriaria* L. leaf were bought from the local market in Konya, Turkey. The leaves from all samples and fruit peel from *J. Regia* L. were air dried at room temperature and aliquots of 20 g were then extracted with methanol using Soxhlet apparatus for about 8 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered by using Whatman filter paper and the residues were evaporated to dryness using rotary evaporator at not more than 40 ^{o}C. Than the extracts were lyophilized and stored until the analysis.

2.2. Determination of total phenolics

The determination of the total polyphenolic content of plant extracts were performed using a ThermoSpectronic Helios-y spectrophotometer, according to previously described methods (Rossi et al., 2011). The content of total polyphenols was expressed as g of gallic acid equivalent (GAE)/kg of extract.

2.3. Radical scavenging activity: spectrophotometric DPPH assay

Stock solutions of all extracts (25 mg/ml of methanol) were diluted 100, 300, 500, 700, 850, 1000-fold with methanol. An aliquot of 100 µl of each solution were added to 2.9 ml of 1,1-

diphenyl-2-picrylhydrazyl (DPPH; $1x10^{-4}$ M in ethanol), shaken vigorously and kept in the dark for 30 min at room temperature. Sample absorbance was measured at 517 nm with UV–vis spectrophotometer (ThermoSpectronic Helios-y, Cambridge, UK). A blank was assessed as the solution assay described above without extract, instead of which methanol was employed. Trolox was used as positive control and prepared with testing solutions as described above for dried extracts. The radical scavenging activities of each sample were calculated according to the following formula for inhibition percentage (Ip) of DPPH:

$$Ip\ DPPH\% = (A_B - A_A) / A_B \times 100$$

where A_B and A_A are the absorbance values of the blank sample and of the test sample respectively, after 30 min. Extracts and Trolox antiradical activity was considered as the concentration providing DPPH 50% inhibition (IC_{50}), calculated from inhibition curves obtained by plotting inhibition percentage against extract concentration (Rossi et al., 2012).

2.4. Antibacterial activity

Microrganisms used for this study were: *Staphylococcus aureus* ATCC 6538P, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027. Cultures for antimicrobial tests were grown in Mueller-Hinton Broth (MHB, Oxoid, Basingstoke, United Kingdom) at 37°C for 24 h.

For antibacterial testing, each extract was dissolved in pure dimethylsulfoxide (DMSO; BDH, Milan, Italy) to obtain a stock solution at a concentration of 250 mg/ml. Overnight broth cultures, adjusted to yield approximately 5×10^8 colony forming units (CFU)/ml were streaked with a calibrated loop on plates containing Mueller Hinton Agar (MHA, Oxoid). Filter paper discs (6 mm diameter; Oxoid) were placed on the inoculated agar surfaces and impregnated with 10 µl of stock solutions. The plates were observed after 18 h at 37 °C. All tests were performed in duplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts

The minimum inhibitory concentration (MIC) of plant extracts was determined in MHB, using a broth dilution micromethod in 96-well round-bottomed polystyrene microtiter plates according to the Clinical Laboratory Standards Institute (2009) guidelines, with some modifications. The stock solutions of each extract were serial twofold diluted in MHB and the final concentration ranged from 5000 to 39 µg/ml. The MIC was considered as the lowest concentration of each extract giving a complete inhibition of visible bacterial in comparison with a control well. The minimum bactericidal concentration (MBC) was determined by seeding 20 µl from all clear MIC wells onto MHA plates and was defined as the lowest extract concentration that allowed no microbial growth after incubation at 37 °C for 18 to 24 h. The data from at least three replicates were evaluated, and modal results were calculated.

2.5. Effect on growth an biofilm formation

The extracts of *J. regia* L. (leaf), *O. europaea* L., *P. granatum* L. and *R. coriaria* L. were selected as the best antibacterial extracts for evaluation of the effect on bacterial growth and biofilm formation. The effect of different concentrations of plant extracts (ranging from 1/2 to 1/16 MIC) on biofilm-forming ability was tested on 96-well polystyrene flat-bottomed microtitre plates (Costar, Corning) as described by Cramton et al., (1999) with some modifications. Briefly, bacterial cultures were grown overnight in 10 ml of Tryptic Soy Broth (TSB) for *L. monocytogenes, E. coli* and *P. aeruginosa* and TSB+1 % glucose for *S. aureus*, diluted in growth medium to 5×10^5 CFU/ml and dispensed (100 µl) into each well of microtiter plate in presence of sub-MIC concentrations (100 µl) of plant extracts or control medium. After incubation 24 h at 37 °C, the effect on (a) planktonic bacterial growth and (b) biofilm formation was evaluated as follows:

(a) planktonic bacterial growth was estimated by measuring the optical density (OD) at 492 nm using a spectrophotometer EIA reader (Bio-Rad Model 2550, Richmond, CA, USA);

(b) biofilm formed on the polystyrene well was washed twice with sterile phosphate-buffered saline (PBS; pH 7.4), dried, stained for 1 minute with 0.1% safranin and then

washed with water. The stained biofilms were resuspended in 200 µl of 30% (v/v) acetic acid and OD was measured by spectrophotometry at 492 nm using a spectrophotometer EIA reader.

The growth or biofilm reduction was calculated as:

$$100 - [\text{mean } OD_{492} \text{ of treated well} / \text{mean } OD_{492} \text{ of control well}] \times 100.$$

2.6. (HP)TLC-bioautographic assay

The extracts of *P. granatum* L. and *R. coriaria* L. were selected for (HP)TLC-bioautographic assay with the aim to determine the most active radical scavenging and antibacterial compounds (Rossi et al., 2011).

For DPPH-(HP)TLC bioautographic assay, sample solutions of 25 mg/ml of *P. granatum* L. and *R. coriaria* L. extracts were prepared. Negative control was set up with methanol (chosen solvent), and positive with Trolox. For *R. coriaria* L., 15 µl of solution were applied in triplicate to (HP) TLC plate of silica gel (Merck, silica gel 60, with fluorescence indicator F254) as 15 mm wide bands with Linomat IV (Camag Muttenz, Switzerland) and then, eluted in a chromatographic chamber at first step with a solvent solution composed as ethyl acetate/acetic acid/formic acid/ water 100/11/11/20 for 4 cm and at second step with toluene/ethyl acetate/acetic acid 100/90/10 for 8 cm. For *P. granatum* L., 15 µl of solution were applied in triplicate to (HP)TLC plate as 15 mm wide band and the eluted with ethyl acetate/methanol/acetic acid/water for 8 cm. After plate development (three chromatograms on the same plate), the first chromatogram was sprayed with an ethanolic solution of 2,2-diphenyl- 1-picryl-hydrazyl radical (DPPH, 20 mg/100ml) to detect the antioxidant fractions, while the other two were visualized at 254 nm (in the case of *R. coriaria* L., sprayed with NP/PEG reagent) to identify the active constituents spots and be able to scratch it out, and extract it, from the eluted silica gel. The spot extract was directly analyzed by HPLC-DAD.

The antibacterial-(HP)TLC-bioautographic assay was performed employing *S. aureus*. The strain was cultured according to the method previously described (Rossi et al., 2011). Stock

192 solutions of *P. granatum* L. and *R. coriaria* L. were applied in triplicate to (HP)TLC plate,
193 processed and eluted ad described in the above paragraph. Negative control was set up with
194 methanol, and positive with chloramphenicol. The developed plates were dried at room temperature
195 for 30 min for complete removal of the solvent and a chromatogram was separated from the other
196 two and treated with *S. aureus* (10^7 CFU/ml) in proper agarized medium distributed over (HP)TLC
197 plate. An aqueous solution of triphenyl tetrazolium chloride (TTC) (20 mg/ml) was added to the
198 medium as growth indicator. The (HP)TLC plates, prepared as described above, were then
199 transferred to Petri dishes as support and incubated overnight at 37°C. Antimicrobial compounds
200 appeared as clear yellow spots against a red coloured background. The other two chromatograms
201 were used to separate and analyze the active compounds as described in the previous paragraph.
202
203 2.7. HPLC analysis
204 The active fractions revealed with DPPH and antibacterial bioautographic assay were
205 scratched out and extracted from silica gel in methanol solution was injected in HPLC. The analysis
206 were performed using a JASCO modular HPLC system (Tokyo, Japan, model PU 2089) coupled to
207 a diode array apparatus (MD 2010 Plus) linked to an injection valve with a 20 µl sampler loop. The
208 column used was a Eclipse-PLUS-C18 (25 µm × 0.46 cm, i.d., 5 µm) at a flow rate of 1.0 ml/min.
209 The mobile phase consisted of solvent solution A (methanol/acetonitrile = 50:50) and B
210 (water/formic acid = 99.5:0.5). The gradient system adopted was chosen according to the molecule
211 to analyze. The gradient was characterized by five steps: 1, starting point at 90:10 v/v (A/B); 2,
212 gradual changing to 50:50 v/v in 30 min; 3, B progressive raise to 0:100 v/v in 5 min; 4, isocratic
213 (0:100 v/v) for 5 min and 5, back to starting point (90:10 v/v) in 5 min. Injection volume was 40 µl
214 (Tacchini et al., 2015). The mass experiment were carried out on a FinniganMAT LCQ
215 (ThermoQuest Corp./FinniganMAT; San Jose, CA) mass spectrometer module, equipped with an
216 ion trap mass analyzer and an ESI ion source electrospray, in negative ion mode. For ESI-MS and
217 MS^2 experiments, the parameters were set as follows: the capillary voltage was 3.5 kV, the

nebulizer (N_2) pressure was 20 psi, the drying gas (N_2) temperature was 300 °C, the drying gas flow was 9 L/min and the skimmer voltage was 40 V. The mass spectrometer was operated in the negative ion mode in the m/z range 100–1500.

2.8. SOS-Chromotest

Genotoxicity and antigenotoxicity assays were performed as described by Prencipe et al., (2014) using exponential-phase culture of *E. coli* PQ37. The induction factor (IF), value that establish the genotoxicity degree of the tested matrix, was obtained by comparing β-galactosidase and alkaline phosphatase activity in treated and untreated cells. The protective effect of *P. granatum* L. and *R. coriaria* L. extracts was, instead, expressed as percentage of inhibition of genotoxicity induced by 4NQO and was evaluated following the formula reported by Bouhlel et al., (2007):

$$\text{inhibition (\%)} = 100 - ((IF_1\text{-}IF_2)/(IF_2\text{-}IF_0)) \times 100$$

where IF_1 is the induction factor in presence of both test compound and mutagen; IF_2 is the induction factor of the mutagen in absence of the tested compound ; and IF_0 is the induction factor of the untreated cells. All the data collected for each assay are the average of three determinations in three independent experiments.

2.9. Statistical analysis

All the experiments were performed in triplicate. All values are expressed as the mean ± SD. The significance of the results was analyzed by one way analysis of variance (ANOVA), with a p value <0.05 considered significant.

3. Results

3.1. Determination of total phenolics

The content of total polyphenols of Turkish plant extracts is reported in Table 1. The most interesting results were obtained for *P. granatum* L. and *R. coriaria* L. extracts: the content of polyphenols were respectively 17.63±0.43 and 25.38±1.03 g $_{\text{gallic acid}}$ /100 g $_{\text{plant extract}}$

3.2. Radical scavenging activity: spectrophotometric DPPH assay

The samples tested by spectrophotometric DPPH assay showed different antioxidant activity (Fig. 1). The most relevant result was exhibited by *R. coriaria* L. extract, which showed an IC_{50} value better than positive control Trolox, followed by *P. granatum* L. extract.

3.3. Antibacterial activity

The antibacterial activity of plant extracts is listed in Table 2. Specifically, disc diffusion testing reported the higher activity of *P. granatum* L. and *R. coriaria* against Gram-positive bacteria (inhibition diameters ranged from 12 to 21 mm) than Gram-negative bacteria (inhibition diameters of 8-18 mm). The other extracts showed slight activity according to following order: *O. europaea* L. > *J. regia* L. (leaf) > *F. carica* L. and *J. regia* L. (fruit peel). The higher efficacy of *P. granatum* and *R. coriaria* was confirmed by the broth dilution method. The MIC values ranged from 78 µg/ml to 625 µg/ml for *L. monocytogenes* and *S. aureus* and from 312 µg/ml to 1250 µg/ml for *E. coli* and *P. aeruginosa*. However, the inhibitory effect of the extracts was bacteriostatic rather than bactericidal except for *S. aureus*, in fact the MBC values were 625-2500 µg/ml.

3.4. Effect on growth and biofilm formation

Despite that, the biofilm production was differently formed in accordance with the strain, *P. granatum* L. and *R. coriaria* L. leaves extract were able to reduce biofilm formation more readily than other extracts. The results revealed that 1/2, 1/4, 1/8 and 1/16 x MIC of *P. granatum* L. and *R. coriaria* L. extracts poorly interfered with the planktonic growth, causing a slight decrease equal to 20-10% respect to the control (Fig. 2), while produced a significant ($p<0.05$) inhibition of biofilm

formation equal to 80-60 % and 90-80 % for *L. monocytogenes* and *S. aureus* respectively (Fig. 3). In contrast, a reduced biofilm biomass inhibition of *E. coli* (40-25 %) and *P. aeruginosa* (30-20 %) was observed. SubMIC doses of *J. regia* (leaf) and *O. oleuropea* showed a lower inhibiting activity with reductions of biofilm formation equal to 50-25% for Gram positive bacteria and 40-10% for Gram negative bacteria than the other extracts.

3.5. (HP)TLC-bioautographic assay and HPLC analysis

The bioautographic analysis demonstrated the most active fractions at R_f= 0.7 for *P. granatum* and at R_f= 0.5 for *R. coriaria* (Fig. 4). Based on the previous literature data, as well as by direct comparison of standards UV spectra obtained by HPLC-DAD and mass spectra obtained by HPLC-MS (Table 3), we confirmed the presence of myricetrin and quercitrin in the active fraction at R_f=0.5 of *R. coriaria* L. extract and the apigenin 4′-O-β-glucoside as main active constituent in the active fraction at R_f=0.7 of *P. granatum* L. extract.

3.6. SOS-Chromotest

A earlier series of experiments carried out on the antigenotoxic activity evaluation pointed out the absence of influence of the different concentrations of *R. coriaria* L. extract on the viability of the *E. coli*. The same phenomenon was not exhibited by *P. granatum* L. extract, that showed an effect on viability at the highest concentrations tested. The SOS chromotest genotoxicity assay examined the ability of the two plant extracts to produce a SOS response. According to Kevekordes et al., (1999), a compound is classified non-genotoxic if its Induction Factor (IF) is <1.5, slightly genotoxic if the induction factor ranges between 1.5 and 2, and genotoxic if the IF value exceeds 2. The test revealed, for both plant, the very low effect of non-citotoxic concentrations on the induction factor of the SOS chromotest, because IF values are lower than 1.5 (Table 4). Considering the experimental data and the literature data, *P. granatum* L. and *R. coriaria* L. extracts were considered non-genotoxic. Dose of 2.5 µg/assay of the directly acting mutagen 4-nitroquinoline-1-

oxide (4NQO) was chosen for the antigenotoxic activity evaluation, since this dose induce a significant SOS system activation and was not toxic. Every concentration of *R. coriaria* L. extract decreased the activation of the SOS system induced by 4NQO, showing inhibition of genotoxicity in a dose dependent manner except for 0.6 and 0.3 µg/ml. Concerning *P. granatum* L., the highest concentration of the extract interfered with *E. coli* viability and the remaining concentrations tested showed antigenotoxic potential in a non-dose-dependent manner (Table 4).

4. Discussion

The scientific interest into biological properties of Turkish wild plants has received much attention in recent years and a lot of papers have confirmed their traditional uses (Güzel et al., 2015; Polat et al., 2013; Turker and Usta, 2008). In this context, the study contributed to enrich the literature data exploring the activity of five Turkish plant extracts. The findings pointed out that the leaves of *P. granatum* L. and *R. coriaria* L. possess interesting antibacterial, antibiofilm and antioxidant potential and are highlighted as bioactive promising plant-materials. Additionally, antigenotoxicity was observed for the non-bacterial-toxic concentrations of both plant extracts.

P. granatum L. and *R. coriaria* L., commonly known as pomegranate and sumac respectively, are recognized for their well documented biological activities including antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory and anticancer activities (Jurenka, 2008; Rayne and Mazza, 2007; Shabbir, 2012). However, while the efficacy of *P. granatum* L. and *R. coriaria* L. fruits is well reported in literature (Fawole et al., 2012; Fazeli et al., 2007), few studies were performed on the antimicrobial activity of leaves extracts. The results of the study emphasize the higher polyphenols content and stronger antibacterial and antioxidant activity of *P. granatum* L. and *R. coriaria* L. leaves extracts compared to the other plant extracts. The bioautographic analysis of these plant extracts revealed bioactive bands in correspondence of flavonoidic fractions, effectively identified by HPLC analysis as myricetrin and quercitrin in *R. coriaria* L. and apigenin 4′-O-β-glucoside in *P. granatum* L.. These components belonging to

flavonols and flavones are a large group of naturally occurring plant compounds that received a great deal of attention because of their antibacterial and antioxidant activity (Cushnie and Lamb, 2011; Gould and Lister, 2006). In this context, Madikizela et al., (2013) demonstrated antimicrobial activity of flavonol glycosides myricetin-3-O-arabinopyranoside, myricetrin-3-O-rhamnoside and quercetin-3-O-arabinofuranoside isolated from *Searsia chirindensis* L.. Furthermore, Sato et al., (2000) suggested the potential use of apigenin and related flavonoids against methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

Regarding the effect on biofilm formation, the extracts of *P. granatum* L. and *R. coriaria* L. leaves produced a significant inhibition of *L. monocytogenes* and *S. aureus* biomass. Biofilm formation is still a worldwide public health concern especially in terms of nosocomial infections and food-borne illness. The research of novel molecules efficacy to prevent the biofilm formation is, therefore, a priority. Once again, the antibiofilm activity of *P. granatum* L. and *R. coriaria* L. has been rarely investigated and the few published papers are focused on the activity of different plant-material (gel, fruit and flower). One of the previous studies confirmed the potential of peel extract of *P. granatum* L. on inhibition of biofilm formation and disruption of preformed biofilm (Bakkiyaraj et al., 2013), and later papers reported the effect of *P. granatum* L. and *R. coriaria* L. on oral biofilm in patients using fixed orthodontic appliances or orthodontic wire (Vahid Dastjerdi et al., 2014a; Vahid Dastjerdi et al., 2014b). In this study, the ability of the plant extracts to prevent the bacterial adherence could be related to the inhibitory effect of its flavonoidic components. Apigenin has been reported as a promising natural anti-biofilm compound against *Streptococcus mutans* (Koo et al., 2003) while myricetin and quercetin have been studied for their inhibition of biofilm formation of *S. aureus* strains, including clinically isolated MRSA strains (Arita-Morioka et al., 2015; Lee et al., 2013). Interestingly, myricetin, quercetin and quercitrin exhibited strong sortase inhibitory activity (Kang et al., 2006; Liu et al., 2015), an enzyme modulating the ability of the bacteria to adhere to host tissue and responsible for anchoring surface protein virulence factors to the peptidoglycan cell wall layer of *S. aureus* (Mazmanian et al., 1999). Generally, the direct

antibacterial activity of different flavonoids may be attributable to up to three mechanisms: cytoplasmic membrane damage, inhibition of nucleic acid synthesis and inhibition of energy metabolism (Cushnie and Lamb, 2011). Additional evidence has also been presented for two new mechanisms: inhibition of cell membrane synthesis and inhibition of cell wall synthesis by D-alanine–D-alanine ligase inhibition (Wu et al., 2008). Although the mechanism behind biofilm inhibition is still unclear, the reason for the observed effects could be due to multiple factors acting in concert rather than alone. The primary adhesion to surfaces is a crucial event for the biofilm formation and it is affected by many factors such as physic-chemical properties of surfaces, bacteria characteristics and environmental components (Simoes et al., 2007). Then it is conceivable that the inhibition of biofilm may be related to the ability of plant extracts/flavonoidic components to inactivate microbial adhesins and enzymes leading to an alteration of the bacterial surface thereby interfering and compromising the cell-substratum interactions, attachment phase and normal biofilm development. The aggregatory effect of flavonols on whole bacterial cells (Cushnie et al., 2007) could also determine a preferred interaction of bacterial cells between themselves rather than with the surface. Further studies are required to clarify the action mechanism(s).

In conclusion, having more effective antimicrobial agents, with no citoxicity or DNA damage and also effectives to prevent or at least interfere with biofilm formation, is a considerable achievement. To the best of our knowledge, this is the first study reporting the antibiofilm activity of the leaf extract of *P. granatum* L. and *R. coriaria* L.. Therefore the present findings provide scientific basis to promote the value-adding of *P. granatum* L. and *R. coriaria* L. leaves as natural antimicrobial/antioxidant agents for control of food-related bacterial biofilms.

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Fig. 1. Radical scavenging activity. IC_{50} with DPPH test.

Fig. 2. Effect of turkish plant extracts at subMIC doses on planktonic bacterial growth. Values are expressed as means ± standard deviations.

Fig. 3. Effect of turkish plant extracts at subMIC doses on biofilm formation. Values are expressed as means ± standard deviations.

Fig. 4. (HP)TLC bioautographic assay of (a) *P. granatum* L. and (b) *R. coriaria* L. extracts.

Table 1
Total polyphenols of Turkish plant extracts.

Plant extract	Parts used	Total polyphenols[a]
Ficus carica L.	leaves	3.29±0.12
Juglans regia L.	leaves	10.50±0.38
Juglans regia L.	fruit peel	4.22±0.43
Olea europaea L.	leaves	8.18±0.33
Punica granatum L.	leaves	17.63±0.43
Rhus coriaria L.	leaves	25.38±1.03

[a] $g_{\text{gallic acid}}$ /100 g of plant extract.

Table 2
Antibacterial activity of Turkish plant extracts.

Plant extract	Parts used	Test	*L. monocytogenes* ATCC 7544	*S. aureus* ATCC6538P	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 9027
Ficus carica L.	leaves	Ø[a]	6	6	<6	<6
		MIC[b]	5000	5000	>5000	>5000
		MBC[c]	>5000	>5000	>5000	>5000
Junglans regia L.	leaves	Ø	10	10	6	6
		MIC	2500	2500	5000	5000
		MBC	>5000	>5000	>5000	>5000
Junglans regia L.	fruit peel	Ø	7	6	<6	<6
		MIC	5000	5000	>5000	>5000
		MBC	>5000	>5000	>5000	>5000
Olea europaea L.	leaves	Ø	12	12	6	6
		MIC	625	625	1250	5000
		MBC	>5000	2500	>5000	>5000
Punica granatum L.	leaves	Ø	12	19	8	10
		MIC	625	156	625	1250
		MBC	>5000	2500	>5000	>5000
Rhus coriaria L.	leaves	Ø	21	20	9	18
		MIC	78	78	312	312
		MBC	>5000	625	>5000	>5000

[a] Ø, Disc diffusion test, inhibition diameters in mm.
[b] MIC Minimum inhibitory concentration expressed in µg/ml.
[c] MBC Minimum bactericidal concentration expressed in µg/ml.

Table 3

Identification of phenolic compounds in *P. granatum* L. and *R. coriaria* L. active fractions.

	$[M-H]^-$(m/z)	MS/MS (m/z)	λ_{max}	Compound
Punica granatum L. $R_f=0.7$	431	269	267	Apigenin 4'-O-β-glucoside
Rhus coriaria L. $R_f=0.5$	463	316	263	Myricitrin[a]
	447	301	263	Quercitrin[a]

[a] Verified against pure standard

Table 4

Genotoxicity of *P. granatum* L. and *R. coriaria* L. extracts by SOS chromotest.

		Genotoxic activity	Antigenotoxic activity
	Dose (µg/ml)	Induction Factor	Inhibition of genotoxicity (%)
4NQO[a]	2.5	2.554	0
NC[b]	0	1.000	0
Punica granatum L.	0.06	1.035	44.510
	0.3	0.754	58.097
	0.6	0.878	51.046
	3	1.109	52.460
	6	1.245	38.729
	30	/[c]	/[c]
	60	/[c]	/[c]
Rhus coriaria L.	0.06	0.942	73.224
	0.3	0.829	77.059
	0.6	0.880	77.007
	3	0.884	77.543
	6	1.350	76.268
	30	0.770	78.359
	60	1.072	83.096

[a] 4NQO, 4-nitroquinoline-1-oxide, positive control of genotoxicity.

[b] NC, negative control (non-treated cells).

[c] The extract exhibited citotoxicity at this concentration.

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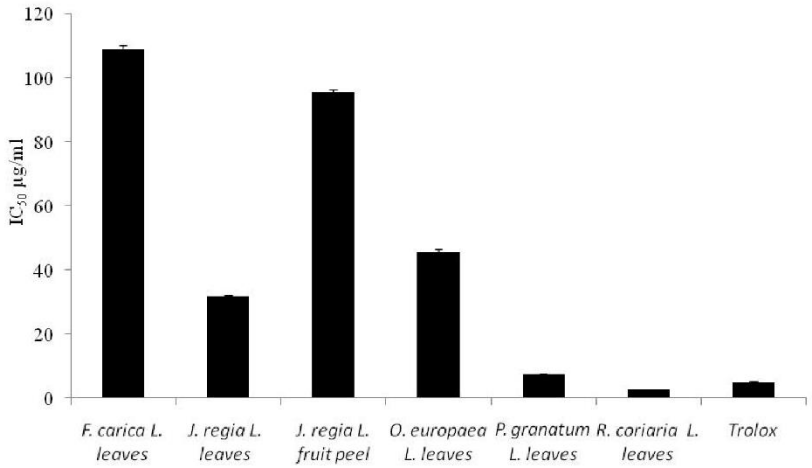
Fig. 1


120
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IC50 µg/ml
F. carica L. leaves
J. regia L. leaves
J. regia L. fruit peel
O. europaea L. leaves
P. granatum L. leaves
R. coriaria L. leaves
Trolox

673 Fig. 2

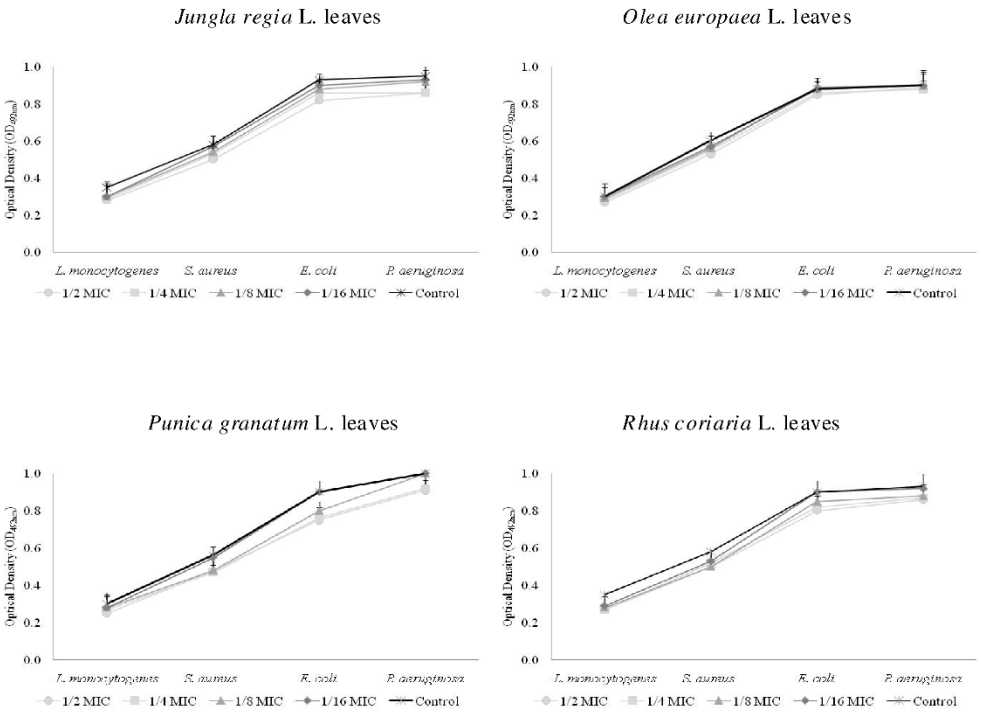

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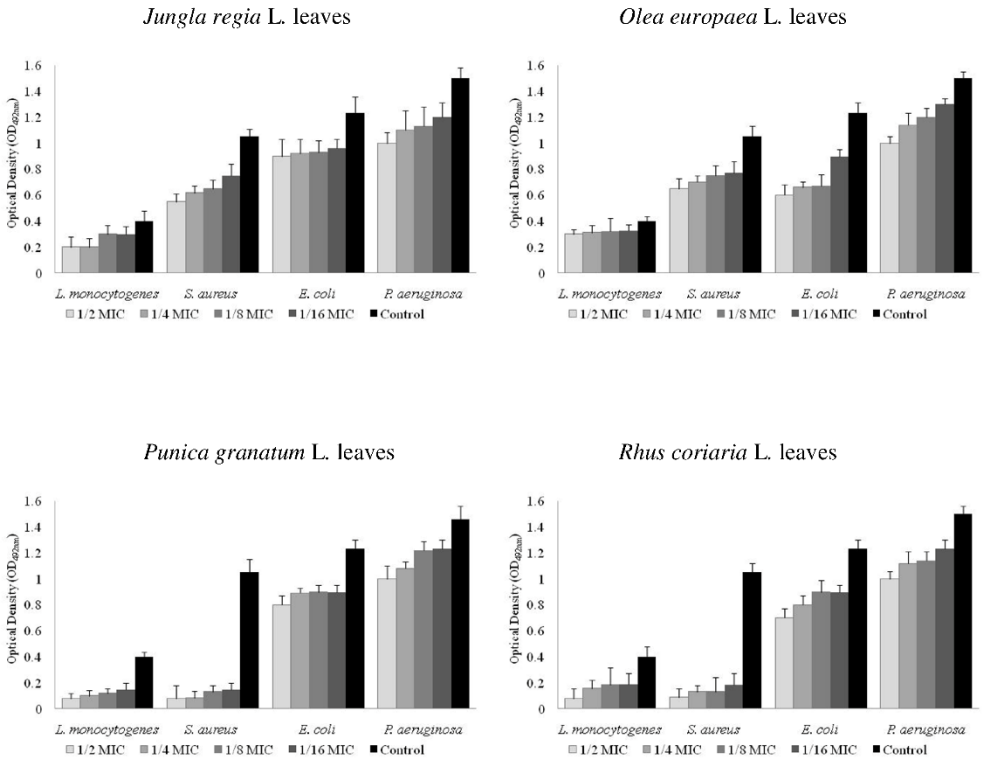
Fig. 3


Jungla regia L. leaves
Olea europaea L. leaves
Punica granatum L. leaves
Rhus coriaria L. leaves
Optical Density (OD_{492nm})
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
L. monocytogenes
S. aureus
E. coli
P. aeruginosa
1/2 MIC
1/4 MIC
1/8 MIC
1/16 MIC
Control

727 Fig.4
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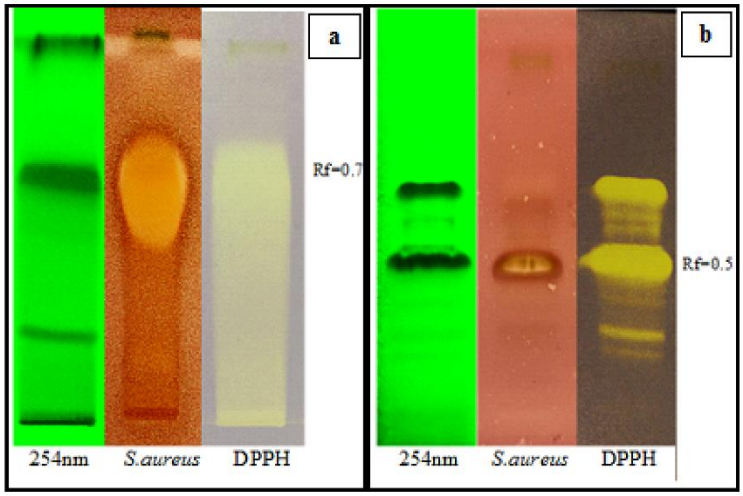

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