

# Accepted Manuscript

Profiling of Turkish propolis subtypes: Comparative evaluation of their phytochemical compositions, antioxidant and antimicrobial activities

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PII: S0023-6438(18)30378-5

DOI: [10.1016/j.lwt.2018.04.063](https://doi.org/10.1016/j.lwt.2018.04.063)

Reference: YFSTL 7074

To appear in: *LWT - Food Science and Technology*

Received Date: 27 November 2017

Revised Date: 11 March 2018

Accepted Date: 19 April 2018

Please cite this article as: Ristivojević, P., Dimkić, I., Guzelmeric, E., Trifković, J., Knežević, M., Berić, T., Yesilada, E., Milojković-Opsenica, Duš., Stanković, Slaviš., Profiling of Turkish propolis subtypes: Comparative evaluation of their phytochemical compositions, antioxidant and antimicrobial activities, *LWT - Food Science and Technology* (2018), doi: 10.1016/j.lwt.2018.04.063.

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1     **Profiling of Turkish propolis subtypes: comparative evaluation of their**  
2     **phytochemical compositions, antioxidant and antimicrobial activities**

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34 **Abstract**

35 Comprehensive analysis of phenolic profiles of botanically different subtypes of Turkish  
36 propolis samples were performed using UHPLC–LTQ/Orbitrap/MS/MS method, and  
37 additionally total phenolic (TPC) and total flavonoid contents (TFC) as well as their  
38 antioxidative activities were evaluated by spectrophotometry. Antimicrobial activity of  
39 Turkish propolis against oral cavity bacteria from the genus *Streptococcus* (*S. pyogenes*, *S.*  
40 *sanguinis*, *S. mutans*) and *Candida albicans* ATCC 10231 was determined by diffusion and  
41 microdilution methods. Extensive fingerprint analysis of Turkish propolis revealed the  
42 presence of fifty one phenolic compounds, with fifteen quantified which confirm their  
43 affiliation to the two subtypes of the European propolis. All analysed samples have shown  
44 antimicrobial potential against all tested bacteria, with *S. pyogenes* being the most sensitive  
45 one. Turkish propolis, especially its orange subtype, can be considered as the high-quality  
46 product due to its rich phenolic and flavonoid content, strong antioxidative and antimicrobial  
47 activities. Turkish propolis could be, therefore, a good raw material for food and  
48 pharmaceutical industry.

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52 **Keywords:** Phenolic profile of three subtypes of Turkish propolis; UHPLC–  
53 LTQ/Orbitrap/MS/MS; Total phenolic and flavonoid content; Antioxidant activity;  
54 Antimicrobial activity.

55

## 56 1. Introduction

57 Propolis is a natural resinous substance collected by honeybees (*Apis mellifera* L.) from  
58 different plant parts such as buds, branches, leaves and exudates (Yesilada, 2015). To date,  
59 two subtypes of propolis originated from *Populus* spp. were identified from Romanian,  
60 German, Serbian, Croatian, Slovenian and French propolis samples using several analytical  
61 techniques in combination with multivariate data analysis by various authors (Andjelković et  
62 al. 2017; Berthrams, Müller, Kunz, Kammerer, & Stintzing, 2013; Chasset, Häbe,  
63 Ristivojević, & Morlock, 2016; Morlock, Ristivojević, & Chernetsova, 2014; Milojković-  
64 Opsenica et al., 2016; Ristivojević et al. 2014; Sârbu, & Moş, 2011). These authors suggested  
65 that all poplar type propolis samples could be categorized under two botanically different  
66 varieties known as orange (O) and blue (B) subtypes depending upon the color of the  
67 separated compounds on HPTLC plate under UV-light after derivatization. In addition to  
68 these findings, Guzelmeric et al. (2018) have confirmed the existence of O- and B-subtypes  
69 of propolis from Turkey, as well as the existence of a new subtype which was mainly  
70 composed of non-phenolic components. Previous studies on Turkish propolis samples have  
71 reported their chemical compositions and several biological effects (antimicrobial and  
72 antioxidant), while in these studies the authors have mainly focused on the geographical  
73 origin without identification of the plants constituents (Keskin, Hazir, Baser, & Kürkçüoğlu,  
74 2001; Koru et al., 2007; Uzel et al., 2005). However, botanical origin of propolis is an  
75 important task due to the fact that its chemical composition depends on the plant resource.  
76 Till now, mainly microscopic pollen analysis was applied to justify the botanical origin of  
77 Turkish propolis (Çelemlî, & Sorkun, 2012). Gas Chromatography-Mass Spectrometry (GC-  
78 MS) was also used by several authors for investigation of chemical composition and  
79 determination of botanical origin of Turkish propolis (Duran et al., 2011). Furthermore,  
80 Popova, Silici, Kaftanoglu, & Bankova, 2005 investigated qualitative and quantitative

81 composition of Turkish propolis using TLC and GC-MS techniques and also determined its  
82 antibacterial activity. Botanical origins of propolis samples collected from different regions in  
83 Turkey were identified by simultaneous analysis of phenolic profile of propolis samples and  
84 plant buds' extracts by HPTLC, for the first time by our group (Guzelmeric et al., 2018).  
85 However, the phenolic composition of three subtypes of Turkish propolis, particularly based  
86 on its botanically different origins has not been investigated in detail so far.  
87 Current paper is continuation of our previous research related to HPTLC phenolic profiles of  
88 Turkish propolis, authentication according to their botanical origins as well as determination  
89 of antioxidative activity (Guzelmeric et al., 2018). The main objective of the present study  
90 was the detailed phenolic profiling of O- and B-subtypes of Turkish poplar type propolis by  
91 ultrahigh-performance liquid chromatography (UHPLC) coupled with hybrid mass  
92 spectrometer, which combines the linear trap quadrupole (LTQ) and Orbitrap MS/MS mass  
93 analyser. In addition, the quality control parameters such as total phenolic content (TPC),  
94 total flavonoid content (TFC), as well as antioxidative activity and antimicrobial activity  
95 against oral cavity bacteria from the genus *Streptococcus* (*S. pyogenes*, *S. sanguinis*, *S.*  
96 *mutans*) and *Candida albicans* were also investigated. The results from this study might solve  
97 a question: Which subtype of Turkish propolis would be a better source of raw material for  
98 pharmaceutical and/or food industry?

99

## 100 **2. Materials and methods**

### 101 *2.1. Chemical and materials*

102 Methanol (HPLC grade), sodium carbonate, potassium chloride, Folin-Ciocalteu reagent, and  
103 filter paper (Whatman No.1) were purchased from Merck (Germany). 2,2-Diphenyl-1-  
104 picrylhydrazyl·(DPPH·) was purchased from Fluka AG (Switzerland). Ethanol (96 vol. %)

105 was purchased from J. T. Baker (Netherlands). Syringe filters (13 mm, PTFE membrane  
106 0.45µm) were purchased from Supelco (USA). Ultrapure water was used in experiments  
107 (ThermoFisher TKA MicroPure water purification system, 0.055µS/cm). Aluminium chloride  
108 and standard phenolic compounds (chlorogenic acid, caffeic acid, vanillic acid, *p*-coumaric  
109 acid, ferulic acid, rutin, luteolin, quercetin, protocatechuic acid, *p*-hydroxybenzoic acid,  
110 cinnamic acid, apigenin, kaempferol, chrysin, pinocembrin, and galangin) were purchased  
111 from Sigma Aldrich (Germany). Streptomycin (stock 20 mg/mL), rifampicin (stock 100  
112 mg/mL and 15µg/disc), ampicillin (stock 25 mg/mL), cefpodoxime (10 mg/disc),  
113 amphotericin B (100 units/disc), pristinamycin (15 mg/disc), clotrimazole (10 mg/disc),  
114 mezlocillin (75 mg/disc) and nystatin (stock 5 mg/mL) were purchased from Sigma Aldrich  
115 (Germany). Resazurin Sodium Salt (> 90% (LC) C<sub>12</sub>H<sub>6</sub>N<sub>4</sub>NaO<sub>4</sub> = 251.17 g/mol) was  
116 purchased from TCI (Belgium).

## 117 2.2. Turkish propolis samples

118 In this study, forty-eight propolis samples [27 samples of orange, 17 of blue and the 4 of the  
119 third subtype propolis] (Guzelmeric et al., 2018), which were obtained from different regions  
120 of Turkey, were investigated (Fig. S1). Extraction procedure was described in our previous  
121 paper (Guzelmeric et al., 2018).

## 122 2.3. Measurement of the absorption spectra of propolis samples

123 The UV-Vis spectra were recorded using a Cintra 6 UV-Visible Spectrometer. Measurement  
124 of the absorption spectra was described in Ristivojević et al. (2017).

## 125 2.4. Estimation of the total phenolic content (TPC), total flavonoid content (TFC) and radical 126 scavenging activity (RSA)

127 Total phenolic content (TPC), and total flavonoids content (TFC) were analysed according to  
128 Kumazawa et al. (2004). The 0.1 mL of EEP and 6.0 mL of deionized water were mixed with  
129 0.5 mL of Folin-Ciocalteu reagent and the solution was incubated 5 min at room temperature.  
130 Then, 1.5 mL of sodium carbonate (20%) was added. After shaking and one hour of  
131 incubation at 40 °C, absorbance was measured at 760 nm. Gallic acid was used as a standard  
132 compound. The results were presented as mean value of three replicate measurements and  
133 expressed as mg of gallic acid equivalents (GAE) per gram of propolis sample.

134 For TFC, 0.5 mL of EEP was diluted with water up to 7.4 mL. Further, 0.4 mL of solution of  
135 aluminium chloride (10%) was added. Solution was shaken and incubated at room  
136 temperature for one hour; afterwards absorbance was measured at 420 nm. Quercetin was  
137 used as a standard. The results were presented as mean value of three replicate measurements  
138 and expressed as mg of quercetin (QE) per gram of propolis sample.

139 The radical scavenging activity (RSA) of the analysed samples was determined according to  
140 previous describes procedure (Ristivojević et al. (2017)). The 0.1 mL of EEP and 4.0 mL of  
141 freshly prepared methanol solution of DPPH· (71 mM) were mixed and then left for 45 min  
142 in the dark. The reduction of the DPPH· radical was measured by monitoring continuously  
143 the decrease of absorption at 517 nm. RSA was calculated as a percentage of DPPH·  
144 discoloration using the equation:

$$145 \quad RSA(\%) = \frac{(A_{DPPH} - A_{sample})}{A_{DPPH}} \cdot 100$$

146 where  $A_{DPPH}$  is the absorbance of methanol solution of DPPH· radical,  $A_{sample}$  is the  
147 absorbance in the presence of propolis extract.

148

149

## 150 2.5. UHPLC–LTQ/Orbitrap/MS/MS

151 Qualitative and quantitative analysis as well as validation parameters of UHPLC–  
152 LTQ/Orbitrap/MS/MS method were described in our previous paper (Ristivojević et al.,  
153 2014). Chromatographic separations were performed using a UHPLC system consisting of a  
154 quaternary Accela 600 pump and Accela Autosampler (Thermo Fisher Scientific). An  
155 analytical Hypersil gold C18-column (50 × 2.1 mm, 1.9 µm particle size; Thermo Fisher  
156 Scientific) was used for separations. The mobile phase consisted of (A) water with 1% formic  
157 acid and (B) acetonitrile. The gradient programme was as follows: 0.0–10.0 min, 5–95% B;  
158 10.0–12.0 min, 95% B; 12.0–12.2 min, 95–5% B; 12.2–15.0 min, 5% B. The injection  
159 volume for all samples was 5 µL and the flow rate was 300 µL/min. The UHPLC system was  
160 coupled to a linear ion trap and Orbitrap hybrid mass spectrometer (LTQ/Orbitrap) equipped  
161 with a heated- electrospray ionisation probe (HESI-II; Thermo Fisher Scientific). The mass  
162 spectrometer was operated in negative mode. Parameters of the ion source were as follows:  
163 source voltage 5 kV, capillary voltage –40 V, tube lens voltage –80 V, capillary temperature  
164 275°C, sheath and auxiliary gas flow (N<sub>2</sub>) 42 and 11 (arbitrary units). The MS spectra were  
165 acquired by full-range acquisition covering 100–900 m/z. A data-dependant scan was  
166 performed for the fragmentation study by deploying collision- induced dissociation (CID).  
167 The normalised collision energy of the CID cell was set at 35 eV.

## 168 2.6. Bacterial strains and growth conditions

169 Antibacterial activity of all propolis samples was tested against *S. mutans*, *S. pyogenes* and *S.*  
170 *sanguinis* isolated from the human oral cavity (Nikolić et al., 2013) and against *Candida*  
171 *albicans* ATCC 10231. The Luria-Bertani (LB) medium (HiMedia, India) was used for  
172 culturing the bacterial strains, while TSB medium (Biomedics, Spain) was used for the  
173 growth of *C. albicans*. The number of viable cells (CFU/mL) was determined for each tested



174 strains at hourly intervals for a period of 8 hours. A single colony of the particular strain was  
175 inoculated in 150 mL of the appropriate growth medium in duplicate and shaken at 200 rpm  
176 and 37 °C. In parallel, optical density (OD) of the cultures was measured at 600 nm using a  
177 UV – 6300 PC double beam spectrophotometer (MRC, Israel). The CFU/mL was obtained  
178 from appropriate dilutions which were plated onto LA and TSA agar plates in triplicate. For  
179 the each time interval, the growth curve was constructed and calibration was performed for  
180 each isolate. The microorganisms were grown to the optical density that matched to the  $1 \times$   
181  $10^8$  CFU/mL concentration of cells.

### 182 2.7. Diffusion assay

183 The initial screening of antimicrobial activity of all Turkish propolis samples was determined  
184 by well diffusion method as previously reported (Dimkić et al., 2016). Sterile molds for the  
185 wells were placed on the solid appropriate medium (LA and TSA) and 6 mL of LA/TSA soft  
186 agar inoculated with 60  $\mu$ L ( $1 \times 10^8$  CFU/mL) of the appropriate strain added. Each of  
187 propolis samples was tested in three different concentrations (1, 0.5 and 0.25 mg/well) in two  
188 repetitions. The Petri dishes were incubated overnight at 37 °C. Antibiotic discs of  
189 cefpodoxime, amphotericin B, pristinamycin, clotrimazole, mezlocillin and rifampicin as well  
190 as ampicillin and streptomycin (0.2 and 0.4 mg/well) as an aqueous solution were used as a  
191 positive control for bacterial isolates and nystatin (0.1 and 0.15 mg/well) for *C. albicans*. As  
192 a negative control, 20  $\mu$ L of methanol was used. The inhibition zone diameters were  
193 expressed in mm and graphically presented.

194

### 195 2.8. MIC assay

196 A broth microdilution method previously published (Ristivojević et al., 2016) was used to  
197 determine the minimum inhibitory (MIC), minimum bactericidal (MBC), and minimum

198 fungicidal concentration (MFC) for 39 selected propolis samples. Final concentration of each  
199 tested propolis sample in the first well was 1 mg/mL, while the concentration of methanol as  
200 a solvent was 10%. Two-fold serial dilutions of the propolis samples were made with LB and  
201 TSB media in 96-well microtiter plates. Besides a negative control (bacterial and fungal  
202 growth control), and a sterility control, the antibiotics streptomycin, rifampicin, ampicillin  
203 and nystatin were used as positive controls. The final concentration of antibiotics in the first  
204 well was 0.4 mg/mL. Each well, except for the sterility control, was inoculated with 20  $\mu$ L of  
205 bacterial and fungal culture ( $1 \times 10^8$  CFU/mL), reaching a final volume of 200  $\mu$ L. At the  
206 end, 22  $\mu$ L of resazurin (oxidation-reduction indicator) was added to each well. The plates  
207 were incubated for 24 h at 37 °C. After incubation, the resazurin colour change reaction was  
208 observed. The MIC values were determined as no change in colour, while MBC and MFC  
209 were obtained by sub-culturing the test dilutions from each well without colour change on  
210 agar plates and incubating for 24 h. The lowest concentration that shows no bacterial growth  
211 was defined as the MBC value. The results were expressed in mg/mL.

### 212 2.9. Statistical analysis

213 The analysis of variance was supported by the Kolmogorov–Smirnov test for the normality of  
214 residuals and Levene’s test for homogeneity of variance. The data obtained were subjected to  
215 analysis of variance (ANOVA) and means separation of MIC, MBC and MFC values, were  
216 accomplished by Tukey’s HSD (honest significant difference) test. Significance was  
217 evaluated at  $P < 0.05$ . All dilutions were tested in duplicate with two repetitions.

218 Statistical analyses were conducted by the general procedures of STATISTICA v.7 (StatSoft,  
219 Inc.) and IBM SPSS Statistics v.20 (SPSS, Inc.).

220

## 221 3. Results and discussion

### 222 3.1. Chemical profiling of propolis samples

#### 223 3.1.1. UV/Vis spectroscopy

224 The UV/Vis spectroscopy was applied to reveal the botanical origin of Turkish propolis, *i.e.*  
225 to verify the presence of three botanically different subtypes. On the Fig. 1 differences in  
226 UV/Vis patterns of O- and B-subtype propolis and specific profile of the third subtype are  
227 indicated. The spectra of analysed samples showed characteristic UV/Vis pattern in the  
228 regions between 200 to 400 nm with peaks attributable to the main classes of phenolics. O-  
229 subtype propolis samples showed two absorption maximums at  $\lambda = 290$  and 325 nm, B-  
230 subtype at  $\lambda = 295$  and 320 nm, while absorption maximum of the third subtype had low  
231 intensity maximum at  $\lambda = 290$  nm (Fig. 1). On the other hand, the UV/Vis absorption spectra  
232 of Serbian O- subtype propolis were characterized with maximums at near  $\lambda = 270$ , 290 and  
233 320 nm, while samples classified as B- subtype have two characteristic absorption maximums  
234 at  $\lambda = 290$  and 316 nm. Ristivojević et al. (2017) and Andjelković et al. (2017) also reported  
235 UV/Vis spectra of two Serbian propolis subtypes and identified two main characteristic  
236 absorption maximums at 291 nm and 314 nm. Same authors compared the UV/Vis spectra of  
237 *Populus tremula*, and *P. x euramericana* with both Serbian propolis subtypes and identified  
238 their botanical origins. UV/Vis spectra of Turkish propolis samples also showed  
239 characteristic absorption bands similar to Serbian, Romanian, and Italian propolis samples  
240 (Fabris, et al., 2013; Isla, Paredes-Guzman, Nieva-Moreno, Koo, & Park, 2005).

241 The three commonly applied assays of routine analysis of propolis are TFC, TPC and RSA. .  
242 Orange subtype of propolis samples were characterized with higher mean value of TPC  
243 ( $486.9 \pm 184.2$  mg/g) comparing to the B- subtype ( $310.6 \pm 201.2$  mg/g), while the lowest  
244 TPC value was measured for the third subtype of propolis samples ( $115.7 \pm 70.5$  mg/g). Large  
245 variations among data are not related only to the plant origin but also to the degree of

246 digestion by  $\beta$ -glycosidase from bees' saliva, and the percent of beeswax mixed with  
247 propolis. It is not unusual to get high variability among the data obtained from naturally  
248 occurring objects, i.e. samples. Turkish propolis showed much higher TPC values in  
249 comparison with the poplar subtype propolis of different geographic origins, *i.e.*, Chinese  
250 (Ahn et al., 2007), Japanese (Hamasaka, Kumazawa, Fujimoto, & Nakayama, 2007), and two  
251 times higher than Portugal (Moreira, Dias, Pereira, & Estevinho, 2008) samples. Above  
252 mentioned authors used maceration process of extraction with methanol and ethanol, while  
253 we in this study used ultrasonic extraction as a more efficient technique which could  
254 significantly influence on TPC and TFC values. Similar to TPC values, the O- subtype ( $265.7$   
255  $\pm 140.4$  mg/g) samples have higher average TFC value in relation to B- subtype samples  
256 ( $185.5 \pm 131.4$  mg/g), and that of the third subtype of propolis ( $109.53 \pm 54.42$  mg/g). The  
257 flavonoids content was much higher comparing to Japanese (Hamasaka et al., 2007), Chinese  
258 (Ahn et al., 2007) and Serbian propolis (Ristivojević et al., 2017).  
259 From the viewpoint of determined specifications with regard to phenolic compounds and  
260 flavonoids, Turkish poplar propolis may be considered as high quality propolis.

261

### 262 3.1.2. UHPLC–LTQ/Orbitrap/MS/MS

263 The qualitative and quantitative profile of phenolics was determined using the UHPLC  
264 system coupled to a LTQ OrbiTrap mass analyzer. UHPLC chromatograms of three subtypes  
265 of Turkish propolis were presented in Fig. 2. Fifteen phenolic compounds were quantified  
266 (Table 1). In all samples of Turkish propolis two benzoic acids derivatives (compounds **1** and  
267 **2**), five phenolic acids (compounds **3-7**) and several flavanols (compounds **10**, **12** and **15**),  
268 flavones (compounds **9**, **11** and **13**), flavanones (compound **14**) and glycosides (compound **8**)  
269 were determined (Table 1). The concentration of almost all above mentioned compounds  
270 were higher in O-subtype of propolis comparing to other two subtypes (Table 1).

271 Compounds **1** and **2** as benzoic acids derivatives yielded two characteristic fragments at  $m/z$   
272 93 and  $m/z$  109 by elimination of  $\text{CO}_2$  and  $\text{CH}_3$  groups from the molecule. The phenolic acids  
273 and their derivatives (compounds **3–16**) share a common fragmentation pathway based on  
274 loss of the  $\text{CO}_2$  group resulting in  $[\text{M}-\text{H}-\text{CO}_2]^-$ ,  $-44\text{Da}$  (Ristivojević et al., 2014).  
275 Compounds **7** and **8** were tentatively identified with specific fragmentation loss of  $\text{CO}_2$  and  
276  $\text{CH}_3$ , respectively. Caffeic acid and its derivatives (compounds **9, 11-13, 15, 16**) showed  
277 characteristic fragments at  $m/z$  179, 161, and 135 (Table 2). Furthermore, *p*-coumaric acid  
278 derivatives (compounds **10** and **14**) produce ions at  $m/z$  163 and 119, corresponding to *p*-  
279 coumaric acid and the fragment obtained after loss of  $\text{CO}_2$ . Compound **10** showed several  
280 more characteristic fragments at  $m/z$  295, 277, 191, 179, 163, 135, 119; it was identified in  
281 both Turkish propolis subtypes (Kečkeš et al., 2013). Compounds **5** and **7** were identified as  
282 main phenolic components in orange and blue subtypes of Turkish propolis.

283 Using LTQ-Orbitrap- $\text{MS}^2$  analysis, the comprehensive fragmentation pathways of flavonoids  
284 were identified, while ten compounds were additionally quantified (Table 2). Nine flavonols  
285 identified in Turkish propolis shared common fragmentation pathway of flavonols that  
286 correspond to retro-Diels–Alder (RDA) reaction (Kečkeš et al., 2013). Compounds **22** and **23**  
287 produce two common ions at  $m/z$  315 and 299. Additionally, in case of compound **18** and  
288 compound **20** ion at  $m/z$  300 was attributed to  $[\text{M}-\text{H}-\text{CH}_3]^-$  (Ristivojević et al., 2014).  
289 Flavonols such as compounds **17, 19** and **24** were recognized by several authors as markers  
290 of O-subtype of propolis from France, Germany, Serbia, and Turkey (Ristivojević et al.,  
291 2014). Based on the HPTLC fingerprinting of Turkish propolis samples analysed in our  
292 previous study (Guzelmeric et al., 2018), these phenols showed orange bands characteristic  
293 for O-subtype propolis. Compounds **17** and **24** were found in O-subtype propolis in higher  
294 amount (Table 1). Compound **25** produced several fragments at  $m/z$  257, 242, 199, and 125,  
295 confirmed by literature data (Leveques et al., 2012; Mišić et al., 2015).

296 Mass spectra of Turkish propolis samples indicated seven flavanonols and their esters and  
297 ethers (Table 2). Compound **26** and its derivatives (**26-32**) were characterised by the same  
298 fragments obtained by loss of the acyl group, yielding ions at  $m/z$  271 and 253, which  
299 correspond to  $[M-\text{acyl}]^-$  and  $[M-\text{acyl}-\text{H}_2\text{O}]^-$ , respectively (Kečkeš et al., 2013).

300 Five flavones (compounds **33-37**) were identified with two commonly ions such as  $m/z$  117  
301 and 151, which corresponded to the RDA fragmentation pathway. Compound **36** showed ions  
302 at  $m/z$  209, 181, and 143 which correspond to  $[M-\text{H}-\text{CO}_2]^-$ ,  $[M-\text{H}-\text{CO}_2-\text{CO}]^-$ ,  $[M-\text{H}-\text{C}_3\text{O}_2-$   
303  $\text{C}_2\text{H}_2\text{O}]^-$ . Compounds **36**, together with **24** and **42** were found in O- subtype in higher amount  
304 than in blue and the third subtypes (Table 1). Compounds **36** was also identified as a  
305 characteristic component of O- subtype propolis from Turkey with a green band on the  
306 HPTLC chromatogram (Guzelmeric et al., 2018) in higher concentration comparing to other  
307 two subtypes (Table 1). Fragment ions,  $[^{1,3}\text{A}]^-$ ,  $[^{1,3}\text{A}-\text{CO}_2]^-$  and  $[^{1,3}\text{B}]^-$  were identified for  
308 compound **34** (Kečkeš et al., 2013; Ristivojević et al., 2014). The molecular ion of **37**  
309 produced fragment ion at  $m/z$  117, possibly originated from  $[^{1,3}\text{B}]^-$ . Compounds **33** and **35**  
310 showed a fragment at  $m/z$  151; these flavonoids were also identified in Serbian and German  
311 propolis samples (Kečkeš et al., 2013; Morlock et al., 2014).

312 Examination of mass spectra of propolis samples revealed that there are six flavanone  
313 derivatives in the Turkish propolis samples (compounds **38-42**) based on the peaks of  
314 fragmentation ions  $[^{1,3}\text{A}]^-$  and  $[^{1,3}\text{B}]^-$ . Pinocembrin and pinobanksin were reported to be the  
315 main components for poplar type propolis (Ristivojević et al., 2014). Compounds **41** and **42**  
316 produced characteristic fragments at  $m/z$  254 and 213 originated by loss of  $\text{CH}_3$  and  $\text{C}_2\text{H}_2\text{O}$   
317 groups, respectively, as previously described in the literature (Kečkeš et al., 2013).

318 Compounds **38**, **39**, and **40** yielded characteristic fragments at  $m/z$  119, which were found in  
319 both orange and blue subtypes of Turkish propolis (Table 2) (Fabre, Rustan, de Hoffmann, &  
320 Quetin-Leclercq, 2001; Ristivojević et al., 2014). As we mentioned in our previous reports,

321 galangin, pinocembrin, chrysin, kaempferol, quercetin, caffeic acid, caffeic acid phenethyl  
322 ester (CAPE), luteolin and apigenin were the main components of O- subtype of Serbian and  
323 Turkish propolis samples (Table 1) (Guzelmeric et al., 2018; Ristivojević et al., 2014).  
324 Recently, the presence of flavonoid glycosides in Portuguese and Serbian propolis samples,  
325 although the number of such reports were quite few (Falcão et al., 2001; Ristivojević et al.,  
326 2014). In the present paper, presence of three glycosides such as compounds **43**, **44**, **45** were  
327 identified in Turkish propolis. Rutin was quantified in B- subtype propolis in higher amount  
328 compared to O- subtype; two ions at  $m/z$  315 and  $m/z$  300 were formed as a result of  
329 elimination of rutinoside and rutinoside-CH<sub>3</sub> units, respectively (Falcão et al., 2013;  
330 Ristivojević et al., 2014). Same fragments were also identified in compound **45** with a  
331 molecular ion peak at  $m/z$  463.0848. Compound **44** was quantified in higher amount in O-  
332 subtype propolis and characterized by a typical fragmentation pattern with three ions at  $m/z$   
333 269, 268, and 151.

334 Phenolic glycerides were found in North Russian, Bulgarian, Swiss, German, Russian, Polish,  
335 Belarusian, Croatian, Serbian as well as Turkish propolis samples and they probably  
336 originated from various *Populus* hybrids (Bankova, Popova, Bogdanov, & Sabatini, 2002;  
337 Bertrams et al., 2013; Falcão et al., 2013; Isidorov, Szczepaniak, & Bakier., 2014). On the  
338 other hand, seven phenolic glycerides were identified in Turkish propolis samples.  
339 Compound **46** and **47** formed a fragment ion at  $m/z$  179 originating from caffeic acid, which  
340 is in accordance with literature data (Svensson et al., 2010). Furthermore, compounds **48-51**  
341 had fragments at  $m/z$  193, 179, 163, and 161 (Table 2), which could be inferred as *p*-coumaric  
342 acid, caffeic acid and ferulic acid esterified to glycerol (Ristivojević et al., 2014).

### 343 3.2. Biological profile of Turkish propolis samples

#### 344 3.2.1. Antioxidative activity

345 Antioxidant capacity of propolis samples was determined by radical scavenging activity. The  
346 average RSA value of Turkish propolis samples was  $55.01 \pm 27.23\%$ . Samples of O- subtype  
347 exerted higher RSA value ( $65.64 \pm 25.88\%$ ) in comparison with the B-subtype ( $42.22 \pm$   
348  $24.42\%$ ) as well as the third subtype of propolis ( $26.49 \pm 6.72\%$ ) (Fig. S2). Higher RSA  
349 value of O- subtype propolis might possibly correlate with higher TPC and TFC values.  
350 These results are in accordance with our previous findings evaluated by HPTLC-DPPH-  
351 assay (Guzelmeric et al., 2018). The RSA values of Chinese (Ahn et al., 2007) and Serbian  
352 types (Ristivojević et al. 2017) were almost identical, while that of Japanese type was  
353 significantly lower (Hamasaka et al., 2004). In our previous study, we identified potential  
354 antioxidative components such as caffeic acid, CAPE, pinobanksin and galangin in both  
355 propolis subtypes (Guzelmeric et al., 2018).

### 356 3.2.2. Antimicrobial assays

357 Before assaying antimicrobial activity, the growth conditions of each strain were determined.  
358 The growth curves were constructed (Fig. S3), based on obtained data from repeated  
359 experiments (Table S1). According to the calibration curves, optical densities which  
360 corresponded to the  $1 \times 10^8$  CFU/mL were determined: 0.30, 0.12, 0.15 and 1.52 for strains *S.*  
361 *mutans*, *S. pyogenes*, *S. sanguinis* and *C. albicans*, respectively.

#### 362 3.2.2.1. Diffusion assay

363 According to the obtained results, *S. sanguinis* was the most resistant strain against all tested  
364 propolis samples. The O- subtype propolis samples showed moderate activity exclusively at  
365 highest concentration against this strain, while B- and the third subtypes of propolis samples  
366 mostly exerted no antibacterial activity against this strain (Fig. 3 and 4). The reference  
367 antibiotic mezlocillin demonstrated a potent antimicrobial activity against *S. sanguinis*, with  
368 31 mm of inhibition zone, while streptomycin and rifampicin showed moderate activity



369 against this pathogen (16 and 13 mm). Other tested antibiotics had no effect against *S.*  
370 *sanguinis*.

371 Turkish propolis samples showed moderate antibacterial activities against *S. mutans* and *C.*  
372 *albicans* strains, while eleven and fifteen propolis samples had no activity against these  
373 strains, respectively. Some O- and B- subtypes of propolis produced inhibition zones larger  
374 than 12 mm, at 0.5 mg/well concentration. In general, *S. mutans* and *C. albicans* were more  
375 sensitive to the O- subtype. These samples also had the highest values for TPC. Among the  
376 reference antibiotics streptomycin and mezlocillin showed the strongest activity against *S.*  
377 *mutans* (25 mm), while rifampicin produced smaller inhibition zone (17 mm). Other  
378 antibiotics, except pristinamycin with the smallest inhibition zone diameter, showed no  
379 antibacterial effect against this strain. Nystatin showed weaker antifungal activity against *C.*  
380 *albicans*, comparing to the many of the tested propolis samples.

381 Among the tested microorganisms, *S. pyogenes* was the most sensitive strain. Samples of the  
382 third propolis subtype had antibacterial effect only against this strain (Fig. 4). Almost all  
383 tested propolis samples produced inhibition zones at 1 mg/well concentration. In general,  
384 samples of O- subtype propolis exerted a higher antimicrobial activity. Rifampicin  
385 demonstrated the highest antibacterial effect against *S. pyogenes*, with 27 mm of inhibition  
386 zone diameter. Amphotericin B and ampicillin had no effect against this strain, while all other  
387 antibiotics showed moderate activity (10-17 mm). Out of all tested samples, the sample 8 had  
388 the strongest activity against *S. pyogenes* and *S. mutans*. Sample 40 had the strongest activity  
389 against *S. sanguinis*, and samples 24 and 25 against *C. albicans*. Samples 40, which possess a  
390 lower TPC value, had the best activity against resistant *S. sanguinis* strain. Higher flavonoid  
391 content might be responsible for the potential bacterial activity.

392 3.2.2.2. MIC assay

393 MIC, MBC and MFC values were determined for the 39 propolis samples (24 samples O-, 14  
394 samples B- and one of the third subtypes) based on well diffusion assay results. MIC values  
395 for the most samples were found in the concentration range from 0.01 to 1 mg/mL (Table 3).  
396 Sample 18 was the only one showing the strongest activity against all strains, with MIC  
397 values lower than 0.10 mg/mL. The majority of O- subtype of propolis samples exerted a  
398 strong antimicrobial activity against various strains, often with MIC values lower than 0.10  
399 mg/mL. The third subtype propolis sample (30) exerted a higher antimicrobial effect against  
400 *S. pyogenes* (0.14 mg/mL), while a weak activity against *C. albicans* (1 mg/mL). Similar  
401 results were also observed in diffusion test. Also TPC, TFC and RSA values were low for this  
402 sample, while cinnamic acid was the main component. MIC values against *S. sanguinis* were  
403 ranging from 0.06 mg/mL (sample 18) to over 1 mg/mL for the sample 45 which had also  
404 low TPC and TFC values. Like in diffusion assay, *S. sanguinis* was the most resistant strain  
405 in this assay. Higher MIC values (0.50 - 1 mg/mL) were recorded for several O- and B-  
406 subtypes of propolis (2, 28, 31, 32, 33, 34, 35, 37, 38, 39, 40, 41, 43 and 45). Among these  
407 samples 37, 38 and 43 were found to contain high concentration of cinnamic acid, in addition  
408 to ferulic and caffeic acids as the main components, while sample 2 was found to be rich in  
409 chlorogenic acid (around 50 times higher than in the others). Other propolis samples had MIC  
410 values lower than 0.50 mg/mL. MIC values were ranged for B- subtypes of propolis samples  
411 against *S. mutans* from 0.03 (sample 3) to 0.75 mg/mL (sample 45). On the other hand, the  
412 lowest MIC values (less than 0.1 mg/mL) were recorded for O- subtype samples (8, 18, 22,  
413 28, 29, 33 and 35). Samples 8, 18, 28 and 29 showed to possess strong activity against this  
414 strain in diffusion assay. Sample 3 had extremely high TPC, TFC and RSA values. Except  
415 caffeic and ferulic acids, *p*-coumaric acid was also presented in a higher concentration in  
416 samples 18 and 28. In general, all tested samples, except samples 15, 40 and 45, had MIC  
417 values lower than 0.50 mg/mL. MIC values against *S. pyogenes* were ranging from 0.01

418 (sample 18) to 1 mg/mL (sample 40). *Streptococcus pyogenes* was the most sensitive strain,  
419 with the lowest MIC values ranging from 0.01 to 0.09 mg/mL, against the most of O- subtype  
420 samples. Sample 30 (the third subtype) also had low MIC value against this strain which was  
421 in accordance with the diffusion assay results. MIC values against *C. albicans* ranged from  
422 0.06 to over 1 mg/mL. The O- subtype sample 2, and B- subtype samples 40 and 45, had the  
423 highest MIC values and absence of antifungal activity in diffusion assay. A few O- subtype  
424 samples (11, 18, 22 and 25) had the lowest MIC values ranging between 0.06 - 0.09 mg/mL,  
425 while some others (2, 4, 7, 17, 5, 20, 30, 37, 40, 43 and 45) had the highest MIC values. The  
426 rest of the samples had shown medium MIC values, less than 0.5 mg/mL. For samples 2, 30,  
427 40, 41, 43, and 45 MBC/MFC were not determined (MBC/MFC > 1 mg/mL) against  
428 particular strains. In general, MBC values were twice and even three times higher than the  
429 MIC values (Table 4) for the most of the samples. The majority of samples had two times  
430 higher MFC than MIC values against *C. albicans*. MFC values for samples 2, 30, 40 and 45  
431 were not found at all, while MBC values for samples 40, 43, and 45 were at 1 mg/mL or  
432 higher. Methanol as solvent did not show any antimicrobial activity. All three tested bacterial  
433 strains exerted resistance against ampicillin, and also *S. pyogenes* against streptomycin.  
434 Rifampicin had a lowest MIC value against *S. pyogenes* (0.006 mg/mL), while higher values  
435 were recorded against *S. mutans* (0.1 mg/mL) and *S. sanguinis* (0.2 mg/mL). Streptomycin  
436 showed highest inhibitory rates against *S. sanguinis* and *S. mutans* (0.025 mg/mL). On the  
437 other hand, MIC value of nystatin against *C. albicans* was 0.4 mg/mL, which was  
438 significantly higher than for all propolis samples.

### 439 3.2.2.3. General observations

440 Only a few studies have investigated the antimicrobial potential of Turkish propolis. Oral use  
441 of propolis as the most common form of application, or in the form of vaginal tablets,  
442 provides an incentive in finding adequate propolis samples as an alternative for the control of

443 selected opportunistic and pathogenic microorganisms tested in this study. *Candida albicans*  
444 is an opportunistic pathogen, which exists in several morphological forms. In case of  
445 immunity collapse, this type of over expression occurs, causing a candidiasis disease that  
446 may be oropharyngeal, vulvovaginal or invasive (Sudbery, Gow, & Berman, 2004). The  
447 presence of *Streptococcus mutans* in the oral cavity is associated with the formation of caries,  
448 gingivitis and chronic periodontitis (Contardo, Díaz, Lobos, Padilla, & Giacaman, 2007).  
449 *Streptococcus sanguinis* is the most common bacterial causative agent of the dental plaque,  
450 and its presence in combination with *S. mutans* is also associated with the formation of caries  
451 and other diseases of the tooth (Borges, Ferreira, Saavedra, & Simões, 2013). *Streptococcus*  
452 *pyogenes* is a trigger of pharyngitis, which most commonly occurs in inflammatory mucous  
453 membranes of the nasal and sinus, oral cavity and tonsils (Lyon, & Caparon, 2003). The  
454 results of antimicrobial activity of Turkish propolis against particular oral microorganisms,  
455 used in this study, are scarce. In one of these studies, a good antimicrobial activity of propolis  
456 samples from Central Anatolia was obtained with an average concentration of 0.1 mg/mL  
457 against *S. mutans* (Arslan, Silici, Percin, Koç, & Er, 2012). Similarly antimicrobial activity of  
458 propolis samples from two different areas in Marmara region of Turkey have been reported  
459 against the beta-hemolytic streptococci by Keskin et al. (2001).

460 Otherwise, antimicrobial effects of various propolis types from other parts of the world have  
461 been investigated by several research groups. Australian propolis showed very strong  
462 antibacterial activity against *Streptococcus* isolates (Nam et al., 2016), while Nigerian  
463 propolis demonstrated potent activity against *S. mutans* (Ophori et al., 2010). The average  
464 inhibition zone of Nigerian propolis was high (24 mm), which is considerably higher than  
465 that of the Turkish propolis (9.3 mm). In another study Iraqi propolis showed activity against  
466 *S. pyogenes* (Hendi, Naher, & Al-Charrakh, 2010) with a similar inhibition zone as it was  
467 observed in the present study.

468 On the other hand, *C. albicans* was found to be resistant to the Iraqi and Serbian propolis  
469 samples (Hendi et al., 2010; Stepanović et al., 2003), while a moderate activity was  
470 determined by the Lebanese propolis samples (Chamandi et al., 2015). Hegazi et al. (2000)  
471 also reported that *C. albicans* isolates were found to be quite resistant to propolis, with MIC  
472 values higher than 1 mg/mL, while propolis samples from the Mediterranean part of Turkey  
473 showed a moderate activity against *C. albicans* (Velikova et al., 2000). A similar antifungal  
474 activity profile has been reported for propolis samples from the other parts of Turkey  
475 (Katircioglu, & Mercan, 2006).

476 In the present study, many of the samples originating from Eastern Anatolia (18 samples)  
477 showed strong or moderate antimicrobial activity against different isolates. More samples that  
478 had similar antimicrobial potential were provided from other regions of Turkey: Marmara (8  
479 samples), Mediterranean (4 samples), Aegean (3 samples), Black Sea (4 samples) and South  
480 eastern Anatolia (1 sample). However, the sample 18 showed the strongest activity against all  
481 tested strains which was comparable with the activity of streptomycin. This sample also had  
482 an extremely high TPC and TFC values, while caffeic and ferulic acids were determined as  
483 the main constituents. We cannot mark more propolis samples which exhibited equally strong  
484 antimicrobial activity against all isolates. The cinnamic acid concentration was the highest  
485 among all tested samples. Ferulic and caffeic acid were also present in almost all samples  
486 with strong antimicrobial activity; these compounds might possibly contribute to the  
487 antimicrobial activity of propolis samples. As a matter of fact, cinnamic, chlorogenic and *p*-  
488 coumaric acids were also quantified in higher concentrations in several samples with strong  
489 antimicrobial activity. According to the previous reports, ferulic (Borges, Ferreira, Saavedra,  
490 & Simões, 2013) and caffeic acids (Mirzoeva, Grishanin, & Calder, 1997) exerted their  
491 antimicrobial effects on the cell membrane, inducing irreversible changes and damage.

492 Accordingly, it is evident that phenolic acids exert higher contribution to the antimicrobial  
493 activity of Turkish propolis samples than flavonoids.

#### 494 **4. Conclusions**

495 Recently, demand for propolis on the market has steadily increasing due to its evidenced  
496 health benefits. However, some propolis products are marketed without examining their  
497 chemical compositions, without identifying the plant sources or determining the type of  
498 propolis. On the other hand, in case when honeybees cannot find possible plant sources  
499 around, they may collect materials such as paint, asphalt and/or mineral oils which would  
500 raise the risk for the human health when consumed due to such toxic contamination and also  
501 reduced the pharmacological effects. For this reason, it is extremely important to analyse the  
502 quality, to determine the chemical composition and the botanical origin of propolis, which  
503 would have direct impact on its health benefits or risks.

504 In this study, the phenolic profiles of Turkish propolis samples from different botanical  
505 origins were evaluated in detail. Moreover, TPC, TFC, antioxidant and antimicrobial  
506 potentials were determined of O-, and B- as well as the third subtypes of Turkish propolis.  
507 Experimental results have shown that particularly O-subtype of propolis originated mainly  
508 from *Populus nigra* could be used as a raw material in pharmaceutical and/or food industry  
509 due to its rich phytochemical composition and a wide range of health benefits.

#### 510 **Acknowledgments**

511 This work was supported by the Ministry of Education, Science and Technological  
512 Development of Serbia, Grant Nos. 173026 and 172017. The funders had no role in study  
513 design, data collection and analysis, decision to publish, or preparation of the manuscript. We  
514 are grateful to Dr Marina Soković for providing the oral cavity isolates from the genus  
515 *Streptococcus*.

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### Figure Captions

**Fig. 1.** UV/Vis spectra of three subtypes of Turkish propolis (A- Orange type, B- Blue type, C- Third type).

**Fig. 2.** Total ion chromatograms (TICs) of three subtypes of Turkish propolis samples, obtained with the LTQ-Orbitrap XL instrument in negative ion mode (A, B- Orange type, C-Third type, D-Blue type).

**Fig. 3.** Antimicrobial potential of the orange subtype samples of Turkish propolis tested by diffusion method at concentrations of 1 (A), 0.5 (B) and 0.25 mg/well (C).

Amp - Ampicillin, Stp - Streptomycin, Rif - Rifampicin, Mez - Mezlocillin, Klo - Clotrimazole, Pri - Pristinamycin, Cef - Cefpodoxime, Amf B - Amphotericin B, and Nys – Nystatin.

**Fig. 4.** Antimicrobial potential of the blue and third (in rectangles) subtypes samples of Turkish propolis tested by diffusion method at concentrations of 1 (A), 0.5 (B) and 0.25 mg/well (C).

Amp - Ampicillin, Stp - Streptomycin, Rif - Rifampicin, Mez - Mezlocillin, Klo - Clotrimazole, Pri - Pristinamycin, Cef - Cefpodoxime, Amf B - Amphotericin B, and Nys – Nystatin.

1 **Table Captions**

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3 **Table 1.** The content of phenolic compounds (expressed in mg/mL as mean  $\pm$  SD) in three  
4 subtypes of Turkish propolis.

5 **Table 2.** Phenolic compounds tentatively identified in Turkish propolis.

6 **Table 3.** The minimum inhibitory concentration (MIC) of Turkish propolis samples  
7 (mg/mL). The mean values and standard error are shown.

8 **Table 4.** The minimum bactericidal (MBC) and fungicidal concentrations (MFC) of Turkish  
9 propolis samples (mg/mL). The mean values and standard error are shown.

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27 **Table 1.** The content of phenolic compounds (expressed in mg/mL as mean  $\pm$  SD) in three  
 28 subtypes of Turkish propolis

No.	Phenolic compounds	Orange type	Blue type	Third type
1	<i>p</i> -Hydroxybenzoic acid	2.24 $\pm$ 1.74	1.44 $\pm$ 1.17	0.46 $\pm$ 0.23
2	Vanillic acid	0.39 $\pm$ 0.26	0.30 $\pm$ 0.15	0.27 $\pm$ 0.11
3	Protocatechuic acid	1.69 $\pm$ 1.01	0.71 $\pm$ 0.24	0.45 $\pm$ 0.19
4	Caffeic acid	34.78 $\pm$ 16.77	24.82 $\pm$ 18.70	3.96 $\pm$ 1.93
5	<i>p</i> -Coumaric acid	4.91 $\pm$ 3.69	3.13 $\pm$ 2.25	0.19 $\pm$ 0.11
6	Cinnamic acid	5.19 $\pm$ 4.67	3.00 $\pm$ 2.24	5.28 $\pm$ 4.21
7	Ferulic acid	19.42 $\pm$ 18.38	9.63 $\pm$ 5.91	1.00 $\pm$ 0.63
8	Rutin	0.36 $\pm$ 0.17	0.47 $\pm$ 0.32	0.16 $\pm$ 0.09
9	Luteolin	1.57 $\pm$ 0.87	1.24 $\pm$ 0.74	0.31 $\pm$ 0.18
10	Quercetin	4.33 $\pm$ 1.56	2.85 $\pm$ 1.44	1.11 $\pm$ 0.75
11	Apigenin	1.56 $\pm$ 0.64	1.05 $\pm$ 0.43	0.54 $\pm$ 0.32
12	Kaempferol	1.76 $\pm$ 0.72	0.92 $\pm$ 0.45	0.44 $\pm$ 0.29
13	Chrysin	2.22 $\pm$ 0.89	1.85 $\pm$ 0.56	1.54 $\pm$ 0.86
14	Pinocembrin	2.81 $\pm$ 1.00	2.16 $\pm$ 0.84	0.94 $\pm$ 0.37
15	Galangin	2.70 $\pm$ 1.39	1.67 $\pm$ 0.40	0.96 $\pm$ 0.51

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**Table 2.** Phenolic compounds tentatively identified in Turkish propolis

No.	Identified compounds	t <sub>R</sub> (min)	Calculated mass [M-H] <sup>-</sup>	Accurate mass [M-H] <sup>-</sup>	Error (ppm)	Fragmentation	Reference
<b>Benzoic acid and its derivatives</b>							
1	<i>p</i> -Hydroxybenzoic acid	5.19	137.02442	137.02230	2.12	109, 93	Natić et al., 2015
2	Vanillin	6.55	151.04007	151.03960	0.47	136	
<b>Phenolic acids and their derivatives</b>							
3	Protocatechuic acid	4.07	153.01970	153.01800	1.7	136 [M-H-H <sub>2</sub> O], 109, [M-H-CO <sub>2</sub> ] <sup>-</sup> , 107	Kečkeš et al., 2013
4	Protocatechuic acid or isomer	5.02	153.0197	153.0183	1.4	136 [M-H-H <sub>2</sub> O], 109 [M-H-CO <sub>2</sub> ] <sup>-</sup>	Kečkeš et al., 2014
5	Caffeic acid	5.18	179.035	179.0336	1.4	161[M-H-H <sub>2</sub> O] <sup>-</sup> , 151, 135 [M-H-CO <sub>2</sub> ] <sup>-</sup>	Kečkeš et al., 2013, Pellati et al., 2011
6	<i>p</i> -Coumaric acid	6.49	163.0401	163.0387	1.4	119 [M-H-CO <sub>2</sub> ] <sup>-</sup>	Kečkeš et al., 2013, Pellati et al., 2011
7	Ferulic acid	6.73	193.0506	193.0495	1.1	179 [M-H-CH <sub>3</sub> ] <sup>-</sup> , 178, 149 [M-H-CH <sub>3</sub> -CO <sub>2</sub> ] <sup>-</sup> , 134	Kečkeš et al., 2013, Pellati et al., 2011
8	Cinnamic acid	8.55	147.0452	147.0449	0.3	103 [M-H-CO <sub>2</sub> ] <sup>-</sup>	Kečkeš et al., 2013
9	3,4-Dimethyl-caffeic acid (DMCA)	8.16	207.0663	207.0645	1.8	179 [M-H-2CH <sub>3</sub> ] <sup>-</sup> , 163 [M-H-CO <sub>2</sub> ] <sup>-</sup>	Pellati et al., 2011
10	<i>p</i> -Coumaroylquinic acid	9.07	337.0929	337.0912	1.7	295, 277, 179, 191 [C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> ] <sup>-</sup> , 161, 135, 119	Weisz et al., 2009
11	Prenyl caffeate	11.26	247.0976	247.0972	0.4	179 [C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> ] <sup>-</sup> , 135 [C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> -CO <sub>2</sub> ] <sup>-</sup>	Gardana et al., 2007, Medana et al., 2008
12	Caffeic acid phenethyl ester (CAPE)	11.60	283.0976	283.0948	2.8	179 [C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> ] <sup>-</sup> , 135 [C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> -CO <sub>2</sub> ] <sup>-</sup>	Kečkeš et al., 2013
13	Caffeic acid cinnamylester	12.19	295.0976	295.0956	2.0	179 [C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> ] <sup>-</sup> , 135 [C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> -CO <sub>2</sub> ] <sup>-</sup>	Pellati et al., 2011
14	<i>p</i> -Coumaric methyl butenyl ester	12.37	231.102	231.101	1.0	163 [C <sub>9</sub> H <sub>7</sub> O <sub>3</sub> ] <sup>-</sup> , 119 [M-H-CO <sub>2</sub> ] <sup>-</sup>	Gardana et al., 2007
15	Benzyl caffeate	12.72	269.0819	269.0811	0.8	179 [C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> ] <sup>-</sup> , 135 [C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> -CO <sub>2</sub> ] <sup>-</sup>	Gardana et al., 2007, Pellati et al., 2011
16	Methyl- <i>O</i> -caffeoylquinic acid	13.21	367.10346	367.10010	3.36	179, 161, 135	Natić et al., 2015
<b>Flavonols</b>							
17	Quercetin	8.54	301.0354	301.0331	2.3	271, 179 [ <sup>1,2</sup> A] <sup>-</sup> , 151 [ <sup>1,2</sup> A-CO] <sup>-</sup> , 121 [ <sup>1,2</sup> B] <sup>-</sup>	Kečkeš et al., 2013, Fabre et al., 2001
18	Rhamnetin	8.88	315.051	315.0486	2.4	300 [M-H-CH <sub>3</sub> ] <sup>-</sup>	Kečkeš et al., 2013, Fabre et al., 2001
19	Kaempferol	8.90	285.0405	285.0395	1.0	267 [M-H-H <sub>2</sub> O] <sup>-</sup> , 241 [M-H-CO <sub>2</sub> ] <sup>-</sup> , 199 [M-H-C <sub>2</sub> H <sub>2</sub> O-CO <sub>2</sub> ] <sup>-</sup> , 151 [ <sup>1,3</sup> A] <sup>-</sup>	Kečkeš et al., 2013
20	Isorhamnetin	8.96	315.051	315.0564	-5.4	300 [M-H-CH <sub>3</sub> ] <sup>-</sup> , 151 [ <sup>1,3</sup> A] <sup>-</sup>	Kečkeš et al., 2013, Fabre et al., 2001
21	Kaempferide	10.50	299.0561	299.054	2.1	284 [M-H-CH <sub>3</sub> ] <sup>-</sup> , 151 [ <sup>1,3</sup> A] <sup>-</sup>	Kečkeš et al., 2013
22	Bis-methylated quercetin	10.59	329.0642	329.0642	0.0	315 [M-H-CH <sub>3</sub> ] <sup>-</sup> , 299 [M-H-2CH <sub>3</sub> ] <sup>-</sup>	Kečkeš et al., 2013
23	Bis-methylated quercetin	10.91	329.0667	329.0654	1.3	315 [M-H-CH <sub>3</sub> ] <sup>-</sup> , 299 [M-H-2CH <sub>3</sub> ] <sup>-</sup>	Kečkeš et al., 2013
24	Galangin	11.30	269.0456	269.0455	0.1	213 [M-H-C <sub>2</sub> O <sub>2</sub> ] <sup>-</sup> , 183 [M-H-C <sub>2</sub> H <sub>2</sub> O-CO <sub>2</sub> ] <sup>-</sup> , 151 [ <sup>1,2</sup> A-CO] <sup>-</sup>	Kečkeš et al., 2013
25	Hesperetin	11.93	301.07176	301.06940		257, 242, 199, 125	Leveques et al., 2012
<b>Flavanonols</b>							
26	Pinobanksin	9.02	271.0612	271.0593	1.9	253 [M-H-H <sub>2</sub> O] <sup>-</sup> , 243 [M-H-CO] <sup>-</sup>	Kečkeš et al., 2013, Pellati et al., 2011
27	Pinobanksin-5-methyl-ether-3- <i>O</i> -acetate	9.17	327.087	327.0851	1.9	285 [M-acetate] <sup>-</sup> , 165 [M-H-acetate-H <sub>2</sub> O-2CO <sub>2</sub> ] <sup>-</sup>	Kečkeš et al., 2013, Pellati et al., 2011
28	Pinobanksin-3- <i>O</i> -acetate	11.67	313.0712	313.0686	2.6	271 [M-acetate] <sup>-</sup> , 253 [M-acetate-H <sub>2</sub> O] <sup>-</sup>	Kečkeš et al., 2013
29	Pinobanksin-5-methyl-ether	11.83	285.0767	285.0749	1.8	271 [M-CH <sub>3</sub> ] <sup>-</sup> , 253 [M-CH <sub>3</sub> -H <sub>2</sub> O] <sup>-</sup> , 239 [M-H-H <sub>2</sub> O-CO] <sup>-</sup>	Kečkeš et al., 2013, Pellati et al., 2011
30	Pinobanksin-3- <i>O</i> -propionate	12.17	327.0869	327.085	1.9	271 [M-propionate] <sup>-</sup> , 253 [M-propionate-H <sub>2</sub> O] <sup>-</sup>	Kečkeš et al., 2013
31	Pinobanksin-3- <i>O</i> -butyrate (or isomer)	13.43	341.1002	341.106	-5.8	253 [M-H-butyrate-H <sub>2</sub> O] <sup>-</sup>	Kečkeš et al., 2013
32	Pinobanksin-3- <i>O</i> -pentanoate (or isomer)	14.20	355.1183	355.1228	-4.5	271 [M-H-pentanoate] <sup>-</sup> , 253 [M-H-pentanoate-H <sub>2</sub> O] <sup>-</sup>	Kečkeš et al., 2013
<b>Flavones</b>							
33	Luteolin	4.14	285.0405	285.0385	2.0	213 [M-H-CO <sub>2</sub> -CO] <sup>-</sup> , 151 [ <sup>1,3</sup> A] <sup>-</sup>	Kečkeš et al., 2013
34	Apigenin	9.53	269.0456	269.0385	7.1	151 [ <sup>1,4</sup> B+2H] <sup>-</sup> , 149 [ <sup>1,4</sup> B] <sup>-</sup> , 117 [ <sup>1,3</sup> B] <sup>-</sup>	Kečkeš et al., 2013
35	Acacetin	11.40	283.0612	283.0593	1.9	151, 107	Kečkeš et al., 2013
36	Chrysin	12.05	253.0506	253.0486	2.0	209 [M-H-CO <sub>2</sub> ] <sup>-</sup> , 181 [M-H-CO <sub>2</sub> -CO] <sup>-</sup> , 143 [M-H-C <sub>3</sub> O <sub>2</sub> -C <sub>2</sub> H <sub>2</sub> O] <sup>-</sup>	Kečkeš et al., 2013
37	Dihydroxyflavone	12.40	253.0506	253.0486	2.0	117 [ <sup>1,3</sup> B] <sup>-</sup>	Kečkeš et al., 2013

<b>Flavanones</b>							
38	Sakuranetin	11.87	285.0769	285.0749	2.0	165 [C <sub>8</sub> H <sub>5</sub> O <sub>4</sub> ] <sup>-</sup> , 119	Kečkeš et al., 2013
39	Naringenin	11.96	271.0612	271.0601	1.1	151 [ <sup>1,3</sup> B] <sup>-</sup> , 119 [ <sup>1,3</sup> A] <sup>-</sup>	Fabre et al., 2001
40	Liquiritigenin	12.11	255.0663	255.0635	2.8	153 [ <sup>1,3</sup> A] <sup>-</sup> , 135 [ <sup>1,3</sup> A-H <sub>2</sub> O] <sup>-</sup> , 119 [ <sup>1,3</sup> A-OH-OH] <sup>-</sup>	Wang et al. 2008
41	Pinostrobin	12.18	269.0819	269.0797	2.2	254 [M-H-CH <sub>3</sub> ], 165 [ <sup>1,3</sup> A] <sup>-</sup>	Kečkeš et al., 2013
42	Pinocembrin	12.46	255.0663	255.0663		213 [M-H-C <sub>2</sub> H <sub>2</sub> O] <sup>-</sup> , 151 [ <sup>1,3</sup> A] <sup>-</sup>	Kečkeš et al., 2013
<b>Glycosides</b>							
43	Rutin	6.23	609.1461	609.1443	1.8	301 [M-H-glycoside] <sup>-</sup> , 300	Kečkeš et al., 2013
44	Apigetrin (Apigenin-7- <i>O</i> -glucoside)	6.69	431.0984	431.0959	2.5	269 [M-H-glycoside] <sup>-</sup> , 268, 151 [ <sup>1,4</sup> B-2H] <sup>-</sup>	Hossain et al., 2010
45	Quercetin 3- <i>O</i> -galactoside	6.88	463.08820	463.08480	3.4	301, 300	
<b>Phenolic glycerides</b>							
46	Caffeoylglycerol	5.5	253.071	253.0702	0.8	179 [C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> ] <sup>-</sup>	Svensson et al., 2010
47	Coumaroylferuoyl glycerol	6.04	413.1212	413.1217	-0.5	235, 193 [C <sub>10</sub> H <sub>9</sub> O <sub>4</sub> ] <sup>-</sup> , 163 [C <sub>10</sub> H <sub>9</sub> O <sub>4</sub> -2CH <sub>3</sub> ] <sup>-</sup>	Ma et al., 2007
48	Dicoumaroyl acetyl glycerol	6.48	425.1224	425.1221	0.3	365, 321, 163 [C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> ] <sup>-</sup>	
49	Dicaffeoyl acetyl glycerol	9.55	457.1122	457.11	2.2	397, 295, 235, 179, 161	
50	Acetyl-coumaroyl--feruloylglycerol	10.58	425.1236	425.1216	2.0	263, 179, 161	
51	Acetyl-diferuloylglycerol	11.46	485.144	485.1421	1.9	425, 381, 207, 193	Shi et al., 2012

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33 **Table 3.** The minimum inhibitory concentration (MIC) of Turkish propolis samples  
 34 (mg/mL). The mean values and standard error are shown.

Subtype of propolis	Sample	MIC			
		<i>S. sanguinis</i>	<i>S. mutans</i>	<i>S. pyogenes</i>	<i>C. albicans</i>
O	2	0.50 <sup>abcd</sup> ± 0.00	0.15 <sup>cde</sup> ± 0.05	0.02 <sup>cd</sup> ± 0.04	> 1.00 <sup>a</sup> ± 0.00
O	4	0.25 <sup>bcd</sup> ± 0.00	0.37 <sup>bcd</sup> ± 0.07	0.14 <sup>bcd</sup> ± 0.06	0.50 <sup>b</sup> ± 0.00
O	7	0.15 <sup>bcd</sup> ± 0.05	0.25 <sup>bcd</sup> ± 0.00	0.25 <sup>bcd</sup> ± 0.00	0.50 <sup>b</sup> ± 0.00
O	8	0.15 <sup>bcd</sup> ± 0.03	0.09 <sup>cde</sup> ± 0.01	0.13 <sup>bcd</sup> ± 0.06	0.25 <sup>cde</sup> ± 0.00
O	11	0.17 <sup>bcd</sup> ± 0.04	0.28 <sup>bcd</sup> ± 0.12	0.14 <sup>bcd</sup> ± 0.05	0.09 <sup>de</sup> ± 0.01
O	12	0.12 <sup>cd</sup> ± 0.00	0.18 <sup>bcd</sup> ± 0.03	0.07 <sup>cd</sup> ± 0.02	0.25 <sup>cde</sup> ± 0.00
O	16	0.25 <sup>bcd</sup> ± 0.00	0.37 <sup>bcd</sup> ± 0.07	0.28 <sup>bcd</sup> ± 0.12	0.31 <sup>bcd</sup> ± 0.10
O	17	0.12 <sup>cd</sup> ± 0.00	0.34 <sup>bcd</sup> ± 0.09	0.08 <sup>cd</sup> ± 0.02	0.50 <sup>b</sup> ± 0.00
O	18	0.06 <sup>d</sup> ± 0.00	0.09 <sup>cde</sup> ± 0.01	0.01 <sup>d</sup> ± 0.00	0.06 <sup>e</sup> ± 0.00
O	21	0.12 <sup>cd</sup> ± 0.00	0.12 <sup>cde</sup> ± 0.00	0.03 <sup>cd</sup> ± 0.01	0.25 <sup>cde</sup> ± 0.00
O	22	0.12 <sup>cd</sup> ± 0.00	0.08 <sup>cde</sup> ± 0.02	0.07 <sup>cd</sup> ± 0.03	0.09 <sup>de</sup> ± 0.01
O	24	0.12 <sup>cd</sup> ± 0.00	0.12 <sup>cde</sup> ± 0.00	0.03 <sup>cd</sup> ± 0.01	0.28 <sup>bcd</sup> ± 0.12
O	25	0.10 <sup>cd</sup> ± 0.01	0.12 <sup>cde</sup> ± 0.00	0.10 <sup>cd</sup> ± 0.05	0.09 <sup>de</sup> ± 0.01
O	26	0.21 <sup>bcd</sup> ± 0.03	0.18 <sup>bcd</sup> ± 0.03	0.15 <sup>bcd</sup> ± 0.05	0.18 <sup>cde</sup> ± 0.03
O	28	0.50 <sup>abcd</sup> ± 0.00	0.07 <sup>de</sup> ± 0.02	0.04 <sup>cd</sup> ± 0.009	0.25 <sup>cde</sup> ± 0.00
O	29	0.18 <sup>bcd</sup> ± 0.03	0.06 <sup>de</sup> ± 0.00	0.14 <sup>bcd</sup> ± 0.06	0.18 <sup>cde</sup> ± 0.03
O	31	0.62 <sup>abcd</sup> ± 0.21	0.13 <sup>cde</sup> ± 0.06	0.05 <sup>cd</sup> ± 0.007	0.12 <sup>de</sup> ± 0.00
O	32	0.75 <sup>ab</sup> ± 0.14	0.10 <sup>cde</sup> ± 0.05	0.13 <sup>bcd</sup> ± 0.06	0.12 <sup>de</sup> ± 0.00
O	33	0.50 <sup>abcd</sup> ± 0.00	0.09 <sup>cde</sup> ± 0.01	0.07 <sup>cd</sup> ± 0.03	0.10 <sup>de</sup> ± 0.01
O	34	0.75 <sup>ab</sup> ± 0.14	0.25 <sup>bcd</sup> ± 0.00	0.04 <sup>cd</sup> ± 0.01	0.12 <sup>de</sup> ± 0.00
O	35	0.53 <sup>abcd</sup> ± 0.27	0.04 <sup>e</sup> ± 0.09	0.03 <sup>cd</sup> ± 0.01	0.12 <sup>de</sup> ± 0.00
O	36	0.25 <sup>bcd</sup> ± 0.08	0.12 <sup>cde</sup> ± 0.00	0.08 <sup>cd</sup> ± 0.02	0.18 <sup>cde</sup> ± 0.03
O	41	0.75 <sup>ab</sup> ± 0.14	0.13 <sup>cde</sup> ± 0.06	0.09 <sup>cd</sup> ± 0.01	0.12 <sup>de</sup> ± 0.00
O	47	0.28 <sup>bcd</sup> ± 0.07	0.37 <sup>bcd</sup> ± 0.07	0.18 <sup>bcd</sup> ± 0.03	0.14 <sup>de</sup> ± 0.03
B	3	0.18 <sup>bcd</sup> ± 0.03	0.03 <sup>e</sup> ± 0.00	0.02 <sup>cd</sup> ± 0.004	0.18 <sup>cde</sup> ± 0.03
B	5	0.31 <sup>bcd</sup> ± 0.06	0.37 <sup>bcd</sup> ± 0.07	0.17 <sup>bcd</sup> ± 0.04	0.50 <sup>b</sup> ± 0.00
B	6	0.21 <sup>bcd</sup> ± 0.03	0.14 <sup>cde</sup> ± 0.06	0.03 <sup>cd</sup> ± 0.00	0.37 <sup>bc</sup> ± 0.07
B	13	0.31 <sup>bcd</sup> ± 0.06	0.18 <sup>bcd</sup> ± 0.03	0.28 <sup>bcd</sup> ± 0.12	0.18 <sup>cde</sup> ± 0.03
B	15	0.18 <sup>bcd</sup> ± 0.03	0.50 <sup>ab</sup> ± 0.00	0.13 <sup>bcd</sup> ± 0.06	0.12 <sup>de</sup> ± 0.00
B	20	0.12 <sup>cd</sup> ± 0.00	0.15 <sup>cde</sup> ± 0.03	0.08 <sup>cd</sup> ± 0.02	0.50 <sup>b</sup> ± 0.00
B	23	0.21 <sup>bcd</sup> ± 0.03	0.37 <sup>bcd</sup> ± 0.07	0.10 <sup>bcd</sup> ± 0.05	0.18 <sup>cde</sup> ± 0.03
B	37	0.62 <sup>abcd</sup> ± 0.21	0.28 <sup>bcd</sup> ± 0.12	0.31 <sup>bc</sup> ± 0.10	0.50 <sup>b</sup> ± 0.00
B	38	0.62 <sup>abcd</sup> ± 0.21	0.15 <sup>cde</sup> ± 0.05	0.07 <sup>cd</sup> ± 0.02	0.25 <sup>cde</sup> ± 0.00
B	39	0.56 <sup>abcd</sup> ± 0.25	0.18 <sup>bcd</sup> ± 0.03	0.14 <sup>bcd</sup> ± 0.05	0.37 <sup>bc</sup> ± 0.07
B	40	0.68 <sup>abc</sup> ± 0.18	0.50 <sup>ab</sup> ± 0.00	1.00 <sup>a</sup> ± 0.00	> 1.00 <sup>a</sup> ± 0.00
B	43	0.68 <sup>abc</sup> ± 0.18	0.14 <sup>cde</sup> ± 0.06	0.32 <sup>bc</sup> ± 0.10	0.50 <sup>b</sup> ± 0.00
B	45	> 1.00 <sup>a</sup> ± 0.00	0.75 <sup>a</sup> ± 0.14	0.25 <sup>bcd</sup> ± 0.00	> 1.00 <sup>a</sup> ± 0.00
B	48	0.37 <sup>bcd</sup> ± 0.07	0.17 <sup>cde</sup> ± 0.04	0.07 <sup>cd</sup> ± 0.02	0.31 <sup>bcd</sup> ± 0.10
M	30	0.25 <sup>bcd</sup> ± 0.00	0.37 <sup>bcd</sup> ± 0.07	0.14 <sup>bcd</sup> ± 0.06	1.00 <sup>a</sup> ± 0.00
Antibiotics	Rif	0.20 <sup>bcd</sup> ± 0.00	0.10 <sup>cde</sup> ± 0.00	0.006 <sup>d</sup> ± 0.00	NT
	Stp	0.02 <sup>d</sup> ± 0.00	0.02 <sup>e</sup> ± 0.00	> 0.40 <sup>b</sup> ± 0.00	NT
	Amp	> 0.40 <sup>abcd</sup> ± 0.00	> 0.40 <sup>bc</sup> ± 0.00	> 0.40 <sup>b</sup> ± 0.00	NT
	Nys	NT	NT	NT	0.40 <sup>bc</sup> ± 0.00

35 \*Values followed by the same letter in the each column and isolate, are not significantly different (P < 0.05),  
 36 according to Tukey's HSD test.

37 O – Orange subtype of propolis, B – Blue subtype of propolis, M – Third subtype of propolis

38 Rif - Rifampicin, Stp - Streptomycin, Amp - Ampicillin, Nys – Nystatin, NT – Not tested.

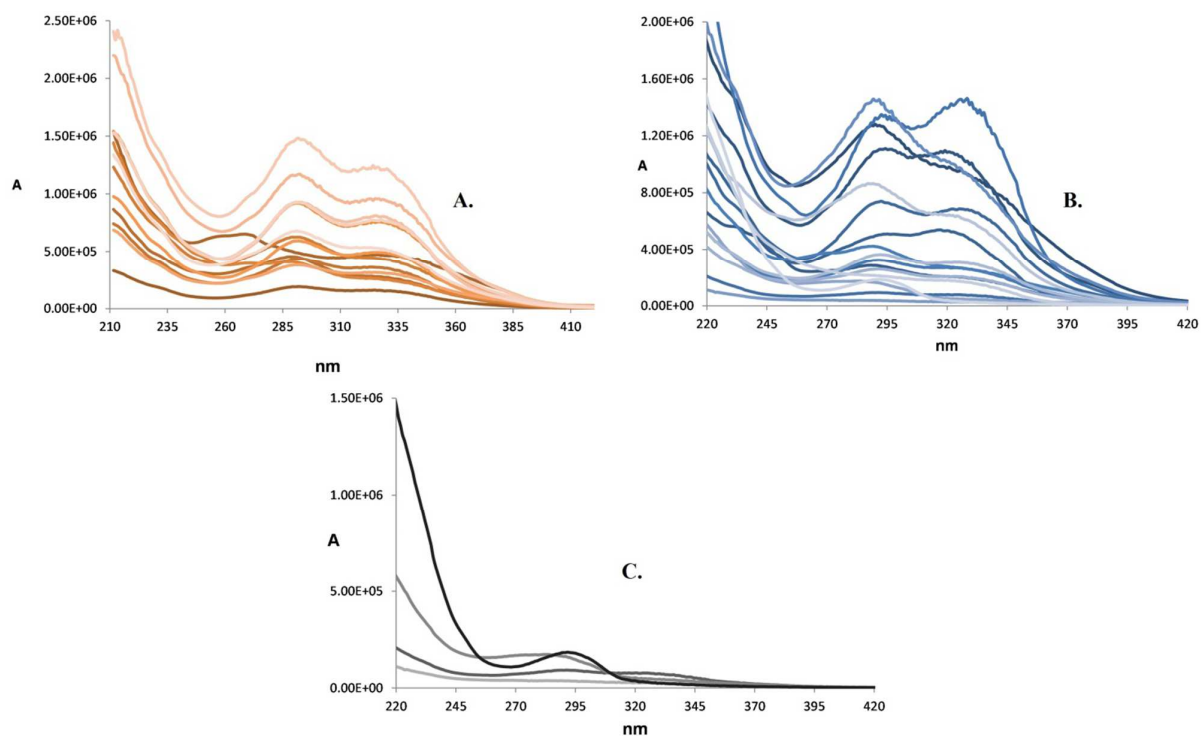
39 **Table 4.** The minimum bactericidal (MBC) and fungicidal concentrations (MFC) of Turkish  
 40 propolis samples (mg/mL). The mean values and standard error are shown.

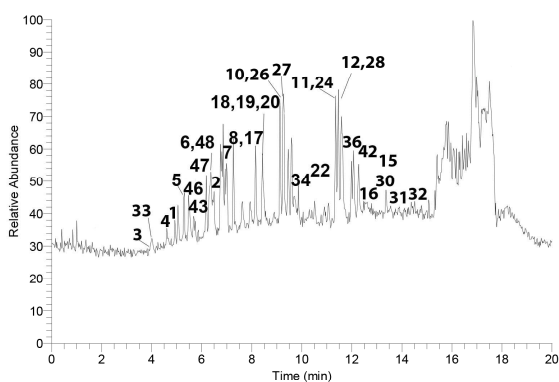
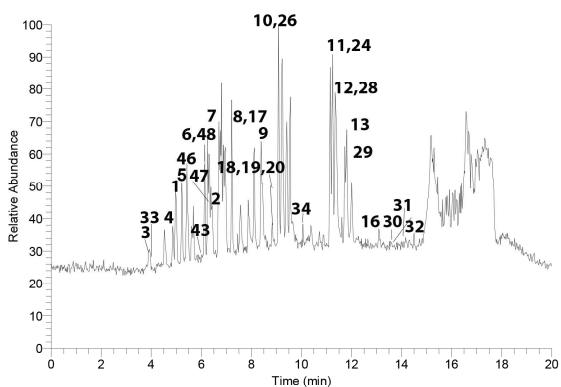
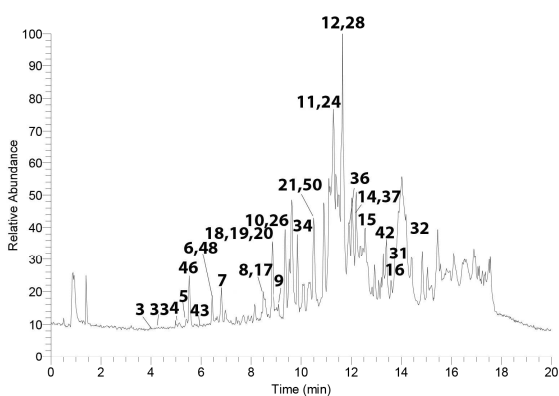
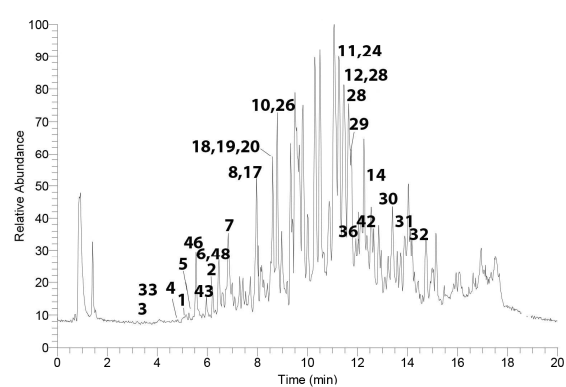
Subtype of propolis	Sample	MBC			MFC
		<i>S. sanguinis</i>	<i>S. mutans</i>	<i>S. pyogenes</i>	<i>C. albicans</i>
O	2	1.00 <sup>a</sup> ± 0.00	0.56 <sup>abc</sup> ± 0.25	0.28 <sup>bc</sup> ± 0.12	> 1.00 <sup>a</sup> ± 0.00
O	4	0.75 <sup>ab</sup> ± 0.14	0.75 <sup>ab</sup> ± 0.14	0.75 <sup>ab</sup> ± 0.14	1.00 <sup>a</sup> ± 0.00
O	7	0.37 <sup>bc</sup> ± 0.07	0.75 <sup>ab</sup> ± 0.14	0.50 <sup>abc</sup> ± 0.00	1.00 <sup>a</sup> ± 0.00
O	8	0.37 <sup>bc</sup> ± 0.07	0.18 <sup>bc</sup> ± 0.03	0.75 <sup>ab</sup> ± 0.14	0.50 <sup>e</sup> ± 0.00
O	11	0.37 <sup>bc</sup> ± 0.07	0.56 <sup>abc</sup> ± 0.25	0.37 <sup>bc</sup> ± 0.07	0.18 <sup>de</sup> ± 0.03
O	12	0.25 <sup>bc</sup> ± 0.00	0.37 <sup>bc</sup> ± 0.07	0.31 <sup>bc</sup> ± 0.10	0.50 <sup>bcd</sup> ± 0.00
O	16	0.50 <sup>abc</sup> ± 0.00	0.75 <sup>ab</sup> ± 0.14	1.00 <sup>a</sup> ± 0.00	0.62 <sup>abcd</sup> ± 0.21
O	17	0.37 <sup>bc</sup> ± 0.07	0.75 <sup>ab</sup> ± 0.14	0.37 <sup>bc</sup> ± 0.07	1.00 <sup>a</sup> ± 0.00
O	18	0.56 <sup>abc</sup> ± 0.15	0.18 <sup>bc</sup> ± 0.03	0.31 <sup>bc</sup> ± 0.10	0.12 <sup>bcd</sup> ± 0.00
O	21	0.25 <sup>bc</sup> ± 0.00	0.25 <sup>bc</sup> ± 0.00	0.31 <sup>bc</sup> ± 0.10	0.75 <sup>ab</sup> ± 0.14
O	22	0.25 <sup>bc</sup> ± 0.00	0.18 <sup>bc</sup> ± 0.03	0.18 <sup>bc</sup> ± 0.03	0.18 <sup>de</sup> ± 0.03
O	24	0.37 <sup>bc</sup> ± 0.07	0.25 <sup>bc</sup> ± 0.00	0.75 <sup>ab</sup> ± 0.14	0.56 <sup>abcde</sup> ± 0.25
O	25	0.37 <sup>bc</sup> ± 0.07	0.25 <sup>bc</sup> ± 0.00	0.75 <sup>ab</sup> ± 0.14	0.18 <sup>de</sup> ± 0.03
O	26	0.50 <sup>abc</sup> ± 0.00	0.50 <sup>abc</sup> ± 0.00	0.37 <sup>bc</sup> ± 0.07	0.37 <sup>bcd</sup> ± 0.07
O	28	1.00 <sup>a</sup> ± 0.00	0.25 <sup>bc</sup> ± 0.00	0.75 <sup>ab</sup> ± 0.14	0.50 <sup>bcd</sup> ± 0.00
O	29	0.62 <sup>ab</sup> ± 0.21	0.37 <sup>bc</sup> ± 0.07	0.50 <sup>abc</sup> ± 0.00	0.37 <sup>bcd</sup> ± 0.07
O	31	1.00 <sup>a</sup> ± 0.00	1.00 <sup>a</sup> ± 0.00	0.56 <sup>abc</sup> ± 0.25	0.25 <sup>cde</sup> ± 0.00
O	32	1.00 <sup>a</sup> ± 0.00	0.53 <sup>abc</sup> ± 0.27	0.37 <sup>bc</sup> ± 0.07	0.25 <sup>cde</sup> ± 0.00
O	33	1.00 <sup>a</sup> ± 0.00	0.37 <sup>bc</sup> ± 0.07	0.18 <sup>bc</sup> ± 0.03	0.25 <sup>cde</sup> ± 0.00
O	34	1.00 <sup>a</sup> ± 0.00	0.50 <sup>abc</sup> ± 0.00	0.50 <sup>abc</sup> ± 0.00	0.37 <sup>bcd</sup> ± 0.07
O	35	0.62 <sup>ab</sup> ± 0.21	0.15 <sup>bc</sup> ± 0.05	0.18 <sup>bc</sup> ± 0.03	0.37 <sup>bcd</sup> ± 0.07
O	36	0.62 <sup>ab</sup> ± 0.21	0.37 <sup>bc</sup> ± 0.07	0.37 <sup>bc</sup> ± 0.07	0.37 <sup>bcd</sup> ± 0.07
O	41	> 1.00 <sup>a</sup> ± 0.00	0.28 <sup>bc</sup> ± 0.12	0.50 <sup>abc</sup> ± 0.00	0.25 <sup>cde</sup> ± 0.00
O	47	0.75 <sup>ab</sup> ± 0.14	0.75 <sup>ab</sup> ± 0.14	0.75 <sup>ab</sup> ± 0.14	0.68 <sup>abc</sup> ± 0.18
B	3	0.50 <sup>abc</sup> ± 0.00	0.06 <sup>c</sup> ± 0.00	0.28 <sup>bc</sup> ± 0.12	0.37 <sup>bcd</sup> ± 0.07
B	5	1.00 <sup>a</sup> ± 0.00	1.00 <sup>a</sup> ± 0.00	0.50 <sup>abc</sup> ± 0.00	1.00 <sup>a</sup> ± 0.00
B	6	0.75 <sup>ab</sup> ± 0.14	0.37 <sup>bc</sup> ± 0.07	0.18 <sup>bc</sup> ± 0.03	0.75 <sup>ab</sup> ± 0.14
B	13	1.00 <sup>a</sup> ± 0.00	0.75 <sup>ab</sup> ± 0.14	0.75 <sup>ab</sup> ± 0.14	0.37 <sup>bcd</sup> ± 0.07
B	15	0.37 <sup>bc</sup> ± 0.07	1.00 <sup>a</sup> ± 0.00	0.75 <sup>ab</sup> ± 0.14	0.25 <sup>cde</sup> ± 0.00
B	20	0.25 <sup>bc</sup> ± 0.00	0.37 <sup>bc</sup> ± 0.07	0.25 <sup>bc</sup> ± 0.00	1.00 <sup>a</sup> ± 0.00
B	23	0.50 <sup>abc</sup> ± 0.00	0.75 <sup>ab</sup> ± 0.14	0.75 <sup>ab</sup> ± 0.14	0.37 <sup>bcd</sup> ± 0.07
B	37	1.00 <sup>a</sup> ± 0.00	0.75 <sup>ab</sup> ± 0.14	0.75 <sup>ab</sup> ± 0.14	1.00 <sup>a</sup> ± 0.00
B	38	0.75 <sup>ab</sup> ± 0.14	0.75 <sup>ab</sup> ± 0.14	0.75 <sup>ab</sup> ± 0.14	0.50 <sup>bcd</sup> ± 0.00
B	39	0.75 <sup>ab</sup> ± 0.14	0.75 <sup>ab</sup> ± 0.14	0.50 <sup>abc</sup> ± 0.00	0.75 <sup>ab</sup> ± 0.14
B	40	> 1.00 <sup>a</sup> ± 0.00	1.00 <sup>a</sup> ± 0.00	> 1.00 <sup>a</sup> ± 0.00	> 1.00 <sup>a</sup> ± 0.00
B	43	> 1.00 <sup>a</sup> ± 0.00	0.37 <sup>bc</sup> ± 0.07	> 1.00 <sup>a</sup> ± 0.00	1.00 <sup>a</sup> ± 0.00
B	45	> 1.00 <sup>a</sup> ± 0.00	> 1.00 <sup>a</sup> ± 0.00	> 1.00 <sup>a</sup> ± 0.00	> 1.00 <sup>a</sup> ± 0.00
B	48	0.75 <sup>ab</sup> ± 0.14	0.50 <sup>abc</sup> ± 0.00	0.75 <sup>ab</sup> ± 0.14	0.62 <sup>abcd</sup> ± 0.21
M	30	0.75 <sup>ab</sup> ± 0.14	1.00 <sup>a</sup> ± 0.00	0.50 <sup>abc</sup> ± 0.00	> 1.00 <sup>a</sup> ± 0.00
	Rif	0.40 <sup>bc</sup> ± 0.00	0.40 <sup>abc</sup> ± 0.00	0.10 <sup>c</sup> ± 0.00	NT
	Stp	0.05 <sup>c</sup> ± 0.00	0.05 <sup>c</sup> ± 0.00	> 0.40 <sup>bc</sup> ± 0.00	NT
Antibiotics	Amp	> 0.40 <sup>bc</sup> ± 0.00	> 0.40 <sup>abc</sup> ± 0.00	> 0.40 <sup>bc</sup> ± 0.00	NT
	Nys	NT	NT	NT	> 0.40 <sup>bcd</sup> ± 0.00

41 \*Values followed by the same letter in the each column and isolate, are not significantly different ( $P < 0.05$ ),  
 42 according to Tukey's HSD test.

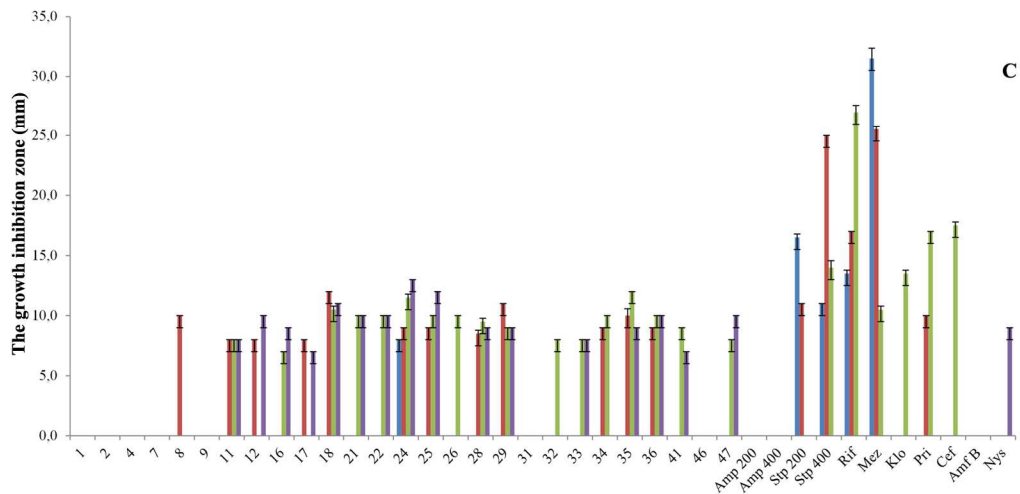
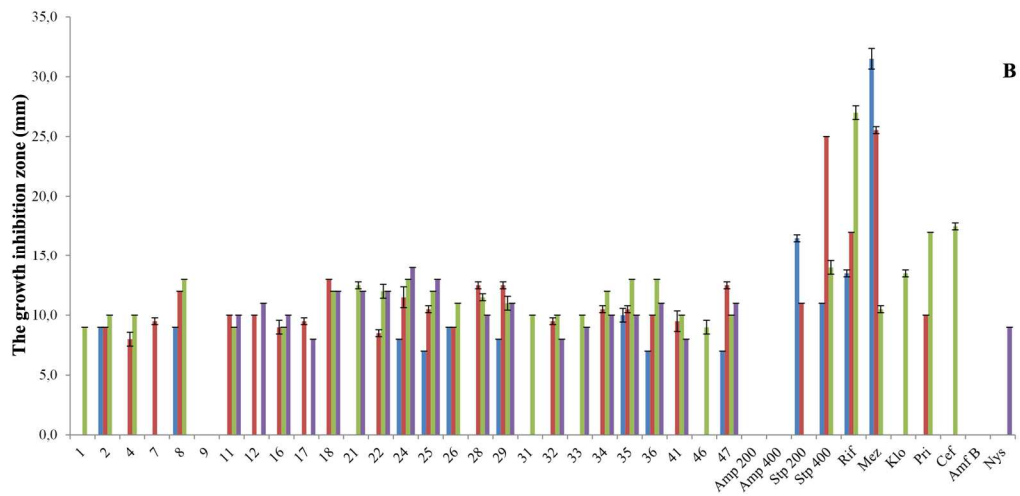
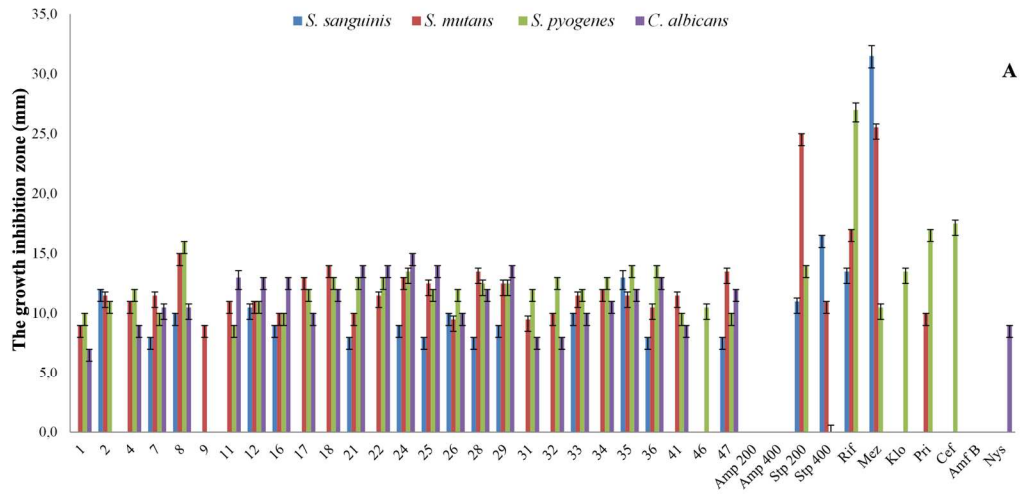
43 O – Orange subtype of propolis, B – Blue subtype of propolis, M – Third subtype of propolis

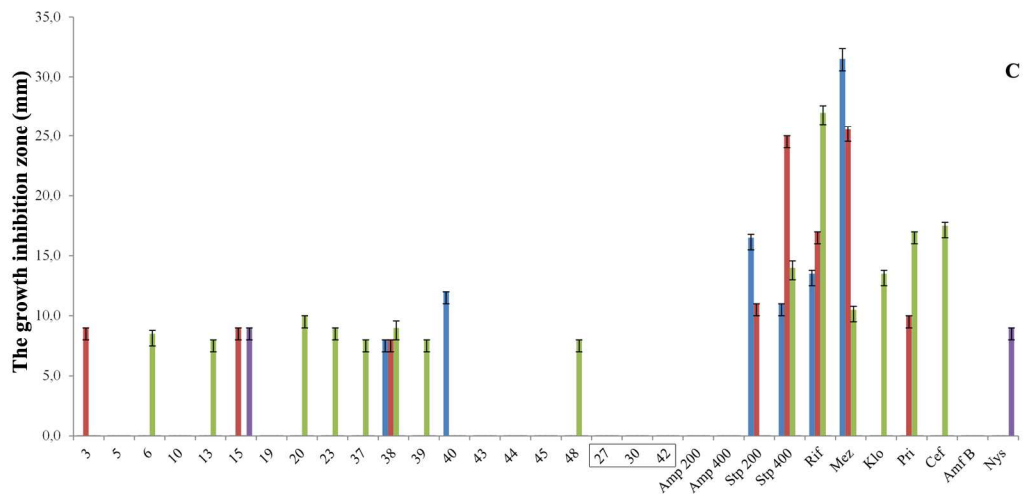
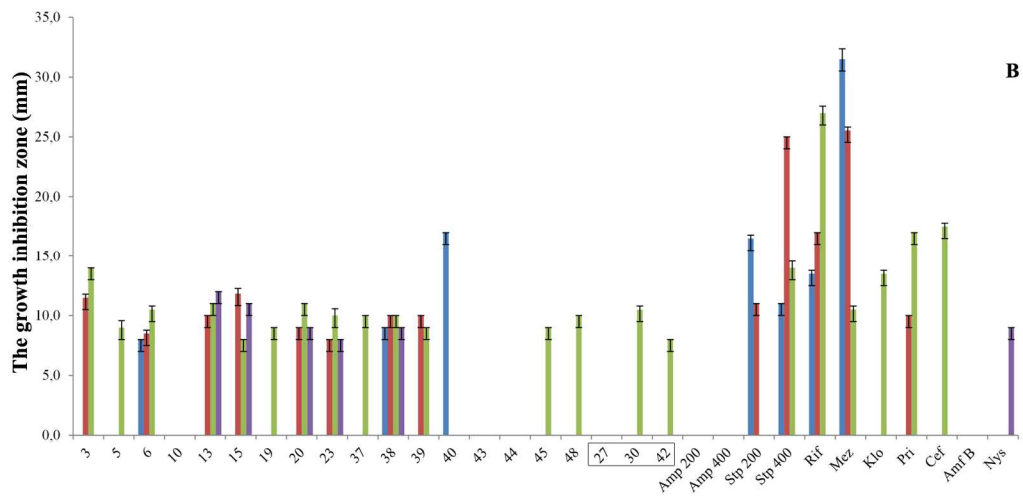
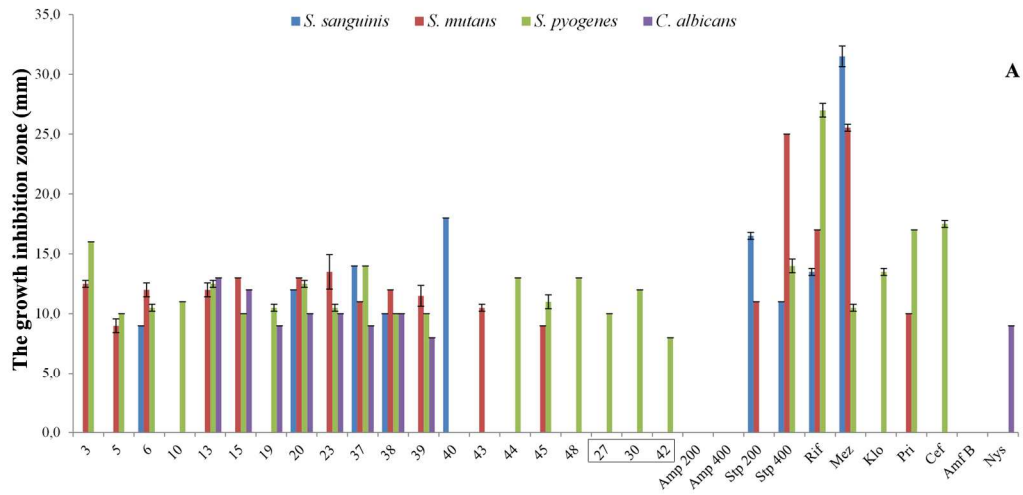
44 Rif - Rifampicin, Stp - Streptomycin, Amp - Ampicillin, Nys – Nystatin, NT – Not tested.



**A****B****C****D**

ACCEPTED MANUSCRIPT







**Highlights**

- Phenolic profiling of three subtypes of Turkish poplar type propolis was studied.
- Quality control parameters of three subtypes of propolis were investigated.
- O-subtype propolis had higher total phenolic and flavonoid contents than B- subtype.
- O- subtype of propolis showed higher antioxidative and antimicrobial activities.