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To cite this article: Leyla Bitis, Ali Sen, Nurten Ozsoy, Seher Birteksoz-Tan, Sukran Kultur & Gulay Melikoglu (2017) Flavonoids and biological activities of various extracts from *Rosa sempervirens* leaves, *Biotechnology & Biotechnological Equipment*, 31:2, 299-303, DOI: [10.1080/13102818.2016.1277956](https://doi.org/10.1080/13102818.2016.1277956)

To link to this article: <https://doi.org/10.1080/13102818.2016.1277956>



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Published online: 11 Jan 2017.



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Flavonoids and biological activities of various extracts from *Rosa sempervirens* leaves

Leyla Bitis^a, Ali Sen^a, Nurten Ozsoy^b, Seher Birteksoz-Tan^c, Sukran Kultur^d and Gulay Melikoglu^e

^aDepartment of Pharmacognosy, Faculty of Pharmacy, Marmara University, Istanbul, Turkey; ^bDepartment of Biochemistry, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey; ^cDepartment of Pharmaceutical Microbiology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey; ^dDepartment of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey; ^eDepartment of Pharmacognosy, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey

ABSTRACT

Rosa sempervirens L. (Rosaceae) growing wildy in Turkey is used in folk medicine for various indications. Here, we report the isolation of four flavonoids and determination of the total phenolic and flavonoid contents and the antimicrobial and antioxidant activities of various extracts from *R. sempervirens* leaves. Antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The total phenolic and flavonoid contents were determined using the Folin-Ciocalteu and AlCl₃ methods, respectively. The antimicrobial activity was evaluated by the broth microdilution method against seven microbial species. The ethyl acetate extract (E) had significant antioxidant activity with half maximal inhibitory concentration (IC₅₀) values of 3.96 in the DPPH and 2.92 µg/mL in the ABTS assay. The total phenolic (203.8 mg gallic acid equivalents/g extract) and total flavonoid (95.81 mg catechin equivalents/g extract) contents of the E extract were significantly higher as compared to other extracts. The E extract exhibited strong antimicrobial activity against *Candida albicans* with a minimum inhibitory concentration of 39 µg/mL. Quercetin 3-xyloside, quercitrin and hyperoside were isolated from the E extract and quercetin, from the chloroform extract, and quercetin and hyperoside were identified for the first time in this species. Quercitrin was found to be a major compound in the E extract. Antimicrobial activity of *R. sempervirens* was also reported for the first time. These results indicate that the E extract has significant antioxidant and antimicrobial activity, probably due to flavonoids as well as other phenolic compounds in the E extract, acting individually or in combination.

ARTICLE HISTORY

Received 14 September 2016
Accepted 28 December 2016

KEYWORDS

Rosa sempervirens;
antimicrobial activity;
antioxidant activity;
flavonoids; polyphenolic
content

Introduction

Rosa sempervirens L. (Rosaceae) is one of the 25 *Rosa* species known to grow in Turkey [1,2]. *Rosa* species are widely distributed not only in Turkey but also in the Balkan region, e.g. in Bulgaria. Some of them are used for various purposes in folk medicine. *Rosa* species have been used in Balkan and Turkish folk medicine for the treatment of dyspepsia, nephritis, haemorrhoids and also as diuretic, expectorant, stomachic and tonic agents [3–5].

Phytochemical studies have shown that the main constituents of *Rosa* spp. are flavonoids, phenolic acids, other phenolics and essential oil (monoterpene alcohols and hydrocarbons) [6–9]. It has also been reported that various extracts and essential oils obtained from different *Rosa* spp. have significant antioxidant and antimicrobial activity [6–10].

Plants are potential sources of natural antioxidants, which have attracted much interest. Numerous

investigations have been carried out in attempts to find natural antioxidant compounds that may take the place of synthetic ones [11]. Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and tert-butylhydroquinone commonly used in the food industry have been reported to cause cancer, as well as toxic effects in the liver [12,13]. For this reason, there is a need for new natural antioxidants to be obtained from natural sources, which are more effective and less toxic.

Some pathogens are resistant to previously discovered effective antimicrobial drugs. Examples of effective antimicrobial compounds that have been isolated from plants are benzoin and emetin. Unlike currently used antimicrobial drugs, compounds with antimicrobial activity found in plants may inhibit the development of microorganisms by different mechanisms and can be used as effective antibiotics against resistant microbial strains [14]. In the context of the witnessed increasing tolerance and escalating levels of drug and antibiotic resistance among

CONTACT Leyla Bitis ✉ leylabitis@marmara.edu.tr

Supplemental data for this article can be accessed at <http://dx.doi.org/10.1080/13102818.2016.1277956>.

pathogenic bacteria and fungi [7,15–17], it is considered a promising approach to turn to the synergistic action of comedications consisting of essential oils in combination with conventional antibiotics and synthetic drugs for prophylaxis and therapy of bacterial infections [7,17]. Thus, there is a need to find new bioactive compounds of plant origin which can be used in the treatment of resistant microbial strains [14].

The present study was carried out to evaluate antimicrobial and antioxidant activities of various extracts obtained from *R. sempervirens* leaves as well as to investigate their chemical composition.

Materials and methods

Plant material

Leaves of *R. sempervirens* L. were collected on 7 June 2014 from Tekirdag province of Turkey (41°34'58"N, 28°8'48"E) and identified by Dr Sukran Kultur. Voucher specimens have been deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 109360).

Extraction and isolation

Dried leaves (550 g) of *R. sempervirens* were first subjected to extraction with petroleum ether and then with ethanol (95°) in a Soxhlet apparatus. The petroleum ether extract (A) was concentrated and extracted with 60% ethanol. The aqueous extract was concentrated and extracted with chloroform (B) in a separatory funnel. The concentrated ethanol (95°) extract was diluted with H₂O and successively extracted with benzene (C), chloroform (D) and ethyl acetate (E) for fractionation.

For the purification of flavonoids from the D and E extracts, silica gel column chromatography, preparative thin-layer chromatography (TLC) and paper chromatography were applied. The structures of the pure compounds were elucidated based on the *R_f* values, colour reactions and spectroscopic methods in comparison with standards or with reference data.

Determination of total phenolic compounds (TPC)

The total content of phenolic compounds in the extracts of *R. sempervirens* leaves was determined by a colorimetric assay, based on the procedure described by Gao et al. [18].

Determination of total flavonoid compounds (TFC)

The total flavonoids content of the extracts of *R. sempervirens* leaves was measured using the AlCl₃ colorimetric assay [19].

DPPH radical-scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical-scavenging activity of the extracts from *R. sempervirens* leaves was evaluated according to the procedure described by Brand-Williams et al. [20].

Total reactive antioxidant potential assay

The total radical antioxidant potential of the extracts was measured using the trolox equivalent antioxidant coefficient assay as described by Cai et al. [21] with minor modifications (see the Online Supplement for details).

Determination of antimicrobial activity

The antimicrobial activities of the extracts were tested against six bacterial species (*Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153) and one yeast strain (*Candida albicans* ATCC 10231) by the broth microdilution method strictly following the recommendations of the Clinical Laboratory Standards Institute. Ciprofloxacin and fluconazole were used as the reference compounds for bacteria and fungi, respectively.

Statistical analysis

Results were expressed as means with standard deviation (\pm SD) from triplicate measurements. Statistical comparisons were performed with Tukey's tests. Differences were considered significant at $p < 0.05$. The correlation coefficient (r^2) between the parameters tested was determined by regression analysis (GraphPad Prism, Version 4.03).

Results and discussion

Isolated compounds

In the present study, four flavonoid compounds were isolated from *R. sempervirens* leaves. These compounds were identified as quercetin (D ext.), quercetin-3-rhamnoside (quercitrin) (E ext.), quercetin-3-xyloside (E ext.) and quercetin-3-galactoside (hyperoside) (E ext.) by comparing their spectral data with those of literature values [22,23] and by TLC comparison with reference standards (Table 1). All these four compounds were isolated, and quercetin and hyperoside were identified for the first time, to the best of our knowledge, in this species. Quercetin 3-rhamnoside was found to be a major compound in the active E extract. Similarly, in a study on the analysis of flavonoids in *R. sempervirens* leaves by high

Table 1. UV data for flavonoids from *R. sempervirens* leaves.

Flavonoids	λ max (nm)					
	MeOH	NaOMe	AlCl ₃	AlCl ₃ /HCl	NaOAc	NaOAc/H ₃ BO ₃
Quercetin	370, 301 sh, 269 sh, 255	321 (dec) 247 sh	458, 333, 304 sh, 272	428, 359, 301 sh, 265	390 (dec) 329, 274, 257 sh	388, 303 sh, 261
Quercetin 3-xyloside	352, 300 sh, 267 sh, 256	395, 327, 271	433, 334, 305 sh, 275	404, 357, 303 sh, 273	376,322 sh, 275	370, 300 sh, 263
Quercitrin	350, 301 sh, 265 sh, 256	393, 326, 270	430, 333, 304 sh, 276	401, 353, 303 sh, 272	372,322 sh, 272	367, 300 sh, 260
Hyperoside	362, 299 sh, 269 sh, 257	409, 327, 272	438,331 sh, 305 sh, 275	405,366 sh, 299 sh, 268	380, 324, 274	377, 298 sh, 262

performance liquid chromatography (HPLC), Cunja et al. [24] reported quercetin-3-rhamnoside as a major compound as well as quercetin-3-arabinofuranoside, quercetin-3-glucoside and quercetin-3-xyloside, kaempferol-3-rhamnoside in the leaves of this species. At the same time, Cunja et al. [24], and Nowak and Gawlik-Dziki [9] reported that quercetin glucosides are found in high amounts in leaves of different *Rosa* species, which is correlated with our present study.

The total phenolic and flavonoid contents of extracts

The total phenolic contents of the B, C, D and E extracts, analysed by using Folin–Ciocalteu's reagent, were 17.49, 62.86, 63.25 and 203.8 mg GAE/g extract, expressed as gallic acid equivalents, respectively. The total flavonoids contents of the B, C, D and E extracts, tested by the AlCl₃ method, were 9.85, 40.87, 38.92 and 95.81 mg CE/g extract, expressed as catechin equivalents, respectively (Table 2). The highest total phenolic and flavonoids contents were found in the E extract obtained from *R. sempervirens* leaves. The average total phenolic content of the E extract (203.8 ± 4.28 mg GAE/g) was found to be close to the values that Ozkan et al. [25] reported for extracts obtained from fresh (276 ± 2.93 mg GAE/g) and dried (248.97 ± 2.96 mg GAE/g) *Rosa damascena* flowers. This value of the E extract was also observed to be higher than the value reported by Nowak and Gawlik-Dziki [9] for *Rosa canina* var. *dumalis* leaves (15.2 ± 0.21 mg GAE/g), which is the highest one in 17 *Rosa* species. A report on the antioxidant activity of *R. sempervirens* leaves collected from different regions of Tunisia

showed that the total flavonoid content of methanol extracts of *R. sempervirens* leaves varies in the range of 0.06–0.37 mg RE/mL, expressed as rutin equivalents [26]. These differences may be explained by the different extraction procedures and the diversity of phenolic compounds. This observation leads to the conclusion that high levels of soluble phenolics in the extracts could be the reason for the strong antioxidant activity.

Antioxidant activity

The IC₅₀ values of the B, C, D and E extracts in the DPPH radical-scavenging assay were 804.1, 56.05, 51.52 and 3.96 µg/mL, respectively. The IC₅₀ values for the ABTS scavenging activity of the B, C, D and E extracts were 201.5, 26.69, 11.97 and 2.92 µg/mL, respectively. The E extract showed strong radical-scavenging activity in both DPPH and ABTS assays when compared to standard antioxidants such as ascorbic acid (2.48 µg/mL) and trolox (3.17 µg/mL) (Table 2). The DPPH and ABTS radical-scavenging activities of antioxidants can be explained by their hydrogen-donating ability [27]. Based on the IC₅₀ values (the effective concentration at which the DPPH radicals were scavenged by 50%), it was observed that, compared to ascorbic acid (IC₅₀ of 2.48 ± 0.24 µg/mL), the E extract of *R. sempervirens* leaves showed considerably high radical-scavenging activity with IC₅₀ of 3.96 ± 0.45 µg/mL. The IC₅₀ value of the E extract was found to be higher than the IC₅₀ value (47.43 ± 0.33 µg/mL) reported in our previous study on the DPPH radical-scavenging activity of ethyl acetate extract from *Rosa agrestis* leaves [6]. The DPPH radical-scavenging activity of the E extract of *R. sempervirens* leaves was

Table 2. Total phenol and flavonoid contents and antioxidant activities of the extracts from *R. sempervirens* leaves.

Extracts/standards	DPPH activity IC ₅₀ (µg/mL)	ABTS activity		TPC (mg GAE/g extract)***	TFC (mg CE/g extract)****
		IC ₅₀ (µg/mL)*	mmol/L TE/g extract**		
B	804.1 ± 13.58 ^a	201.5 ± 7.71 ^a	1.84 ± 0.25 ^a	17.49 ± 0.10 ^a	9.85 ± 0.87 ^a
C	56.05 ± 1.87 ^b	26.69 ± 0.58 ^b	15.31 ± 0.94 ^b	62.86 ± 0.80 ^b	40.87 ± 1.73 ^b
D	51.52 ± 1.20 ^b	11.97 ± 0.26 ^c	28.84 ± 0.77 ^c	63.25 ± 1.44 ^b	38.92 ± 1.41 ^b
E	3.96 ± 0.45 ^c	2.92 ± 0.05 ^d	140.4 ± 1.70 ^d	203.8 ± 4.28 ^c	95.81 ± 5.60 ^c
Ascorbic acid	2.48 ± 0.24 ^c				
Trolox		3.17 ± 0.03 ^d			

Note: Values are means from three replicates ± SD. Different superscript letters in the same row or column indicate significant differences ($p < 0.05$).

* IC₅₀ value was obtained by interpolation from linear regression analysis.

** Expressed as mmol/L trolox equivalents per gram extract.

*** Expressed as milligram gallic acid equivalents per gram extract.

**** Expressed as milligram catechin equivalents per gram extract.

Table 3. Antimicrobial activities of the extracts from *R. sempervirens* leaves.

Extracts ^b / standards	Micro-organisms ^a MIC (µg/mL)						
	S.a.	S.e.	E.c.	K.p.	P.a.	P.m.	C.a.
B	– ^c	–	–	–	–	–	–
C	–	–	–	–	–	–	312
D	–	–	–	–	–	–	–
E	–	–	–	–	–	–	39
Ciprofloxacin							
Fluconazole							1

^aS.a.: *Staphylococcus aureus*; S.e.: *Staphylococcus epidermidis*; E.c.: *Escherichia coli*; K.p.: *Klebsiella pneumoniae*; P.a.: *Pseudomonas aeruginosa*; P.m.: *Proteus mirabilis*; C.a.: *Candida albicans*.

^bB: chloroform fraction of petroleum extract; C: benzene extract; D: chloroform extract; E: ethyl acetate extract.

^cNot active (≥ 1250 µg/mL).

higher than that of 50% aqueous methanol extracts of *Rosa foetida* J. Herrm., *R. hemisphaerica* J. Herrm., *R. pulverulenta* Bieb. and *R. canina* L. with IC₅₀ values of 1.085, 0.986, 1.020 and 0.950 mg/mL, respectively [28]. It was evident that the *R. sempervirens* leaves extract is a stronger free-radical scavenger than the other extracts in our study. Based on the IC₅₀ values (the effective concentration at which the ABTS^{•+} radicals were scavenged by 50%), the ABTS^{•+} radical-scavenging activity of the E extract of *R. sempervirens* leaves was nearly equal (with an IC₅₀ value of 2.92 ± 0.05 µg/mL) in comparison with trolox (3.17 ± 0.03 µg/mL). In agreement with the present study, Ghazghazi et al. [26] demonstrated that the IC₅₀ values of methanol extracts of *R. sempervirens* leaves collected from different regions of Tunisia varied in the range of 1.1–10 µg/mL. According to Rice-Evans et al. [29], the potential scavenging ability of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitutions. These results demonstrated that the E extract of *R. sempervirens* leaves has proton-donating ability and could serve as a free-radical scavenger.

In the present study, the total phenolic and flavonoid content were found reasonably correlated with the IC₅₀ of DPPH ($r^2 = 0.621$ and 0.722 , respectively) and ABTS ($r^2 = 0.636$ and 0.733 , respectively). The IC₅₀ of ABTS was also highly correlated with that of DPPH ($r^2 = 0.998$) and the contents of phenolics exhibited good correlation with the contents of flavonoids ($r^2 = 0.990$). This result was in agreement with previous reports showing that the phenolic compounds contribute significantly to the antioxidant activity. In addition, the results showed that the content of phenolic compounds increased with increasing the polarity of the solvent.

Antimicrobial activity

Only the C and E extracts showed moderate and strong antifungal activity against *C. albicans*, with MIC values of 312 and 39 µg/mL, respectively. None of the extracts had antibacterial activity against *S. aureus*, *S. epidermidis*, *E. coli*,

K. pneumoniae, *P. aeruginosa* or *P. mirabilis* (Table 3). The E extract of *R. sempervirens* leaves in the present study exhibited excellent antifungal activity against *C. albicans*. It has been suggested that the antimicrobial and antioxidant activities of some plant extracts are positively associated with the total phenolic and flavonoid contents of the extract [30]. Daglia [31] claimed that flavan-3-ols, flavonols and tannins have higher antimicrobial activity in comparison with other polyphenols. Furthermore, flavonols have been reported to have fungicidal activity based on recent studies. Parveen and Ghalib [32] reported that the methanol extract of *Xylosma longifolium* leaves, including quercetin-3-rhamnoside as well as other flavonoids, shows high activity against *C. albicans*. Similarly, in another study, Rashed and Butnariu [33] found that the methanol extract of *Eriobotrya japonica* stems exhibits good activity against *C. albicans* and quercetin 3-O- α -rhamnoside as well as other compounds were isolated from this plant. Therefore, the presence of quercetin 3-O- α -rhamnoside found as a major compound in combination with other phenolic compounds in the E extract might be responsible for the antifungal activity observed in our study. Moreover, it is well known that phenolic compounds have good antioxidant and antimicrobial activities.

Conclusions

The results from this study indicate that the ethyl acetate extract of *R. sempervirens* leaves had strong antioxidant and antifungal activity. Therefore, this extract may be considered as a potential source of natural antioxidants and a promising protective agent against oxidative damage by scavenging free radicals owing to endogenous antioxidants, such as phenolic compounds. Also, it might be used as an antifungal agent for killing pathogenic fungi.

Disclosure statement

The authors declare that there are no conflicts of interest.

Funding

This work was supported by the Research Fund of the University of Istanbul [grant number 1660/ 30042001].

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