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Antioxidant and Antibacterial Active Constituents of *Rhus coriaria*

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Abstract : Agents that could act as alternatives to antibiotics in the treatment of several infections can be produced from medicinal plants. The aim of this study is evaluation of *Rhus coriaria* (sumac) extracts as potential new sources of antimicrobial and antioxidant activity. The active constituents like, alkaloids, glycosides, phenol and terpenoids of sumac were extracted and separated using GC-MS. The antioxidant activity of *R. coriaria* extract and its constituents was determined using DPPH and β-carotene-linoleic acid scavenging activity assays. Experimental, *in vitro*, evaluation of the activities of *R. coriaria* extract and its constituents as well as some antibiotics against *E. coli*, *S. aureus*, *P. vulgaris*, *Shigella* spp., *Staph. aureus* and *P. aerugenisis* were performed by agar well diffusion and a modified macro-broth dilution assays, respectively. *R. coriaria* extract showed a higher content of Phenols (41.8%) compared to the other active constituents (glycosides; 19.4%, alkaloids; 17.5 and terpenoids; 11.5%). Antioxidant activity showed a range of (72.70-87.9%) for *R. coriaria* extract compared to lower antioxidant activity of its active constituents. However, phenols showed higher range of antioxidant activity (70.1-75.8%) compared to Glycosides (65.7-67.6%), Alkaloids (53.4-58.4%) and Terpenoids (50.7-51.3%), respectively. Methanol extract of *R. coriaria* and its active constituents showed varying ranges of antibacterial activity at 100 and 200 mg mL^{-1} according to its antioxidant activity. The MIC_{50} values of these products against bacterial strains varied from 3.0 of terpenoids against *Staph. aureus*, 3.12 of phenols against *E. coli*, *S. aureus*, *P. vulgaris*, *Shigella* spp. and *P. aerugenisis*, 3.25 of alkaloids against *Staph.aureus*, 3.4 of glycosides against (*Shigella* spp.) to 5.25 μL mL^{-1} of total *R. coriaria* against *P. aerugenisis*. Total *R. coriaria* extract and its active constituent's phenol and glycosides were the most effective as antioxidant and antibacterial agents compared to alkaloids and terpenoids. The antibacterial activity of these compounds may relate to its total antioxidant activity. Therefore, *R. coriaria* extract and its constituents could act as bactericidal agents against bacterial infection and as a natural preservative in food against food borne diseases.

Key words: Antibacterial, antimicrobial, antioxidant, medicinal plants, sumac, *Rhus coriaria*

INTRODUCTION

Many plants species reported to have pharmacological properties via various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, tirpenes (El-Kamali and El-Amir, 2010; Lalitha *et al*., 2010; Hussain *et al*., 2011) and about 80% of the world's inhabitants relying mainly on traditional medicines based on herbal plants for their primary health care (Owolabi *et al*., 2007).

Recent studies showed that in plants there are bioactive compounds that gained increasing interest as potential therapeutic agents with antimicrobial activities (Ahmad and Beg, 2001; Alagesaboopathi, 2011; Pavithra *et al*., 2010) these agents comparing to conventional antibiotics have fewer side effects, better patient tolerance, relatively less expensive and a long history of use and renewability in nature (Vermani and Garg, 2002). In this connection, sumac the common name for a genus *Rhus* spp. that contains over 250 individual species of flowering plants in the family Anacardiaceae. *Rhus coriaria* is commonly used as a spice by grinding the dried fruit with salt and is also widely used as a medicinal herb in the mediterranean and middle east, particularly for wound healing (Sezik *et al*., 1991).

A variety of biologically active phytochemicals of *R. coriaria* used in herbal medicines as antibacterial, antidiarrheic, antidysenteric, antihepatoxic, antiseptic, antispasmodic, antiviral, astringent, candidicide,

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hepatoprotective, hepatotonic, protisticide, analgesic, antigastric, anti-inflammatory, antioxidant and antiulcer (Nasar-Abbas *et al*., 2004). It was reported that organic solvent extracts of *R. coriaria* (petroleum ether, ether, acetone, ethanol and methanol) exhibited a broad antibacterial spectrum against Gram positive, Gram negative, acid fast and spore-forming bacteria (Khalil, 1996). Most studies on *R. coriaria* extract showed that the activity of *R. coriaria* against intestinal bacteria may related to tannin content, which can be dissolved easily in ethanol (Gulmez *et al*., 2006).

The antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections (Hsueh *et al*., 2005; Lin *et al*., 2005; Mora *et al*., 2005). It also useful as replacements for synthetic preservatives such as parabens, butylated hydroxytoluene (BHT) and butylated hydroxanisole (BHA) that reported as cancer causing agents (Wangensteen *et al*., 2004). Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs (Pavithra *et al*., 2010). Our objective in this study is to evaluate the antioxidant and antimicrobial profile of active constituents from *R. coriaria*.

MATERIALS AND METHODS

Materials: All chemicals were purchased from Sigma Aldrich Co (Milano, Italy). All the chemicals and reagents were of analytical grade.

Preparation of *R. coriaria* extracts: Fruit of *R. coriaria* brought from the local spice store (Othaim Markets) in Riyadh, Saudi Arabia and ground well into a fine powder in a mixer grinder. Twenty five gram of dried plant powder was extracted in a soxhlet apparatus using 250 mL of solvent until the last portion of the extract became colorless. Solvents of all extracts were removed under low vacuum by using rotary evaporation. Crude extracts were maintained at +4°C until use (Thakare, 2004).

Extraction: Terpenoid were extracted with Petroleum ether for 24 h. The dried extracts were stored in a refrigerator at 4°C (present terpenoids) (Harborne, 1984). The dried residue after defatted with petroleum ether retains in a soxhlet and extracted with methanol 90%. For extraction phenol the methanol extract acidify with 2 m H_2SO_4 (pH<3) and partition in a separating funnel with $CHCl_3$ (Harborne, 1984). Glycosides were extracted with n-butanol according to Okonta and Aguwa (2007). Alkaloids were extracted by sonication 10 g of the powder after suspended in 400 mL of surfactant solution sodium dodecyldsulfate (SDS) for 2.5 h in an ultrasonic bath at a constant temperature of 25°C (Djilani *et al*., 2006).

Studies of the *in vitro* antioxidant activity

GC-MS analysis of *R. coriaria* active constituents: GC-MS analysis of the active constituents were performed with GC, using a Hewlett Packard 6890 gas chromatograph equipped with a Hewlett Packard 5973 mass selective detector in the electron impact mode (70 eV). Mass range was from 35-450 m/z. n-Alkenes were used as reference points in the calculation of the Kovats Indices (KI). Identification of the active constituents was done by comparison of their relative retention index and mass spectra with those of NIST library data of the GCMS system and literature data (Adams, 1995).

2,2-Diphenylpicrylhydrazyl (DPPH) assay: The radical scavenging ability of the total *R. coriaria* extract and active constituents against DPPH were evaluated as previously described (Brand-Williams *et al*., 1995). In the presence of an antioxidant that can donate an electron to DPPH, the purple color, typical for free DPPH radical decays and the change in absorbency at λ = 517 nm was measured. The test provides information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate and on the mechanism of antioxidant action. Total *R. coriaria* extract was redissolved in methanol and various concentrations (10, 50, 100, 500 and 1000 μg mL^{-1}) of the extract, 125 μL prepared DPPH (1 mM in methanol) and 375 μL solvent (methanol) were added. After 30 min incubation at 25°C, the decrease in absorbance was measured at λ = 517 nm. The radical scavenging activity was calculated from the equation:

$$\text{Radical scavenging activity (\%)} = \frac{\text{ABS}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Beta-carotene-linoleic acid assay: The antioxidant activity of the total *R. coriaria* extract and active constituents were evaluated, using the β-carotene bleaching method as described by Mothana (2011). Total of 1 mL of a 0.2 mg mL^{-1} β-carotene solution in chloroform was added to flasks containing 0.02 mL of linoleic acid and 0.2 mL of Tween-20. The chloroform was removed at 40°C using a rotary evaporator. The resultant mixture was immediately diluted with 100 mL of distilled water and mixed for 1-2 min to form an emulsion. A mixture prepared similarly but without β-carotene, was used as a blank. A

control containing 0.2 mL of 80% (v/v) methanol instead of extract was also prepared. A 5 mL aliquot of the emulsion was added to a tube containing 0.2 mL of the sample extract at 1 mg mL^{-1}. Rutin (1 mg mL^{-1}) was used as a standard. The tubes were placed in a water bath at 40°C for 2 h. Absorbance was read at 470 nm at 15 min intervals, using a UV-visible spectrophotometer (UV mini-1240, Shimadzu, Japan). The antioxidant activity was calculated using the equation:

$$\text{Antioxidant activity (\%)} = \frac{(Ab_{s0} - Ab_{st})}{(Abs^{*}_{0} - Abs^{\circ}_{t})} \times 100$$

where, Abs_0 and Abs^*_0 are the absorbance values measured at 0 time of incubation for sample extract and control, respectively. Abs_t and Abs^*_t are the absorbance values for sample extract and control, respectively, at t = 120 min.

Determination of *in vitro* anti-bacterial effect Concentration of total extracts and active constituents of *R. coriaria*: Concentration of total methanol extract and active constituents (alkaloids; glycosides; phenols and terpenoids) of *R. coriaria* were used 25, 50 and 100 mg mL^{-1} of phenols and terpenoids, 25, 50, 100 and 200 mg mL^{-1} of alkaloids and glycosides (The concentration 25-100 mg mL^{-1} of alkaloids and glycosides gave no results. Therefore, higher concentrations (200 mg mL^{-1}) were then used and proceeded in order to follow the effectiveness of the plants extracts). Glycosides and phenols dissolved in Distill Water (DW) Alkaloids dissolved in D.W:methanol (7:3). Terpenoides dissolved in dimethylsulfoxid (DMSO) (Jasmine *et al*., 2007).

Test microorganisms: Total extracts and active constituents from *R. coriaria* fruit were tested for antimicrobial activity against six microorganisms, Gram-positive *Staphyloccocus aureus* (ATCC12600), *Streptococcus aureus*, Gram-negative *Escherichia coli* (ATCC 8677), *Pseudomonas aeruginosa* (ATCC 9721), *Proteus vulgaris* and *Shigella* spp. (CIP 5451).

Inoculum preparation: The test microorganisms were maintained at 4°C on nutrient agar slants. Active cultures for each bacterial species were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth. The inoculated tubes were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh nutrient broth to achieve optical densities corresponding to 10^6 CFU mL^{-1} (Duraipandiyan *et al*., 2006).

Broth dilution assay: the Minimum Inhibitory Concentration (MIC) values were determined by using a modified macro-broth dilution technique (Oyeleke *et al*., 2008). Overnight culture of bacteria grown in nutrient broth cultures were diluted 100 folds in NB (100 µL bacterial cultures in 10 mL NB which contained 10^5 CFU mL^{-1} of bacteria). Gradually increasing volumes of the extracts were added to test tubes containing the bacterial cultures to know the inhibitory concentration in a particular tube inhabiting the bacterial growth. The tubes were incubated at 37°C for 18-24 h. The tubes were examined for visible turbidity and optical density of cultures was determined at 620 nm using NB as control. The lowest concentration that inhabited visible growth of the tested organisms was recorded as the MIC. After incubation, the concentration with no visible growth was seen and recorded as the MBC.

Agar well diffusion assay: The agar well diffusion method was used to test the antimicrobial effect of *R. coriaria* methanolic extracts in different stages of germination. (Perez *et al*., 1990; Okeke *et al*., 2001). All media plates (9 cm in diameter) were prepared with nutrient agar. After agar solidification, the well (7 mm in diameter) was cut from the agar to produce a total of four wells per agar plate. For test, three doses of extract (25, 50, 75 µg $well^{-1}$) were use. Standard antibiotics (HIMEDIA Co. India) streptomycin (30 µg), ciprofloxacin (10 µg), doxycycline (30 µg), ampicillin (10 µg) and ofloxacin (5 µg), Tobramycin (10 mcg), were used as positive control. 100 µL (105 cfu) of each diluted microbial suspension were inoculated on nutrient agar plates using sterile cotton swab. The extracts, active constituents of *R. coriaria* and positive control (streptomycin, ciprofloxacin, doxycycline, ampicillin and ofloxacin) were added separately to each well of agar plate and allowed to diffuse at room temperature for 15-20 min. After incubation at 37°C for 24 h, all plates were examined for zones of growth inhibition and the diameter of these zones was measured. The assay was repeated three times for each extract. The antimicrobial effects were recorded as the mean diameter of the resulting inhibition zones of growth in millimeter.

Data analysis: Excel statistical software was used to compare the level of antioxidant activity (TE) to antimicrobial activity (inhibition zones in mm). Regression analysis was generated and the R^2 calculated. Experiments include two factors with three replication in design Completely Random Design (CRD) and tested by F-test (p-value and LSD at level (0.05 and 0.001) (AL-Rawy and Kalafallah, 1980).

RESULTS

Water, acetone and methanol were tested for its extraction efficiency, only methanol extract of *R. coriaria* showed an antibacterial activity where the extract of the three solvent showed no antifungal activity.

Analysis of active constituents in *R. coriaria* extract: The methanol *R. coriaria* extract was analyzed simultaneously by GC and GC-MS methods. The compounds detected, together with their relative percentages are given in Fig. 1 in order of their elution on a HP-5 m sec capillary column. Four compounds were identified of the *R. coriaria* extract. Phenols (41.8%), glycosides (19.4%), alkaloids (17.5%) and terpenoids (11.3%) were the major components comprising the 90% of the *R. coriaria* extract.

Free radical scavenging and antioxidant activity of the *R. coriaria* extract: The potential antioxidant activity of the *R. coriaria* and its active constituents (alkaloids; glycosides; phenols and terpenoids) were investigated on the basis of inhibition of linoleic acid oxidation and of DPPH radical scavenging activity. As shown in Table 1, total *R. coriaria* extract demonstrated radical scavenging activity (68.7 and 87.9%) particularly at the highest concentrations 500 and 1000 μg mL^{-1}. In addition to that, the mean antioxidant activity (72.7%), based on the β-carotene bleaching rate of the *R. coriaria*. However, active constituents showed variety in both radical scavenging and antioxidant activities.

Antibacterial activity of *R. coriaria* and its constituents: The antibacterial activity obtained by broth and agar well diffusion assay is presented in Table 2-3. The concentration of 25 and 50 mg mL^{-1} of all total methanol extract and four active constituents (alkaloids, glycosides, phenols and terpenoids) of fruits of *R. coriaria* were not effective against studied bacteria (*E. coli*, *S. aureus*, *P. vulgaris*, *Shigella* spp., *Staph aureus* and *P. aurogenosa*) while they were effective when increased concentration to 100 and 200 mg mL^{-1} (Table 2-3).

R. coriaria possessed a good antibacterial activity in 100 mg mL^{-1} concentrations. Inhibitory activity of total extract, glycosides and phenols of *R. coriaria* was of greater extent as compared to normal antibiotics. While, terpenoids showed little inhibition activity compared to total extract, glycosides, phenols and most of control antibiotics (Table 2). Also, alkaloids extract did not showed an antibacterial activity against *P. aeruginosa* in 100 mg mL^{-1} concentration. However, a slight antibacterial activity was recorded against *P. aeruginosa* in a concentration of 200 mg mL^{-1} with inhibitory zone ranged from 10-16 mm. All solvents were also used for the test as control but showed no inhibited action against pathogens signifying that it serves as a diluant. The statistical analysis showed significant differences after treating the microorganisms with *R. coriaria* extracts ($p < 0.001$) compared with control and antibiotic.

The results of antibacterial activity (MIC and MBC) are presented in Table 4. The methanol extract of *R. coriaria* showed higher antibacterial activity in this test with bacteriostatic activity in the concentration range 3.25-5.25 μL mL^{-1}, while bactericidal concentrations were in the range of 6.12-10.0 μL mL^{-1}. Active constituents showed a variation in the values of MIC and MBC. Glycosides showed higher antibacterial activity with MIC and MBC values ranging from 3.12-5.0 μL mL^{-1} and 4.25-12.0, respectively, followed by alkaloids (MIC; 1.5-3.25; MBC; 10.5-12.5), phenols (MIC; 1.58-3.12; MBC; 4.25-12.3); terpenoids (MIC; 1.25-3.0; MBC; 6.25-12.5).

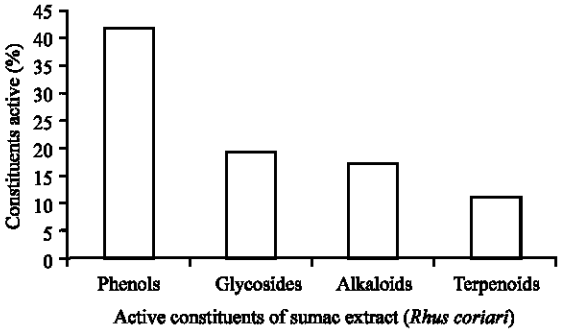

Fig. 1: Active constituents of *R. coriaria*

Table 1: Free radical scavenging activity and antioxidant activity of *R. coriaria* extract and active constituents

	Radical scavenging activity (%)					Total antioxidant activity (%)
Parameter	10	50	100 (μg mL^{-1})	500	1000	1000 (μg mL^{-1})
Plant species						
R. coriaria extract	13.9	34.5	52.3	68.7	87.9	72.7
Active constituents						
Phenols	11.5	31.5	48.5	58.0	75.8	70.1
Glycosides	9.8	28.5	41.2	53.4	65.7	67.6
Alkaloids	8.7	18.7	36.7	48.6	53.4	58.4
Terpenoids	7.5	15.8	31.5	42.3	51.3	50.7
Ascorbic acid	19.1	85.8	92.3	95.0	94.8	-
Rutin						91.8

Table 2: Zone of Inhibition shown by methanol extracts of *R. coriaria* and active constituents (100 µg mL^{-1})

	Inhibition zones (mm)					
Parameter	*E. coli*	*S. aureus*	*P. vulgaris*	*Shigella* spp.	*Staph. aureus*	*P. aerugenisis*
Constituents						
R. coriaria methanol extract	25.8	25.5	28	25	38	39
Active constituents						
Phenols	23.0	23.0	26	23	32	32
Glycosides	21.0	21.0	24	22	30	28
Alkaloids	15.0	17.0	21	18	19	0
Terpenoids	9.0	12.0	10	11	12	19
Antibiotics						
ST	21.0	25.0	21	18	25	16
CF	11.0	15.0	19	21	16	25
DO	25.0	35.0	31	30	24	28
AM	11.0	12.0	13	15	18	15
OF	23.0	18.0	26	22	24	29
TOB	18.0	19.0	18	22	20	18
Control						
Solvents	0.0	0.0	0	0	0	0

Data is a mean of three replications. -: No inhibition observed, ST: Streptomycin (30 µg), CF: Ciprofloxacin (10 µg), DO: Doxycycline (30 µg), AM: Ampicillin (10 µg), OF: Ofloxacin (5 µg),TOB: Tobramycin (10 mcg), Technique used was agar well diffusion assay. Extracts and active constituents (100 µg well^{-1}). LSD (0.001) = 1.53

Table 3: Zone of Inhibition shown by methanol extracts of *R. coriaria* and active constituents (200 µg mL^{-1})

	Inhibition zones (mm)					
Parameter	*E. coli*	*S. aureus*	*P. vulgaris*	*Shigella* spp.	*Staph.aureus*	*P. aerugenisis*
Constituents						
R. coriaria methanol extract	30	42	38	28	45	46
Active constituents						
Phenols	26	35	31	25	38	36
Glycosides	22	29	27	23	35	31
Alkaloids	18	23	25	21	29	15
Terpenoids	16	21	23	18	23	25
Antibiotics						
ST	21	25	21	18	25	16
CF	11	15	19	21	16	25
DO	25	35	31	30	24	28
AM	11	12	13	15	18	15
OF	23	18	26	22	24	29
TOB	18	19	18	22	20	18
Control						
Solvents	0	0	0	0	0	0

Data is a mean of three replications. -: No inhibition observed, ST: Streptomycin (30 µg), CF: Ciprofloxacin (10 µg), DO: Doxycycline (30 µg), AM: Ampicillin (10 µg), OF: Ofloxacin (5 µg),TOB: Tobramycin (10 mcg), Technique used was agar well diffusion assay. Extracts and active constituents (200 µg well^{-1}). LSD (0.001) = 4.83

Table 4: Antibacterial activity expressed as minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC_{50}), in µL mL^{-1}, of the studied *R. coriaria* active constituents, determined by the microdilution method

	T. extract		Phenols		Glycosides		Alkaloids		Terpenoids	
Bacterial species	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	4.25	10.0	3.12	4.25	3.12	6.25	1.90	10.5	1.25	11.30
S. aureus	3.25	9.25	3.12	5.00	3.12	5.00	3.20	11.5	2.25	9.50
P. vulgaris	4.25	8.25	3.12	6.25	3.12	4.80	2.25	12.5	1.50	10.50
Shigella spp.	3.90	10.6	3.12	11.25	3.40	12.00	2. 50	12.5	2.25	12.50
Staph. aureus	3.70	6.12	1.58	12.30	3.12	7.00	3.25	12.5	3.00	6.25
P. aerugenisis	5.25	7.00	3.12	6.25	3.12	4.25	1.50	12.5	1.90	10.50

Shigella spp. showed a higher resistance than other bacteria in this test, while *P. aerugenisis*, *P. vulgaris* and *E. coli* showed higher sensitivity when treated with total methanolic *R. coriaria* compared to the tested active constituents.

Statistical analysis of the serial dilution results showed that the antibacterial effect of the *R. coriaria* methanolic extract against studied bacteria (*E. coli*, *S. aureus*, *P. vulgaris*, *Shigella* spp., *Staph.aureus* and *P. aurogenosa*) was greater than that of antibiotics used ($p<0.001$). When compared with the *R. coriaria* active constituents, only terpenoids showed significant low antibacterial effect on bacterial strains ($p<0.05$) (Table 5).

Table 5: Comparison of the statistical analysis results of the antibacterial activity of *R. coriaria* extract and its active constituents at 200 mg mL^{-1} concentration by antibiotics ($p<0.05$)

Mean inhibition zone (Extract/active constituents) (mm)	Mean inhibition zone of antibiotics					
	Streptomycin (21 mm)	Ciprofloxacin (17.83 mm)	Doxycycline (24.5 mm)	Ampicillin (14 mm)	Ofloxacin (23.7 mm)	Tobramycin (19.2 mm)
R. coriaria: 25.7	p<0.001	p<0.001	p<0.001	p<0.05	p<0.001	p<0.001
Active constituents						
Phenols: 29.3	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
Glycosides: 26.3	p<0.001	p<0.001	p<0.001	p<0.01	p<0.001	p<0.001
Alkaloids: 19.3	p<0.001	p<0.001	p<0.01	p<0.01	p<0.01	p<0.01
Terpenoids: 12.2	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

Table 6: Correlation between antioxidant level (as % inhibition of BCB) versus antimicrobial activity (inhibition zone in mm) of *R. coriaria* extracts and its active constituents

Parameters Antioxidant (%) vs inhibition zone (mm)	Microorganisms					
	E. coli (R^2)	*S. aureus* (R^2)	*P. vulgaris* (R^2)	*Shigella* spp. (R^2)	*Staph. aureus* (R^2)	*P. aerugenisis* (R^2)
R. coriaria: 72.7 vs. 25.7	0.34**	0.34**	0.41**	0.36**	0.31**	0.34**
Phenols: 70.1 vs. 29.3	0.35**	0.31**	0.35**	0.32**	0.31**	0.34**
Glycosides: 67.6 vs. 26.3	0.30**	0.29**	0.27**	0.29**	0.28**	0.28**
Alkaloids: 58.4 vs. 19.3	0.19*	0.14*	0.26*	0.23*	0.21*	0.27*
Terpenoids 50.7 vs. 12.2	0.19*	0.18*	0.15*	0.20*	0.25*	0.27*

R-values are significant ($p<0.05$), the correlations are significantly according to microorganism and type of extract *$p<0.01$, **$p<0.001$ $Y = 3E\text{-}05x+8.6732$, $R^2 = 0.298$

Relationships between antioxidant and antibacterial activity of *R. coriaria* and its constituents: The relation between antioxidant level (% inhibition of BCB) and antimicrobial activity (inhibitions zones in mm) of *R. coriaria* extract and its active constituents against the six tested microorganisms was studied. Antioxidant activity, with an R^2 of 0.30, explained only 30% of the variation in antimicrobial activity. Correlation coefficients between antioxidant and antimicrobial activity for individual microorganisms were shown to significantly correlated (Table 6). Greater antimicrobial activity against all studied bacteria was observed in total *R. coriaria* extract with antioxidant level of 72.7% ($p<0.001$).

In active *R. coriaria* constituents, higher antibacterial activity appeared against all studied bacteria in phenol and glycosides with antioxidant activity levels (70.1; 67.6, $p<0.001$), respectively. However, less antimicrobial activity against bacteria was obtained in alkaloids and terpenoids with lower antioxidant levels (58.4; 50.7; $p<0.01$), respectively (Table 6).

DISCUSSION

In the last decade much interest has occurred to search for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes (Wangensteen *et al*., 2004). One of these very important compounds are antioxidants (Lampart-Szczapa *et al*., 2003). In plants, the term antioxidant often refers to a wide range of phenolic compounds that vary from simple phenolic acids to highly polymerized compounds such as tannins, phenolic compounds and polyphenols are categorized into 15 main classes with over 8,000 identified compounds. The largest category is the flavonoid group, comprising 13 classes with over 5,000 compounds (Fine, 2000). Antioxidants that retard the oxidation process may additionally exhibit antimicrobial activity (Cutter, 2000; Puupponen-Pimia *et al*., 2001).

Since medicinal herbs are cheaper than drugs and produce fewer side effects, their application has been increased in recent years (Gangoue-Pieboji *et al*., 2007). Studies on the antibacterial effect of *R. coriaria* extract showed a strong antibacterial sensitivity against both gram-positive and gram-negative intestinal bacteria which may related to its tannin content (Rayne and Mazza, 2007).

In the present study, the active constituents of *R. coriaria* extract were analyzed simultaneously by GC and GC-MS methods. Phenols (41.8%), glycosides (19.4%), alkaloids (17.5%) and terpenoids (11.3%) were the major components comprising the 90% of the extract. The abundance polyphenols may explain the utility of *R. coriaria* in the treatment of infectious diseases (Sudharameshwari and Radhika, 2007).

R. coriaria extract demonstrated radical scavenging activity particularly at the highest concentrations. In addition to that, the mean antioxidant activity based on the β-carotene bleaching rate of the *R. coriaria* was 72.7%. However, active constituents showed variety in both radical scavenging and antioxidant activities. The data obtained matched with those who reported that methanol extracts of *R. coriaria* exhibited superoxide radical scavenging activity *in vitro* (Candan and Sokmen, 2004).

The antibacterial activity of total methanol extract, glycosides and phenols of *R. coriaria* was of greater extent as compared to normal antibiotics. Very limited literature is available on the mechanism for the antimicrobial activity of herbs and spices (Nasar-Abbas *et al*., 2004). As for the compounds in *R. coriaria* which may be responsible for the antimicrobial activity, more than 120 volatile constituents have been identified by using gas chromatography and mass spectroscopy, of which terpenoids and aliphatic compounds were found occurring more frequently in six different varieties of *R. coriaria*. Main constituents of *R. coriaria* are terpene hydrocarbons (i.e. α-pinene, β-cary-ophyllene and cembrene), oxygenated terpenes (i.e., α-ter-pineol, carvacrol and β-caryophyllene alcohol) as well as farnesyl acetone, hexahydrofarnesyl acetone and aliphatic aldehydes (Brunke *et al*., 1993).

Statistical analysis of the serial dilution results showed that the antibacterial effect of *R. coriaria* methanolic extract against studied bacteria (*E. coli*, *S. aureus*, *P. vulgaris*, *Shigella* spp., *Staph. aureus* and *P. aurogenosa*) was greater than that of antibiotics used ($p<0.001$). The data matched with Sagdic *et al*. (2003) who reported that the plant extract showed zone of inhibition ranging from 13-22 mm. Also, dried methanol extracts of *R. coriaria* fruits showed inhibition zone of >15 mm against bacterial species (Bonjar, 2004), this ascribed to the tannins in the ethanolic extracts, with MICs in the range of 10-26 mg mL^{-1} depending on the bacterial species (Nimri *et al*., 1999). Tannins are quite potent antibiotics, the inhibition of extracellular enzymes (cellulase, pectinase, laccase, xylanase, etc.), nutrient deprivation of substrates (metal complexation, protein insolubilization) and action on microbe's membranes (inhibition of oxidative phosphorylation) are involved in tannin toxicity (Lattanzio *et al*., 2008). Moreover phenols and glycosides of *R. coriaria* rich in anthocyanins and hydrolysable tannins, gallic acid, anthocyanin fraction contained cyanidin, peonidin, pelargonidin, petunidin and dolphinidin glucosides and coumarates (Kosar *et al*., 2007).

In studying the antioxidant level (% inhibition of BCB) to antimicrobial activity (inhibitions zones in mm) of *R. coriaria* extract we found that greater antimicrobial activity against all studied bacteria was observed in total extract with antioxidant level of 72.7% ($p<0.001$) and for active *R. coriaria* constituents, higher antibacterial activity appeared against all studied bacteria in phenol and glycosides with antioxidant activity levels (70.1; 67.6, $p<0.001$), respectively. In the other hand, less antimicrobial activity against bacteria was obtained in alkaloids and terpenoids with lower antioxidant levels (58.4; 50.7; $p<0.01$), respectively. These results confirm the relationship between antioxidant and antimicrobial activities as previously reported by Cutter (2000), Hao *et al*. (1998) and Puupponen-Pimia *et al*. (2001).

The results obtained in this study prove that *R. coriaria* extracts show high anti-bacterial activity and that traditional using of this spice may help in protecting from several bacterial diseases spontaneously and may aid in control of bacterial growth in foods.

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