Rhus Coriaria L. (Sumac) Leaves as an Antibacterial Agent

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Abstract

Background

Rhus coriaria L. has various medicinal effects, exhibiting antidiabetic, anti-inflammatory, anticancer, antioxidant, antiviral, anti-ischemic, hypolipidemic and antibacterial activity. Its antibacterial activity has been extensively studied, revealing terpenoids, flavonoids and hydrolysable tannins as the main active compounds. Most of these studies have been performed on fruit, and a few on leaves and seeds.

Methods

Extract of *R. coriaria* leaves was fractionated into six fractions by high-performance liquid chromatography (HPLC). Two active fractions (Rc1 and Rc2) were subfractionated into fractions Rs1–3 and Rs4–6, respectively. The active subfraction Rs5 was subjected to LCMS analysis. Antibacterial activity of the extracts was assessed by well and disk-diffusion methods, and minimal inhibitory concentration (MIC) by tetrazolium (TTC) test and minimal bactericidal concentration (MBC) by drop test were determined. Subfraction Rs5 was further incorporated into an eye drop formulation and tested for antibacterial activity, and leaf extract was also tested against the fungus *Botrytis cinerea*.

Results

Crude leaf extract, fractions Rc1 and Rc2 and subfractions Rs1–6 showed antibacterial activity, with MIC values ranging from 0.2–3.1 mg/mL and MBC values from 0.3–6.2 mg/mL, depending on the fraction and the bacteria. Subfraction Rs5 had the strongest antibacterial activity with a MIC of 0.4 mg/mL and MBC of 0.6–2 mg/mL, depending on the bacterium being tested. Rs5 incorporated into an eye drop formulation also showed antibacterial activity. The leaf extract further showed antifungal activity against the agricultural pest *Botrytis cinerea*.

Conclusions

The crude leaf extract and its fractions exhibited antibacterial and antifungal activity. The antibacterial activity increased in the fractions and subfractions of the leaf extract compared to the crude extract, suggesting enrichment of the active antibacterial compounds during purification. Leaves, which are much more readily available, and in greater quantities than fruit, throughout the year, may therefore serve as an inexpensive and effective source for an antibacterial product that can be used in the food and agricultural industries.

Introduction

A field survey was conducted from 2010–2014 in the Mediterranean region to identify plants with medicinal activity based on knowledge derived from folk medicine. More than 1,100 plants were screened, and 20 of them were found to have potential antibacterial activity (1). Of these, the plant *Rhus coriaria* L., family Anacardiaceae, common name Sumac, was selected for further testing due to its strong antibacterial activity, and because it is an edible plant, widely used as a spice, that is endemic to the Mediterranean region. The fruit is the main part of the Sumac plant used for human consumption (2, 3, 4). More than 200 compounds from the *R. coriaria* plant have been identified and reported, many of them for their association to medicinal activity (3, 5). The most common of these are flavonoids and tannins, but phenolic acids, conjugated phenolic acids, anthocyanins, organic acids, terpenoids and many more have been found as well (4, 7, 8, 9, 10, 11, 12, 13, 14, 15). Most of these studies were performed with fruit, but a few have investigated Sumac leaves and seeds as well. Those studies found terpenoids, flavonoids and hydrolysable tannins as possible active compounds (4, 7, 9, 16). *R. coriaria* has a number of medicinal effects, exhibiting antidiabetic, anti-inflammatory, anticancer, antioxidant, antiviral, anti-ischemic, hypolipidemic and antibacterial activities (3, 5, 17). The antibacterial activity of *R. coriaria* was discovered using different fruit extracts: methanolic, ethanolic and water.

Ethanolic extract showed antibacterial activity against *Salmonella* spp. (18), acne vulgaris (19), *Staphylococcus aureus* biofilm formation (20), and strains of *Brucella* (21). Nimri et al. (22) showed antibacterial activity of ethanolic fruit extracts against 14 different bacteria. Alwan et al. (23) showed antibacterial effects of water and ethanolic extracts of *R. coriaria* against three strains of *Pseudomonas aeruginosa* and two strains of *Escherichia coli* isolated from human epithelial cells. Methanolic extracts of *R. coriaria* also exhibited antibacterial activity against the bacteria *Bordetella bronchiseptica, Bacillus pumilus, Staphylococcus epidermidis*, and *Klebsiella pneumoniae* (24), as well as *E. coli, S. aureus*, and *Brucella* spp. (25). Again, most of those studies used extracts from the fruit of *R. coriaria*, whereas only a few addressed the antibacterial effect of its leaves (7, 9, 26). Here we focus on the antibacterial activity of this plant's leaves, with the rationale that the leaves are much more readily available, and in greater quantities, than fruit throughout the year. Thus, leaves might serve as an available and effective source for the preparation of antibacterial products to be used in the food industry, including food packaging.

Methods

Preparation of plant material

R. coriaria leaves (2 g), collected in the wild from the environs of Jerusalem and the Judean foothills, were cut with scissors and then ground with a mortar and pestle with 4 mL of 60% ethanol and liquid nitrogen. The dry samples were weighed, and 60% ethanol was added to the ground leaves to a final concentration of 50 mg/mL. The sample was then transferred to a 15-mL tube and centrifuged at 6000 rpm (10483 relative centrifugal force, RCF) for 10 min. The supernatant was filtered through a 0.45-µm filter into Eppendorf tubes and used as a stock solution for further separation and analysis.

Analytical and preparative HPLC analysis and fractionation

The sample profile was obtained from an UltiMate 3000 HPLC system coupled with WPS-3000T autosampler, HPG-3400 pump, and 3000 Rapid Separation Diode Array Detector (Thermo Fisher Scientific). Separation was performed on a Raptor ARC-18 LC column, 2.7 µm, 150 x 4.6 mm (Restek), using water/methanol (25:75, v/v) as the diluent, and an injection volume of 5 µL. The mobile phase consisted of Solution A (0.1% acetic acid) and then Solution B (100% methanol) at a flow rate of 1.5 mL/min. Six fractions were collected based on HPLC-detectable peaks. To obtain higher amounts of material in each fraction, preparative HPLC (Agilent Technologies 1260 Infinity II LC Systems, with multiple wavelength detector 1260 MWD VL, and a Kinetex 5 µm EVO C18 100A column, 250 x 21.2 mm from Phenomenex) was performed. The mobile phase consisted of Solution A followed by Solution B, with an injected sample concentration of 5 mg/mL and injection volume of 10 mL. The profile was determined at both 280 nm and 220 nm, and was found to be the same (only 220 nm is shown).

LCMS analysis of the fractionated extracts of *R. coriaria* leaves

Each of the samples of dried material was dissolved in 60% ethanol/water to 1 mg/mL, and 1 mL of sample was injected twice into a Dionex Q Exactive Plus liquid chromatography- mass spectrometry system (LCMS) (Thermo Fisher Scientific) consisting of a workstation with the Xcalibur v. 4.0 software package combined with Dionex SII LC control software, UltiMate SRD-3400 solvent rack and degasser, pulseless chromatography pump (UltiMate HPG-3400RS rapid separation pump), WPS-3000RS autosampler, column compartment TCC-3000RS, and photodiode array detector DAD-3000RS. Eluent flow was then guided to the Q Exactive Plus Orbitrap high-resolution high-mass-accuracy MS. Mass detection was performed as a full MS scan with low-energy collision-induced dissociation (CID) from 100 to 1500 m/z in either negative (first injection), or positive (second injection) ionization mode with electrospray (ESI) interface. Sheath gas flow rate was 30, auxiliary gas flow rate was 7, and sweep gas flow rate was 1 (arbitrary units). Electrospray voltage was 3500 V (-3500 for negative ESI) with a capillary temperature of 275 °C. Mass resolution was set to 140,000. Samples were separated on a Kinetex C8 reversed-phase column, 100 x 2.1 mm, particle size 2.6 mm, pore size 100 Å (Phenomenex). The mobile phase consisted of Solvent A (0.5% ACS-grade acetic acid in LCMS-grade water, pH 3–3.5), and Solvent B (100% acetonitrile, LCMS grade), at a flow rate of 0.20 mL/min. Gradient mode was used for all analyses, starting at 95% A and 5% B, to 5% A and 95% B over 30 min, maintained for 8 min, and a return to the initial ratio over 4 min. An 8-min equilibration interval was included between subsequent injections. The average pump pressure using these parameters was typically around 2900 psi for initial conditions.

Putative formulas of polyphenols and other compounds were determined by analysis of isotope abundance in the high-resolution mass spectral data with Xcalibur v. 4.0 software and determining the best-fitting empirical formula. Quantification was based on external standards of commercially available compounds. Database searches were performed using Reaxys (www.reaxys.com; RELX Intellectual Properties SA).

Antibacterial activity

The bacteria used in this study were: *Escherichia coli* ATCC 8739 DyoPak 4th pass, *Escherichia coli* K-12MG1655*, Klebsiella pneumoniae* derived from CDC 1100192, *Staphylococcus aureus* ATCC 6538 DyoPak 4th pass, and *Bacillus subtilis* ATCC 6633 DyoPak 4th pass.

Materials

Antibiotic–antibacterial solution (100×) consisted of 10,000 U penicillin, 10 mg streptomycin and 25 µg amphotericin B per mL (Sigma antibiotics mix). The following media were used: BBL^{TM} Mueller Hinton II broth from BD (MH), Mueller Hinton agar (MHA) plates (NOVAmed), Luria broth (LB), LB agar plates (hylabs), ATCC medium: 778 Davis and Mingioli glucose minimal medium (MM), YPD broth (Sigma), and YPD agar with 2% glucose. 2,3,5-Triphenyltetrazolium chloride (TTC) was from Sigma, CT0998B blank discs were from Oxoid.

Disk diffusion test

Well and disk diffusion methods were used as described in the literature (22). Briefly, inoculum was suspended in MH broth and standardized to match a 0.5 McFarland standard (corresponding to approximately 1.5 x 10^8 CFU/mL). The inoculum was suspended overnight in MH and adjusted to a turbidity equivalent to OD_{600} = 0.5. Antibiotic mix and all antibacterial agents were diluted to 3.1–100 mg/mL in the MH broth. Disks (6 mm diameter) were brought to room temperature for 1 to 2 h. Drops (10 µL) from the specified dilutions of antibacterial agents were applied to a disk and left for 10–15 min for absorption and evaporation. MHA plates were warmed to room temperature and divided into sections. The bacterial suspension was swirled to ensure thorough mixing, and a sterile cotton-tipped swab was inserted into the suspension which was then evenly inoculated over the top of the MHA plates. The discs containing the test compounds at the specified concentrations were placed on the inoculated plates. The plates were placed for 3 h at 4 ^oC then incubated at 37 ^oC for 18–24 h. The next day, the zones of growth inhibition were measured by caliper using reflected light. All experiments were performed in triplicate and at least three independent experiments were performed for each bacterium and each extract.

MIC and MBC tests

The MIC test was performed in 96-well plates. Bacterial suspension was grown on LB agar plates and incubated for 18–24 h at 37 ^oC. Three to five colonies were selected from the fresh agar plate and transferred using a sterile loop into a sterile tube containing 5 mL LB broth solution. The tubes were incubated to a turbidity of 0.5 at OD_{600}. Twofold serial dilutions of the antibiotics mix, as well as of all antibacterial agents, corresponding to a final concentration range of 0.08–10 mg/mL, were prepared in MM.TheMM solution was made up of Davis and Mingioli glucose minimal medium (K_2HPO_4, 7 g; KH_2PO_4, 3 g;sodium citrate·$3H_2O$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $(NH_4)2SO_4$, 1 g), distilled water to 1 L, 15 g agar, pH adjusted to 7.0. Filter-sterilized glucose (10%) was added to the medium to a final concentration of 0.2%. The bacterial suspension, adjusted to 1 x 10^8 CFU/mL (OD_{600} = 0.5) was diluted 1:100. A 50-µL aliquot of each dilution of the antibacterial agents being tested was distributed into wells of a 96-well plate according to the prepared plan, including a positive control (50 µL bacterial isolate + 50 µL sterile broth) and a negative control (100 µL sterile broth). Each well containing antibiotic solution was inoculated with 50 µL of bacterial suspension. The final inoculum was about 5 x 10^5 CFU/mL. A 10-µL sample from the positive control well was removed into a sterile Eppendorf tube holding 990 µL of the broth. Plates were incubated for 18–24 h at 37 ^oC. MIC was measured using the tetrazolium (TTC) test that measures cell viability (27), and MBC was measured using the drop test (28), where 10 µL was transferred from the well to a LB plate, and MBC was defined as the first droplet in which no live bacteria were found.

Inhibition of *Botrytis cinerea* growth in the presence of *R. coriaria* crude leaf extract

The pathogenic fungus *Botrytis cinerea*, and the plant *Vitis vinifera* (grape vine) were chosen for this test. The developed formulation was prepared for spray application to prevent secondary damage to the plant (such as burning). The ingredients of the formula in which *R. coriaria* leaf extract was dissolved were: glycerol as the stabilizer, water, an emulsifying mixture of mineral oil (surface) emulsified at 4% + 94% water + 2% emulsifier (glycerin monostearate), and 5% dimethyl sulfoxide (EOS). Glycerin monostearate (500 mg), 2 g EOS oil and 1 g glycerol were stirred at 80 ^{o}C. The prepared *R. coriaria* leaf extract was added with 5 g warm water. After 2 min of stirring, it was left to emulsify for 2 min while being chilled to 20 ^{o}C. The control formula was made in the same way without the *R. coriaria* extract.

The fungus *Botrytis cinerea* was grown on agar plates (standard PDA protocol). A disc-shaped leaf of *Vitis vinifera* was placed in the middle of the plate. The formulation with and without *R. coriaria* extract was sprayed on the plated leaf. Fungal-growth inhibition was observed after 24 and 72 h, compared to the control plate on which the fungus grew without the extract.

Results

Activity and fractionation of *R. coriaria* leaf extract

Initial analysis of the antibacterial effects of different parts of the *R. coriaria* plant showed that a crude extract of the leaves inhibits bacterial growth by 51%, whereas with extracts of the fruit and flowers, inhibition was 40% (Table 1). We further fractionated the leaf crude extract into six fractions (F10–F15, Fig. 1a). Antibacterial test (Table 1) suggested that fractions 10 and 14 are most active, and they were designated Rc1 and Rc2, respectively. In the preparative HPLC, Rc1 was present in vials 3 and 4 and eluted at 5–10 min; Rc2 was present in vials 9–11 and eluted at 25–30 min (Fig. 1b, c). Rc1 was then subfractionated into Rs1–3, and fraction Rc2 into Rs4–6 (Supplementary Fig. 1).

Table 1 Inhibition of *B. subtilis* growth in the presence of crude extracts of *R. coriaria* fruit/flower and leaves, and fractions of the leaf extract

Antibacterial agent **0.5 mg/mL**	Fruit/ flower crude extract	Leaf crude extract	Leaf subfraction 10 **(Rc1)**	Leaf subfraction 11	Leaf subfraction 12	Leaf subfraction 13	Leaf subfraction 14 **(Rc2)**
Bacterial growth (%)	60	46	38	49	54	39	1

Antibacterial activity

The antibacterial activity of the crude leaf extract and its fractions Rc1 and Rc2 and subfractions Rs1–6 was determined by MIC assay and disk diffusion test against the bacteria *K. pneumoniae, E. coli, B. subtilis* and *S. aureus*. All fractions showed antibacterial activity, with MIC values ranging from 0.2–3.1 mg/mL, depending on the fraction and on the bacterium (Table 2). Subfraction Rs5 had the strongest antibacterial activity with a MIC of 0.4 mg/mL. The MBC values were in the range of 0.3–6.2 mg/mL depending on the fraction and on the bacterium (Table 2), with subfraction Rs5 again showing the strongest antibacterial activity against the four bacteria tested, with MBC of 0.6–2 mg/mL. Figure 2 shows bacterial viability by TTC test in the presence of different concentrations of subfraction Rs5. All tests were performed with triplicates, at least three times, and the averages are presented.

Table 2 MIC and MBC values (mg/mL) of *R. coriaria* crude leaf extract, fractions Rc1 and Rc2, and subfractions Rs1–6

Antibacterial agents	*Escherichia coli*		*Staphylococcus aureus*		*Bacillus subtilis*	*Klebsiella pneumoniae*
	MIC	MBC	MIC	MBC	MBC	MBC
Crude extract	0.8	2.0	0.2	0.3	1.25	2.5
Rc1	0.8	4.5	2.1	2.0	2.5	5
Rc2	0.4	3.1	0.4	0.6	1.25	2.5
Rs1	1.6	4.5	3.1	3.1	1.25	2.5
Rs2	1.6	3.1	1.6	3.1		
Rs3	1.6	6.2	1.6	1.6		
Rs4	0.8	3.1	0.8	0.8		
Rs5	0.4	2.0	0.4	0.6	0.8	1.6
Rs6	0.8	6.2	0.4	0.4		

LCMS analysis of leaf subfraction Rs5

The analysis was run four times and all four samples showed nearly identical qualitative composition, with the same peaks. Detailed analysis was performed and the identified compounds are presented in Table 3. All of the compounds had a carbon–hydrogen–oxygen composition and some were found in the *R. coriaria* database.

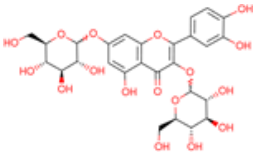
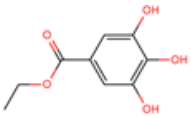
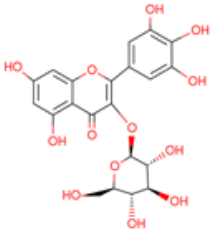
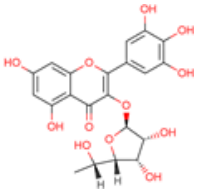
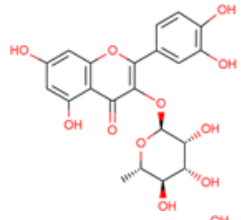
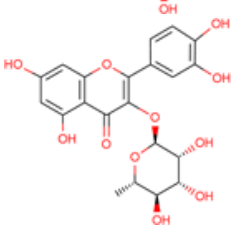
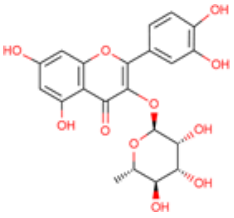
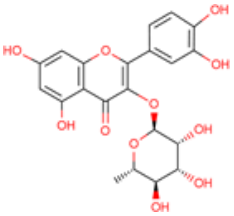
Table 3 Compounds identified in *R. coriaria* subfraction Rs5

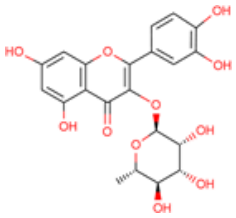
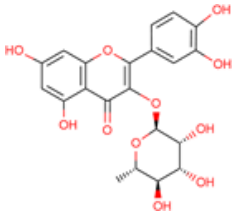
Development of an antibacterial formulation in eye drops

A formulation was developed with eye drops, enabling the use of high concentrations of subfraction Rs5 in low volumes, with a negligible systemic effect. Subfraction Rs5 was added to 1 mL of commercial eye drops which did not contain antibacterial compounds, at a final concentration of 200 mg/mL, and 10 µL were put on a disk. The effect of the eye drops on *E. coli*, *B. subtilis* and *P. aeruginosa*, with and without the addition of subfraction Rs5, was examined. The bacteria were grown on nutrient agar plates. Paper discs were placed on the plate and 10 µL of the eye drop solution, with or without Rs5, was dripped onto the disc. A clear zone of inhibition could be seen around the discs with subfraction Rs5 (Fig. 3).

Inhibition of *Botrytis cinerea* growth in the presence of *R. coriaria* crude leaf extract

The initial formulation was designed to obtain a method for treating pathogenic microorganisms in agriculture. The activity of the prepared formulation was tested on plant tissue for its ability to eliminate the pathogenic fungus *Botrytis cinerea*, which is known to be harmful to agricultural crops, mainly tomato, melon, cucumber, zucchini, and grape vine. We selected grape vine for the tests. The developed formulation, which was prepared for spray application, had to be stable, provide uniform distribution over the plant, have emulsion capacity and cause no secondary damage to the plant (such as burning). None of the formula ingredients at the used doses (i.e., glycerol, water, an emulsifying mixture of mineral oil and dimethyl sulfoxide), either individually or combined, were toxic or damaging to the fungus when added without the leaf extract. In addition, the preparation, with and without *R. coriaria* leaf extract, applied to grape leaf tissue without fungal infection, did not show any leaf damage or burning, and the distribution was uniform. When the formulation with the *R. coriaria* extract was sprayed on the fungus *Botrytis cinerea*, fungal growth inhibition was observed, compared to the control plate on which the fungus grew without the extract (Fig. 4).

	Name	Possible structure	Remarks
1	Quercetin diglucoside ($C_{27}H_{30}O_{17}$)		Not reported from genus *Rhus* (reaxys.com)
2	Ethyl gallate ($C_9H_{10}O_5$)		Reported from *R. coriaria* (reaxys.com)
3	Myricetin glucoside ($C_{21}H_{20}O_{13}$)		Reported from *R. coriaria* (reaxys.com)
4	Myricetin rhamnoside ($C_{21}H_{20}O_{12}$)		Reported from *R. coriaria* (reaxys.com)
5	Quercitrin ($C_{21}H_{20}O_{11}$)	 	Reported from *R. coriaria* (reaxys.com)
6	Ethyl digallate ($C_{16}H_{14}O_9$)		Reported from *Acer rubrum* (reaxys.com)
7	Ethyl digallate ($C_{16}H_{14}O_9$)		Reported from *Arctostaphylos uva-ursi* (reaxys.com)
8	Theaflavin ($C_{16}H_{14}O_9$)		Reported from black tea leaves (reaxys.com)

			
9	Baccatune ($C_{16}H_{14}O_9$)		Reported from *Sapium baccatum* and eucalyptus bark (reaxys.com)
10	Other possible composition could be $C_{26}H_{44}O_6$ with either a sesquiterpene glycoside, or a polyhydroxylated steroid (less likely) structure		Compound with molecular formula $C_{23}H_{48}O_8$
11	Mainly sesquiterpenes and sesquiterpene lactones		Masses of a compound with molecular formula $C_{25}H_{36}O_4$

Discussion

We evaluated and characterized the antibacterial activity of leaves of *R. coriaria*, as well as possible antifungal applications. The antibacterial activity of *R. coriaria* fruit is well known, and many studies have recommended using the fruit extract as an antimicrobial agent in food to extend its shelf life. Because *R. coriaria* leaves are abundant throughout the year, leaf extract may provide an inexpensive and effective antibacterial substance for the food industry.

Antibacterial activity of the crude leaf extract, as well as of its fractions and subfractions—both analytical and preparative—was observed. The antibacterial activity was higher in the fractions and subfractions compared to the crude extract, suggesting enrichment of the active antibacterial compounds during purification. This increase in antibacterial activity confirms the effectiveness of *R. coriaria* as an antibacterial herb and suggests the existence of new bioactive compounds in the fractionated material that could be enriched by further purification. The most active subfraction was Rs5, with MIC values of 0.4–3.1 mg/mL and MBC values of 0.6–2 mg/mL, depending on the bacterium tested. These results are in accordance with other published studies, such as Nimri et al. (22) who found MIC values of 1.95–31.25 mg/mL, and Raodah et al. (29) who reported a range of 0.5–20 mg/mL, depending on the bacterium tested; in the latter study, a higher concentration of the extract was needed for inhibition of gram-negative bacteria. Nostro et al. (26) showed MIC values of 0.78–3.12 mg/mL, and suggested the advantages of using the leaf extract as a natural antibacterial agent for controlling food-related bacterial infections.

The most active subfraction (Rs5) was analyzed by LCMS, revealing compounds with a phenolic skeleton that likely confer the antibacterial activity of *R. coriaria* leaf extract. These compounds were either flavonoids, tannins, or antioxidants. In-vitro antibacterial activity of *R. coriaria* extracts has been previously suggested to be due to the presence of tannins (30). While some of the compounds identified in *R. coriaria* had been reported in the literature (3, 5, 6, 7, 16, 17) and were in the reaxys.com database, we identified the presence of the compound quercetin diglucoside ($C_{27}H_{30}O_{17}$) in *R. coriaria* for the first time. The other major compounds found were: quercitrin, myricetin glucoside, myricetin rhamnoside, ethyl gallate, ethyl digallate, theaflavin, and baccatune. In future work, this subfraction should be further purified to pinpoint the compounds with antibacterial activity.

We developed a formulation for non-systemic use of Sumac leaf extract by humans, where the active fraction was dissolved in eye drops. We also found subfraction Rs5 to be active against the fungal plant pathogen *Botrytis cinerea*, suggesting its potential agricultural application for plant disease protection.

In conclusion, crude extract, fractions and subfractions of *R. coriaria* leaves exhibit strong antibacterial activity. This is the first time that the leaves of *R. coriaria* have been extensively studied for this purpose. Comparing to the crude extract, the antibacterial activity of the fractions and subfractions of the leaf extracts noticeably increased, suggesting enrichment of the active antibacterial compounds following fractionation and subfractionation. Most compounds were either flavonoids, tannins, or antioxidants. Quercetin diglucoside was reported in *R. coriaria* for the first time. Therefore, *R. coriaria* leaves may be a promising ingredient for commercial antibacterial use in the food and agricultural industries.

Declarations

1. **Ethics approval and consent to participate**: “Not applicable”: The study does not involve human participants, nor animals, therefore ne ethics approval is needed.

2. **Consent for publication**: “Not applicable”

3. **Availability of data and materials**: “Data sharing not applicable to this article as no

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5. **Competing interests**: “The authors declare that they have no competing interests.”.

6. **Authors' contributions**:

GJ- Carried out the antibacterial tests, participated in its design and coordination, wrote the manuscript.

HK- Carried out the Purification and all HPLC tests, participated in its design and coordination, proofread, and edited the manuscript.

ER- Carried out the antibacterial tests, eye drop tests, participated in its design and coordination, proofread, and edited the manuscript. NA- Carried out toxicity tests, proofread, and edited the manuscript.

HH- Carried out the antibacterial and antifungal tests and participated in its design and coordination.

IR - Carried out the LCMS tests, proofread, and edited the manuscript.

GM- Carried out the antibacterial and antifungal tests.

MZ- Carried out the Purification and all HPLC tests.

NS- Carried out the Purification and all HPLC tests.

DB- Carried out the antibacterial tests, eye drop tests.

AP- Carried out the LCMS tests.

BF- Conceived of the study, participated in its design and coordination, and helped to draft the manuscript, proofread, and edited the manuscript.

All authors read and approved the final manuscript.

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References

1. Joseph, G., Faran, M., Raskin, I., Lila. M.A., Fridlender B. Medicinal Plants of Israel: A Model Approach to Enable an Efficient, Extensive, and Comprehensive Field Survey. J Biodiversity, Bioprospecting Dev. 2014;01(03):1–8.
2. Sezik E, Tabata M, Yesilada E, Honda G, Goto K, Ikeshiro Y. Traditional medicine in Turkey I. Folk medicine in Northeast Anatolia. J Ethnopharmacol. 1991;35:191–6.
3. Abdul-Jalil TZ. R. coriaria (Sumac): A Magical Spice. In: Herbs and Spices. IntechOpen; 2020. p. 39–52.
4. Gabr S, M E-M, Al-Ghadir A. Antioxidant and Antibacterial Active constituents of Rhus coriaria. Biotechenology [Internet]. 2014 [cited 2021 Jun 19];13(2):37–45. Available from: https://www.researchgate.net/publication/281244555_Antioxidant_and_Antibacterial_Active_constituents_of_Rhus_coriaria
5. Sakhr K, El Khatib S. Physiochemical properties and medicinal, nutritional and industrial applications of Lebanese Sumac (Syrian Sumac - Rhus coriaria): A review. Heliyon [Internet]. 2020;6(1):e03207. Available from: https://doi.org/10.1016/j.heliyon.2020.e03207
6. Abu-Reidah IM, Ali-Shtayeh MS, Jamous RM, Arráez-Román D, Segura-Carretero A. HPLC-DAD-ESI-MS/MS screening of bioactive components from R. coriaria L. (Sumac) fruits. Food Chem. 2015 Jan 1;166:179–91.
7. Regazzoni L, Arlandini E, Garzon D, Santagati NA, Beretta G, Maffei Facino R. A rapid profiling of gallotannins and flavonoids of the aqueous extract of R. coriaria L. by flow injection analysis with high-resolution mass spectrometry assisted with database searching. J Pharm Biomed Anal. 2013 Jan 18;72:202–7.
8. Kossah R, Nsabimana C, Zhang H, Chen W. Optimization of extraction of polyphenols from syrian sumac (R. coriaria L.) and chinese sumac (Rhus Typhina L.) fruits. Res J Phytochem. 2010;4(3):146–53.
9. Kirby CW, Wu T, Tsao R, McCallum JL. Isolation and structural characterization of unusual pyranoanthocyanins and related anthocyanins from Staghorn sumac (Rhus typhina L.) via UPLC–ESI-MS, 1H, 13C, and 2D NMR spectroscopy. Phytochemistry. 2013 Oct 1;94:284–93.
10. Ünver A, Özcan MM. Fatty acid composition of seed and pericarp of sumach (R. coriaria L.) grown wild in different regions of Turkey. J Food, Agric Environ. 2010;8(1):31–3.
11. Singh O, Ali M, Akhtar N. New Antifungal Xanthones from the Seeds of R. coriaria L. Int Res J Pharm. 2011;2(1):188–94.
12. Morshedloo MR, Maggi F, Tavakoli Neko H, Soleimani Aghdam M. Sumac (R. coriaria L.) fruit: Essential oil variability in Iranian populations. Ind Crops Prod. 2018 Jan 1;111:1–7.
13. Farag MA, Fayek NM, Reidah IA. Volatile profiling in R. coriaria fruit (sumac) from three different geographical origins and upon roasting as analyzed via solid-phase microextraction. PeerJ. 2018;5121(7):1–16.
14. Shabana MM, El Sayed AM, Yousif MF, El Sayed AM, Sleem AA. Bioactive constituents from Harpephyllum caffrum Bernh. and R. coriaria L. Pharmacogn Mag. 2011;7(28):298–306.
15. Kizil S, Turk M. Microelement contents and fatty acid compositions of R. coriaria L. and Pistacia terebinthus L. fruits spread commonly in the south eastern Anatolia region of Turkey. Nat Prod Res. 2010;24(1):92–8.
16. Zalacain A, Prodanov M, Carmona M, Alonso GL. Optimisation of extraction and identification of gallotannins from sumac leaves. Biosyst Eng. 2003 Feb 1;84(2):211–6.
17. Elagbar ZA, Shakya AK, Barhoumi LM, Al-Jaber HI. Phytochemical Diversity and Pharmacological Properties of Rhus coriaria. Chem Biodivers. 2020;17(4):2020.
18. Radmehr B, Abdolrahimzade M. Antimicrobial effects of Sumac (R. coriaria L.) extract in minced meat. Planta Med. 2009;75:1068.
19. Mohammed SAS, Anhar AAA, Rana MJ. Antimicrobial activity of Palestinian medicinal plants against acne-inducing bacteria. African J Microbiol Res. 2013;7(21):2560–73.
20. Kırmusaoğlu S, Yurdugul S KE. Effects of fermented sumac on the formation of slime layer of Staphylococcus aureus. Balkan Med J. 2012;29:84–7.

21. Motaharinia Y, Rezaee MA, Hazhir MS, Zandi F, Shapouri R, Hakhamaneshi MS, et al. Evaluation of the antibacterial activity of Zataria multiflora Boiss., R. coriaria L. (sumac), Mentha piperita L., and Ocimum basilicum L. extracts on Brucella strains isolated from brucellosis patients. Turkish J Med Sci. 2012;42(5):816–22.
22. Nimri LF, Meqdam MM, Alkofahi A. Antibacterial Activity of Jordanian Medicinal Plants. Pharm Biol [Internet]. 1999;37(3):196–201. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=iphb20
23. Alwan, B. Ismail, S. Alwan A. Effect of R. coriaria crude extract on inhibition and adhesion of pseudomonas aeruginosa and E. coli clinically isolated from patients infected with acute urinary tract inflammation. Prim Educ Coll J. 2009;60:757–64.
24. Shabbir A. Rhus Coriarialinn, a plant od Medicinal, nutritional and industrial importannce: a review. J Anim Plant Sci. 2012;2(2):505–12.
25. AlJaber G. Antibacterial effect of two phenolic extracts from Rhus coriaria. BRJ. 2008;22:22–32.
26. Nostro A, Guerrini A, Marino A, Tacchini M, Di Giulio M, Grandini A, et al. In vitro activity of plant extracts against biofilm-producing food-related bacteria. Int J Food Microbiol. 2016;238(December):33–9.
27. Rahman M, Kühn I, Rahman M, Olsson-Liljequist B, Möllby R. Evaluation of a Scanner-Assisted Colorimetric MIC Method for Susceptibility Testing of Gram-Negative Fermentative Bacteria. Appl Environ Microbiol. 2004;70(4):2398–403.
28. Naghili H, Tajik H, Mardani K, Razavi Rouhani SM, Ehsani A, Zare P. Validation of drop plate technique for bacterial enumeration by parametric and nonparametric tests. Vet Res forum an Int Q J [Internet]. 2013;4(3):179–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25653794%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4312378
29. Raodah M, Al Ali R, Faleeha HH. The Antioxidant and Antimicrobial of Syrian Sumac (Rhus coriaria) Fruit Extracts. J Nat Sci Res. 2014;4(11):36–40.
30. Rashid TS, Sijam K, Kadir J, Saud HM, Awla HK, Zulperi D HE. Screening for active compounds in R. coriaria L. crude extract that inhibit the growth of Pseudomonas syringae and Ralstonia solanacearum. Indian J Agric Res. 2016;50(1):15–21.

Figures

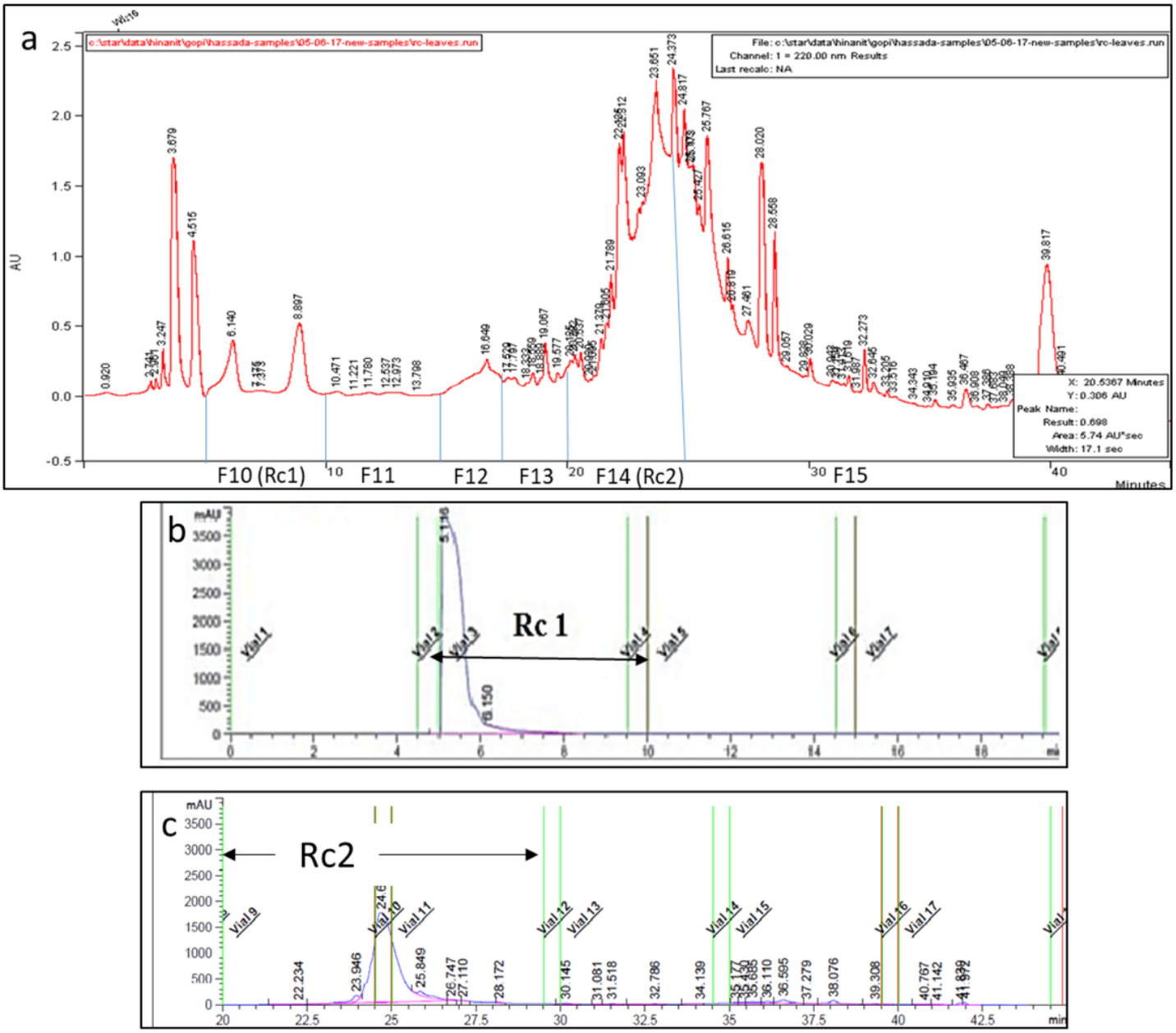

Figure 1

HPLC profile of R. coriaria leaves with the active fractions. (a) Analytical HPLC profile of leaf extract fractions F10–F15. (b) Preparative HPLC fraction Rc1. (c) Preparative HPLC fraction Rc2

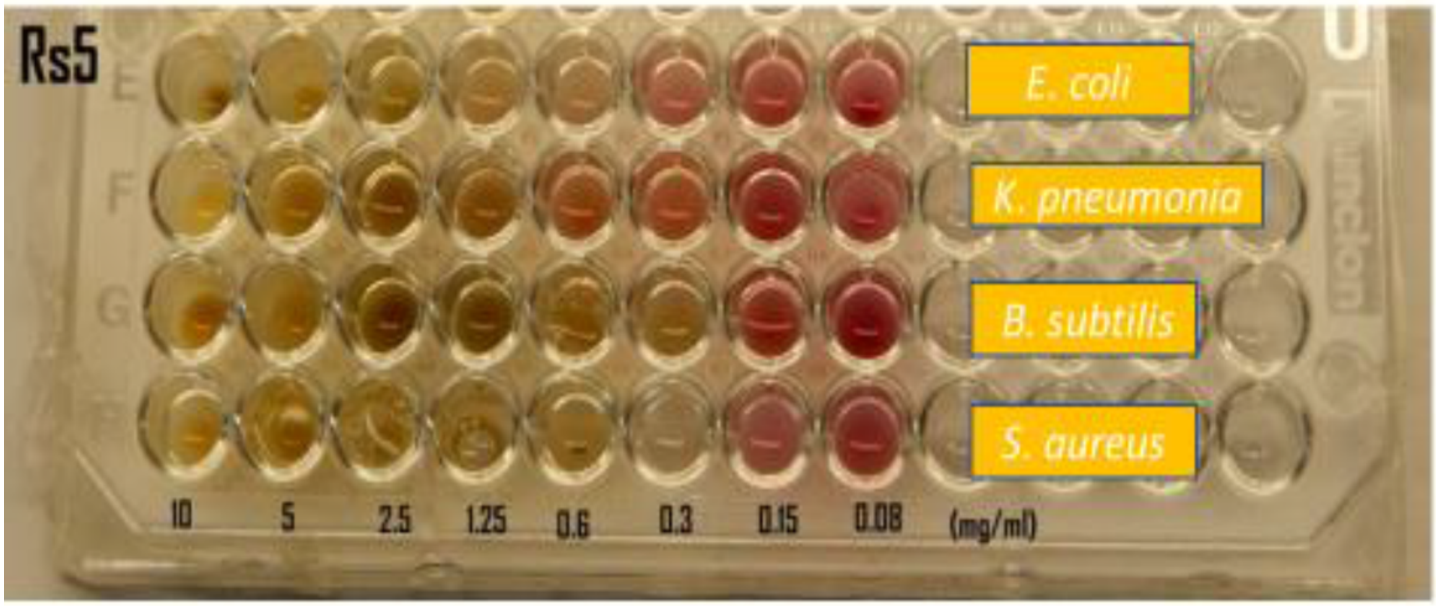

Figure 2

Bacterial viability by TTC test in the presence of different concentrations (0.08–10 mg/mL) of leaf extract subfraction Rs5

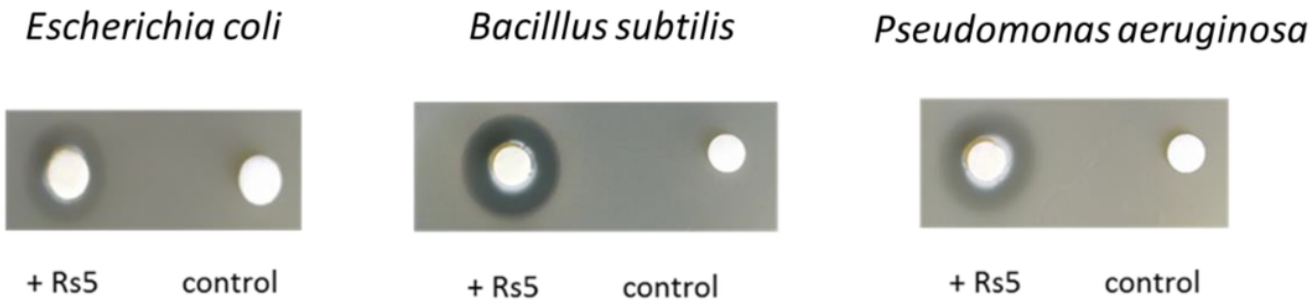

Figure 3

Inhibition of bacterial growth by a commercial eye drop formulation containing subfraction Rs5. Inhibition zone with (+ Rs5) and without (control) subfraction Rs5 (200 mg/mL) by disk diffusion test.

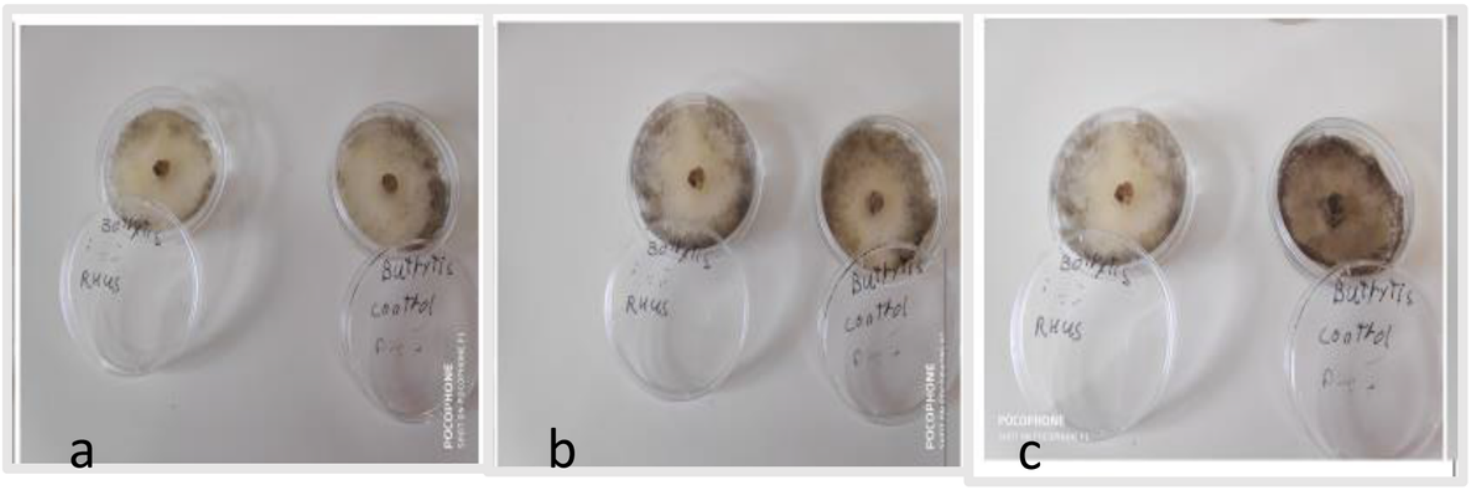

Figure 4

Inhibition of Botrytis cinerea growth in the presence of R. coriaria crude leaf extract. (a) Plates with Botrytis cinerea at time zero; on the left: in the presence of R. coriaria leaf extract, on the right: fungus only (control). (b) Plates with Botrytis cinerea after 24 h of incubation in the presence of leaf extract (left), and alone (right). (c) Plates with Botrytis cinerea after 72 h of incubation in the presence of leaf extract (left), and alone (right)

Supplementary Files

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