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Chloroform-Methanol Extraction Antimicrobial Potential of *Rheum Ribes* Originating from Elazig/Aricak Province

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Highlights:

- R. ribes is known "iskin and is found in the Middle East and Turkey.
- R.ribes has medicinal applications due to important biochemical compounds
- R.ribes,strong antimicrobial properties were detected.

Keywords:

- *R. ribes*
- Antimicrobial activity
- Minimum inhibition concentration
- Probiotic bacteria

ABSTRACT:

Rheum ribes (R. ribes) has a variety of medicinal applications due to the presence of anthraquinone derivatives and other compounds. It was aimed to determine the potential antimicrobial effects on some gram-negative/positive pathogens, and lactic acid bacteria by agar well diffusion test following minimum inhibition concentrations (MIC) with liquid extract samples of *R. ribes*. Growth concentrations of *R. ribes* extract doses (14.17-0,89 mg/L) were applied to indicator microorganisms. MIC method used microbial density values compared to the control group. Result of the Agar well diffusion test, the best antibacterial effects were detected on *L. monocytogenes* and *S. aureus* and following *B. subtilis* (zone diameter of 18.72 and 18.32 mm, respectively). The *R. Ribes* extract showed a higher inhibitor effect than tetracycline antibiotic against *L. monocytogenes*. Similarly, *S. aureus* and *E. faecalis*., *S. paratyphi* A were more affected by *R. Ribes* extract than tetracycline antibiotic. The MIC test result, the highest inhibitory effects of *R. ribes* extract at a concentration of 0,89 mg/L for *S. Paratyphi* A strain, 3.54 mg/L for *K. pneumonia*, and 3.54 mg/L for *E. coli* RSSK 09036 were determined as 50.81%, 60.45%, and 60.40%, respectively. The highest inhibition effects of *R. ribes* at 14.17 mg/L (0.5 dilution concentration) concentration were determined at the rate of 80.12% for *Bacillus clausii* and 96.04% for *B. subtilis*. In the present study, it is thought that the differences between the antimicrobial effect and MIC tests seen in gram-positive, negative and probiotic bacteria may be related to the surface tension effect of the extract.

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INTRODUCTION

Natural products can be isolated from various plant species. Plants are important in the production of phytocomplexes, which have therapeutic characteristics that can be used to treat or prevent health issues (Kavaz et al., 2022). These products have unique structures and functions such as anti-obesity, anti-cancer, anti-diabetes, anti-hypertension, anti-inflammatory, antioxidant, antimicrobial and anti-Alzheimer's. Moreover, they can be used as a dye or cream formulation in the cosmetic industry and as a drug formulations in the pharmaceutical industry (Bati et al., 2020; Gundogdu et al., 2019). According World Health Organization (WHO) report, the number of aromatic plants applied for therapeutic purposes is around 20 000 on average, and they have also been used to add features such as taste, smell, and color to foods since human history (Reichling et al., 2009; Anand et al., 2021). Since they are used as raw materials in many fields such as the cosmetics, pharmaceutical and food industry, medicinal plants and their specific essential oils have been studied in many research areas, especially since 1940, in terms of their antimicrobial effects and important results have been achieved (Faydaoglu and Surucuoglu, 2013).

Bacterial infections and resistance to antibiotics have led to health problems with the emergence and spread of difficult, uncontrolled clinical symptoms with traditional drugs worldwide (Mayyas et al., 2021). Many infectious agents, which can be successfully treated with any of the few drugs, have gained resistance to almost all these drugs in some cases (McEwen and Collignon, 2018). It has been reported in many studies that plants have antimicrobial effects due to their ability to synthesize aromatic substances, most of which are phenols or oxygen-derived compounds (Sakkas and Papadopoulou, 2017). The investigation of medical plants used in the complementary treatment of multiple drug-resistant bacteria has recently become important as it is appropriate, accessible and acceptable by the local population. According to the WHO report, medical plants can potentially meet the needs of communities and improve access to basic health services safe, quality and culturally (WHO, 2019). This can make significant contributions to basic health services in the prevention and management of infectious diseases caused by drug-resistant bacteria (Gadisa and Tadesse, 2021).

R. ribes belongs to the Polygonaceae family. It has been widely used as a food source, medicinal and complementary medicine since ancient times in Turkey and the world (Bati et al., 2020). In many studies, The roots of *R. ribes* include compounds like s-chrysophanol, rhein, aloe emodin-8-O-glucoside, fizcion, aloeemodin, physcion-8-O-glucoside, sennoside A, rhaponticin, and emodin as well as traditionally treat urinary tract infections, diabetes, stomach ulcers, nausea, vomiting, haemorrhoid, measles, and expectoration (Ozturk et al., 2007; Yildirim et al., 2020; Keshavarzi et al., 2021). *R. ribes* has a variety of medicinal applications due to the presence of anthraquinone derivatives and other compounds. *R. ribes* is known in Turkey as “iskin, usgun or uckun” and is found in the Middle East and the highlands of Turkey. Many plant species grown in Turkey are used to reduce or eliminate the symptoms of various diseases (Cinar-Ayan et al., 2021). In this study, *R. ribes* samples were collected from the vicinity of Saman Village in the Aricak district of Elazig province. It was aimed to determine the potential antimicrobial effects on some gram-negative/positive pathogens, and lactic acid bacteria by agar well diffusion test following MIC test with liquid extract samples of *R. ribes*.

MATERIALS AND METHODS

R. ribes Plant Extraction

R. ribes plant was collected from Saman Town, Aricak County, Elazig/Turkey, coordinates as 38°33'08.6"N–40°05'39.8"E. The collected *R. ribes* was immediately taken to –80°C cold chain, until analysis was done. The area where the plant was collected and the images of the plant are given in

Figure 1. 150 grams of *R. ribes* samples were dried in an oven at 60 °C for 24 hours. It was then ground in an IKA brand mill. 100 grams of ground plant samples were taken and 500 mL of Methanol Chloroform mixture (1:1 v/v) was added and kept in a desiccator for three days. The extract obtained after evaporation at 35 °C was prepared with 10 ppm distilled water (Tufekci et al., 2018).

Figure 1. Turkey / Elazig / Aricak / Saman town images by *R. ribes*

Microorganisms and Media

The antimicrobial effect of *R. ribes* was investigated against indicator microorganisms were obtained biotechnology laboratory of Seafood Faculty at Cukurova University. The microorganisms were Gram-negative (*Salmonella Parathypi* A NCTC13, *E. coli RSSK 09036, Klebsiella pneumonia ATCC700603*) and Gram-positive (*Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* ATCC7677 *Enterococcus faecalis* ATCC29212, *Bacillus subtilis* B-354, MRSA (Methicillin-Resistant *S. aureus,* Honey isolate), *Lactobacillus reuteri ATCC 55730, Streptococcus agalactiae (human ısolate) and Bacillus clausii (enterogermina).*

All of the indicator microorganisms were subcultured in their particular medium overnight before study. MRS Broth (*Lactobacillus* Broth acc. to De Man, Rogosa and Sharp: MRS, Merck 1.10661**,** MRS Agar (*Lactobacillus* Agar acc. to De Man, Rogosa and Sharpe) Merck 1.10660 were used for growth of lactic acid bacteria, Tryptic Soy Broth (TSB) Merck 1.05459) for pathogenic microorganisms, Mueller Hinton Agar Merck 1.05437 for agar test, Mueller Hinton Broth (Merck 1.10293) for MIC test.

Antibacterial Assays

Agar well diffusion assay

Agar well diffusion test, similar to the procedure used in disk-diffusion method, analyzed the antibacterial activity of *R. ribes*. Indicator bacteria were subcultured overnight in tryptic soy and MRS broth at 37 °C. 90 mm diameter of Mueller–Hinton agar plates were inoculated with a standardized inoculum of the test microorganism, corresponding to 0.5 McFarland turbidity standard, and left to dry at room temperature for 30 min. Then, in the dried plate was punctured aseptically with a sterile cork borer to create a 6 mm-diameter hole, and 100 µL of plant extract was added (18.89 mg/L) solution into the wells of each plate. The test microorganism is then placed on an appropriate agar plate, and the

incubation process is continued. The studied microbial strain's development is impeded by the antimicrobial agent dispersed in the agar medium (Magaldi et al., 2004; Valgas et al., 2007). The diameters of the inhibitory zones were measured in millimetres by a digital calliper (Mitutoyo 500-181-30, 0-150 mm). The procedure was adopted by the Clinical and Laboratory Standards Authority (CLSI, former NCCLS,) as a consensus standard (CLSI 2006, 2018a; EUCAST 2019b).

MIC test

MICs obtained with the spectrophotometric microdilution method for *R. ribes* against indicator microorganisms were measured at 600 nm in a 96-well plate reader (Multiskan™ FC Microplate Photometer thermofisher.com). 150 µL of dual-strength MHB and 150 µL of Dual-strength *R. ribes* extract were added to the first wells. The range of antimicrobial compound concentrations was performed in twofold dilution as serial dilutions. Then each prepared well was inoculated with 15 µL standardized microbial cultures adjusted to 0.5 McFarland scale. Control wells were not added *R. ribes* extract. After well-mixing, the inoculated 96-well microtitration plate was incubated (without agitation) under suitable conditions for 18-24 hours depending upon each test microorganism group then, sample density was detected by plate reader. Then, MIC results were calculated as mg/ml (CLSI, 2012). MIC was expressed as the highest dilution inhibiting growth (turbidity max in well is low) and among these MIC dilutions, those with positive detection of nonviable cells > 99% in the medium was considered as MBC (Lalitha, 2004).

RESULTS AND DISCUSSION

Agar Well Diffusion Assay

Gram-positive bacteria

The effects of *R. ribes* extracts on gram-positive bacteria are shown in Table-1. The zone diameter interpretation procedure adopted in France is used for semi-quantitative *in vitro* susceptibility testing of certain pathogenic bacteria by the agar disk-diffusion test procedure (Bauer et. al., 1966; CLSI, 2006). In table 1, Zone Diameter Control Zone Interpretive Standards (mm) for Neomycin ≤12, 13 – 16,≥17 and for tetracyline ≤14, 15 – 18, ≥19 were reported as Resistant - Intermediate-Susceptible respectively. As seen in Table 1, the best antibacterial effects were detected on *L. monocytogenes* and *S. aureus* and following *B. subtilis* (zone diameter of 18.72 and 18.32 mm, respectively). The *R. Ribes* extract showed a higher inhibitory effect than tetracycline antibiotic against *L. monocytogenes*. Similarly, *S. aureus* and *E. faecalis* were more affected by *R. ribes* extract than tetracycline antibiotic. The *R. ribes* showed an inhibition zone on other positive microorganisms, MRSA and *S. agalactiae. B. clausii,* and *Lactobacillus reuteri* for 15.26, 9.75 mm zone diameters, respectively. *R. ribes* extract was found to have an effect on all gram-positive bacteria tested in the research group. In fact, suppression of the probiotic group is not desired, but the synergy of natural products on probiotics should be perceived positively. Table 1. Both probiotic as *B. clausii,* and *Lb. reuteri* were not affected by standard antibiotics but were affected by ribes.

Gram-negative bacteria

In this study, the antibacterial effect of *R. ribes* extract on gram-negative bacteria was determined, as shown in Table-1; the most effect on *S. Parathypi* A (19.65 mm zone diameter) It appeared to be sensitive when compared to tetracycline, followed by *E. coli* and *K. pneumonia* (11.42 and 11.15 mm zone diameter, respectively) they appeared to be resistant to *R. ribes* extract when compared to tetracycline and neomycin antibiotics. *R. ribes* extract showed an effect on all three species of gram-negative bacteria tested in the research group.

Table 1. Antimicrobial zone diameters of *R. ribes* extract on indicator microorganisms. Agar well diffusion assay, and supplemental Tables CLSI (CLSI 2018a) procedural standards were taken as reference in the results

		Inhibitor Zone diameter (mm)		
Microorganism	Species	*R. ribes*	TE-30	NE-30
Gram-positive	*Staph. aureus* ATCC 29213	18.32	15.36	20.32
	Bacillus subtilis B 354	18.23	22.31	12.00
	MRSA(Methicillin Resistant Staph)	17.52	20.21	NE
	Enterococcus faecalis(ATCC29212	15.33	9.53	20.00
	Streptococcus agalactiae (human isolate	11.25	22.34	NE
	Listeria monocytogenes (ATCC7677)	18.72	15.76	20.12
Gram- negative	*Salmonella parathypi* A NCTC13	19.65	21.39	NE
	E.coli RSSK 09036	11.42	12.63	22.00
	Klebsiella pneumonia (ATCC700603)	11.15	12.56	20.00
Probiotic	*Bacillus clausii (enterogermina)*	15.26	NE	NE
	Lactobacillus reuteri ATCC 55730	9.75	NE	NE

*NE: No Effected

MIC Test

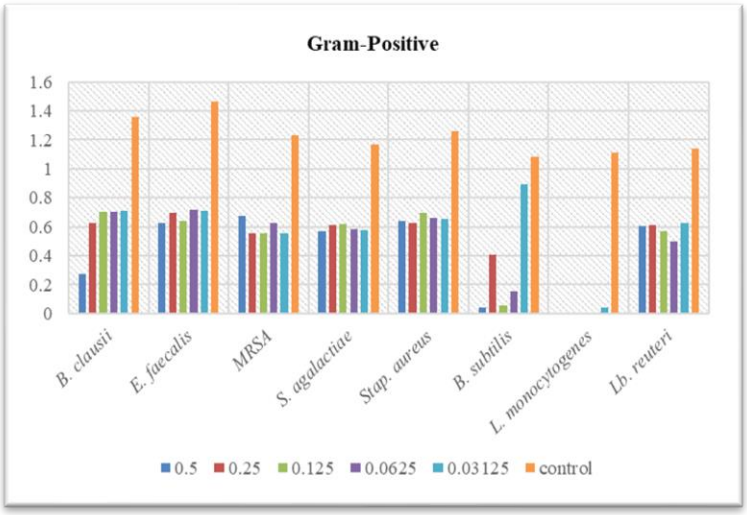
Gram-positive microroganisms

The growth concentrations of *R. ribes* doses applied to Gram-positive bacteria at 600 nm compared to the control group were given in Figure 1. The highest inhibition effects of *R. ribes* at 14.17 mg/L (0.5 dilution concentration) concentration were determined at the rate of 80.12% for *B.clausii* and 96.04% for *B. subtilis*. The inhibition effect was determined as 57.42% for *E. faecalis* strain at 14.17 mg/L concentration. MRSA had 55.05% at 3.54 mg/L concentration while *S. agalactiae* species had 51.20% at 14.17 mg/L concentration. *R. ribes* extract showed the highest inhibition effect on *S. aureus* at 7.71 mg/L concentration of 50.35%, and *L. monocytogenes* at 1.78 mg/L concentration of 99.28%. *L. reuteri* was found to have a maximum inhibitory effect of 56.40% at a concentration of 1.78 mg/L.

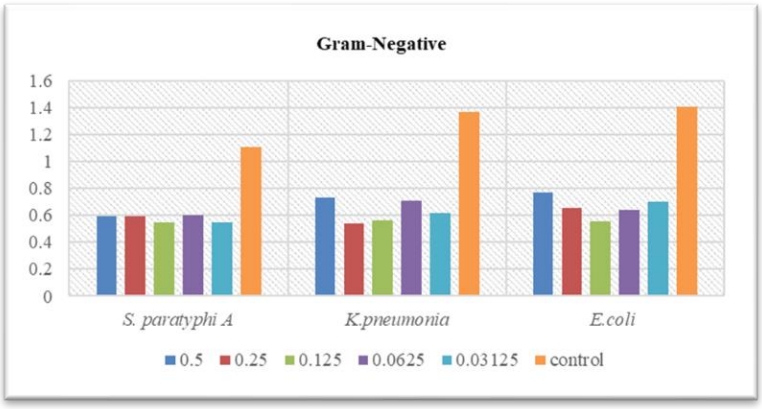
Gram-negative microorganisms

The growth densities of *R. ribes* doses applied to Gram-negative bacteria at 600 nm compared to the control group are given in Figure 2. The highest inhibitory effects of *R. ribes* extract at a concentration of 0.89 mg/L for *S. Paratyphi* A strain, 3.54 mg/L for *K. pneumonia* and 3.54 mg/L for *E. coli* RSSK 09036 were determined as 50.81%, 60.45% and 60.40%, respectively.

The antibacterial, antiallergenic, antioxidant, antithrombotic, and anti-inflammatory effects of phenolic chemicals on plants are significant (Al-Khayri et al., 2022). The presence of anthracene compounds in the subsurface sections of rheum species makes them important therapeutic plants. One of the most vital raw pharmaceuticals in the Middle East comes from the Polygonaceae plant *R. ribes* It is locally known as “iskin or ucgun”, is one of the most important raw drug sources, mostly grown in some of the Middle Eastern countries (e.g. Lebanon and Iran) and eastern Turkey. For example, due to the presence of anthracene derivatives occurring in the underground parts of the plant, it is also used as an antipsoriatic and oriental laxative drug in some countries (Ozturk et al., 2007). It is used in the treatment of assorted diseases such as hypertension, diabetes and ulcers, as well as traditionally consumed as a digestive and helminth preventative (Cinar-Ayan et al., 2021). However, there is little scientific or clinical evidence about the biological activity of similar herbal extracts.



(A)



(B)

Figure 2. Growth concentrations of *R. ribes* extract doses (14.17 - 0.89 mg/L) applied to indicator microorganisms by spectrophotometric (at 600 nm) method compared to the control group. 0.5, 0.25, 0.125, 0.0625, 0.03125 dilutions were 14.17, 7.71, 3.54, 1.78, 0.89 mg/L (Panel A): gram-positive microorganisms, (Panel B): gram-negative microorganisms

In this study, antimicrobial effects of the extract obtained from *R. ribes* plant collected from Elazig/Aricak Saman Village on pathogenic gram-positive/negative specific strains and some probiotic bacteria were investigated. The maximum inhibition concentration of bacteria showing antimicrobial activity was determined. According to the results obtained by the agar well diffusion method in our study, the highest zone spacing was seen in *L. monocytogenes* (18.72 mm) in gram-positive bacteria and *Salmonella parathypi* (19.65 mm) in gram-negative bacteria. In addition, other gram-positive bacteria**s**, *S. aureus*, *Bacillus subtilis*, MRSA, *E. faecalis*, *B. clausii S. agalactiae* and *Lb. reuteri* strains showed antimicrobial activity in the 18.32–9.75 mm zone range, while gram-negatives showed antimicrobial activity in the zone range of 11.4–11.15 mm in *E. coli* RSSK and *K. pneumonia*. As a result of the MIC tests, the extract on gram-positives; It was determined that *Bacillus clausii*, *Bacillus subtilis* and *E. faecalis* showed the highest inhibition rate of 80.12% and 96.04%, 57.42%, respectively. MRSA strain 55.05%, *S. agalactiae* 51.20%, *S. aureus* 50.35%, *L. monocytogenes* 99.28% showed maximum inhibition effect. The highest inhibition effect was determined in *Lb. reuteri* probiotic strain with a value of 56.40%. However, it was observed that *R. ribes* extract had a maximum inhibition effect of 50.81% for *S. Paratyphi* A, 60.45% for *Klebsiella pneumonia*, and 60.40% for *E. coli* RSSK 09036 on gram-negatives. In this present study, it is thought that the differences between the antimicrobial effect and MIC tests seen in gram-positive/negative bacteria may be related to the

surface tension effect of the extract (concentrate-dilution). In the analysis performed on *R. ribes* extracts collected from the HajeOmaran mountains in Iraq, it showed antimicrobial effect in parallel with our study. However, while the most effective bacteria in their study was *E. coli*, when compared with the current study, it was determined that the bacteria with the most effective antimicrobial effect in our study showed the highest antimicrobial effect on *S. parathypi* and then on *L. Monocytogenes* (Alaaddin et al., 2007). It can be estimated that this is due to the growth of *R. ribes* species in different locations and indirectly from the amount of polyphenols and chemical components.

Keser et al. (2020), in their research on the antimicrobial and anticancer properties of methanol/ ethanol/water *R. ribes* extracts showed antimicrobial effect on *C. albicans E. coli, L. monocytogenes, K. pneumonia, S. aureus, P. vulgaris, B. subtilis, B. megaterium,* bacterias and were effective on PC-3, A2780, HCT-116 and MCF-7, cancer cell lines (Keser et al., 2020). In an article by Meydan et al. in 2022, they investigated the lipid peroxidation, DNA damage prevention activities, antimicrobial, antidiabetic and of the ZnO Nano Particles/*R. ribes* complex, which is created when the *R. ribes* plant interacts chemically with ZnO. They stated that the complex developed as a result of the study prevented DNA damage and lipid peroxidation compared to the control groups. In addition, they determined that ZnO NanoParticles/*R.ribes* complex formed zones varying between 21 ± 4.5 and 8 ± 3.0 against gram-negative and gram-positive microorganisms. They stated that the strongest antibacterial effect of the complex formed with *R. ribes* was on *B. subtilis* ATCC 6633 strain (21 ± 4.5). They also found that *B. cereus* (8 ± 4.0), *S. aureus* (14 ± 1.0), *E. faecalis* (9 ± 1.0) and *E. coli* (9 ± 2.0) had antimicrobial effects (Meydan et al., 2022).

Abelmoschus esculentus (okra) plant flower extract showed the maximum effect on *S. aureus*, similar to the results obtained in our study, and showed a strong antimicrobial effect on *E. coli* and *S. typhimurium* bacteria (Kavaz Yuksel et al., 2022). *In vitro* study examining the antimicrobial effect of *Lavandula angustofilia* plant extract, its effects on gram-positive-negative bacteria were investigated, and results similar to our study were obtained (Ozdemir et al., 2022). In yet another *in vivo* study, DNA damage, MDA levels, antioxidant, and biochemical parameters in rat brain tissues, with experimental obesity induced with a high-calorie diet, were therapeutically effected by *R. ribes* plant root extracts (Bati et al., 2020).

A study with Alzheimer's rats, hydro-alcoholic extract (obtained from *R. ribes*) improved cognitive functions by eliminating memory deficits due to bilateral NBM lesions (Zahedi et al., 2015). Raafat et al. (2021) stated in an *in vivo* study that the *R. ribes* extract they applied to alloxan-induced diabetic mice showed protective properties against diabetic neuropathy, one of the most important diabetes-related complications (Raafat et al., 2021). As a result of, due to the compounds they contain, many plants can demonstrate antimicrobial action in foods and living things. Depending on the microbial strain, polyphenols can demonstrate highly varied antibacterial activity against bacteria that cause food deterioration and foodborne pathogens. A specific strain of Gram-positive (or Gram-negative) bacteria can be affected by the same polyphenols (Kavaz Yuksel et al., 2021a,b). The data obtained show that *R. ribes* extract should be supported by many *in vivo* models and clinical studies and the possible synergistic effects of its combinations with different chemical agents or antibiotics should be investigated.

CONCLUSION

According to the results obtained from this study, it was determined that the *R. ribes* extract showed a significant antimicrobial effect on the specific gram-positive/negative bacterial strains investigated, and also gave activating properties to some probiotic bacteria. It is estimated that

consuming such foods is protective against many diseases of microbial origin, since the side effects of herbal extracts are minimal. It has been observed that *R. ribes* species can be used as a sedative, curative and/or symptomatic in the field of complementary medicine due to its many health-beneficial properties. In order to better understand the antimicrobial properties of *R. ribes*, it is thought that this study will be supported by different plant combinations and may contribute to scientific data with *in vivo* and *in vitro* studies.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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