



**TITLE:** Antimicrobial activity of sour cherry

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## Antimicrobial Activity of Sour Cherry

**KEYWORDS:** *sour cherry, chemical composition, antimicrobial, juice*

**ABSTRACT:** *Sour cherry is a plant traditionally used as food. Its health care potency has been discovered recently. In this study, we have investigated the antimicrobial activity of sour cherry towards different pathogens by micro dilution method according to Clinical and Laboratory Standards Institute (CLSI).. The chemical composition of dried juice and ethanol extract was determined by High performance liquid chromatography method. Results showed that juice and extract exhibit antibacterial activity, but have no antifungal and antialgal activity against tested pathogens. In terms of break point, better results were obtained against Gram positive bacteria. *Rhodococcus equi* was the most susceptible specie to both juice and extract. Juice showed better results to: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Sallmonella Typhymurium* and *Acinetobacter lwoffii*, but for all other investigated species extract showed superior activity.*

### INTRODUCTION

The modern trends in nutrition suggest the limitation of synthetic foods or the substitution with natural ones (1). Sour cherries (*Prunus cerasus* L.) represent fruit that is commonly used in human nutrition. Fruits of sour cherry are rich in nutritional ingredients. Those ingredients have many beneficial effects to human health, such as antioxidant, anti-inflammatory, antiviral and antiproliferative to cancer cells (2-4). Cherry fruits have a big potential as tasteful, harmless and healthy antimicrobial agents. A growing resistance to conventional antimicrobial drugs among humans□, domestic and wild animals□ (5) pathogens obligates us to use them rationally (6, 7) and to find new harmless antimicrobial substances that can be mixed into foods and feeds. The aim of this study was to evaluate antimicrobial activity of sour cherry extract and juice on pathogenic zoonotic species. All of them could be spread among humans and animals in various paths, including the transmission by food and feed (8-11).

### MATERIALS AND METHODS

#### Plant material

Sour cherry fruits were purchased from individual producers from Novi Sad, Serbia and processed fresh.

Juice was obtained by squeezing cherry fruits through the sterile gauze and then evaporated to dryness on rotatory-vapor. In order to evaluate antimicrobial activity of fruits' remains, we have separately investigated juice and extract obtained from pomace. Pomace (300 g) was extracted at the room temperature on a shaker device (3500 rpm) two times: the first time for 30 minutes with 300 ml of solution and the second time for 15 minutes with 150 ml of solution. Solution was a mixture containing 80% ethanol, 19.95% sterile water and 0.05% acetic acid. The obtained extracts were combined and evaporated to dryness into the rotatory-vapor.

### Chemical analysis

Identification and quantification of bioactive compounds was carried out by HPLC analysis in a Shimadzu Prominence (Shimadzu, Kyoto, Japan) chromatographic system, which consisted of LC-20AT binary pump, CTO-20A thermostat and SIL-20A autosampler connected to the SPD-20AV UV/Vis detector (Shimadzu, Kyoto, Japan).

### Microorganisms

All investigated micro-organisms are important human and animal pathogens that can, among other ways, be transmitted by food and feed. In order to investigate both Gram positive and Gram negative bacterial, fungal and algal human and animal relevant pathogens we have selected species presented in Table 1.

Gram positive bacteria									
<i>Staphylococcus aureus</i> (Sa)	<i>Staphylococcus aureus</i> ATCC 11632 (Sa ATCC)*	<i>Streptococcus agalactiae</i> (Sag)	<i>Rhodococcus equi</i> (Re)	<i>Rhodococcus equi</i> ATCC 6939 (Re ATCC)*	<i>Trueperella pyogenes</i> (Tp)	<i>Arcanobacterium haemolyticum</i> (Ah)	<i>Enterococcus</i> sp. (E)		
Gram negative bacteria								Fungus	Alga
<i>Pseudomonas aeruginosa</i> (Pa)	<i>Pseudomonas aeruginosa</i> ATCC 10145 (Pa ATCC)*	<i>Acinetobacter lwoffii</i> (A)	<i>Escherichia coli</i> (Ec)	<i>Salmonella Enteritidis</i> (Se)	<i>Salmonella Typhimurium</i> ATCC 14028 (St ATCC)*	<i>Klebsiella pneumoniae</i> (K)	<i>Candida albicans</i> (Ca)	<i>Prototheca wickerhamii</i> (Pw)	

Table 1. Microorganisms tested in our study (\*-referent (American Type Culture Collection [ATCC]) strain)

### Antimicrobial susceptibility testing

Concentrations of juice and extract were from 100 mg/ml to 0.78 mg/ml, by using twofold dilution. Antimicrobial effects were determined by micro-dilution broth method by the 2012 CLSI Guidelines (12).

### Statistical analysis

Based on experimental results, using the Excel software package (Microsoft Office 2007), graphs were generated to show the percentage of surviving bacteria depending on the corresponding concentrations of the tested substances. The function, which was used to approximate the experimental results, was determined using the Trendline supplement from the Microsoft Excel program. An antimicrobial activity is expressed as following effects: break point-no bacterial growth, minimal bactericidal concentration (MBC)-99.9%, minimal inhibitory concentrations (MIC99, 90 and 80)-99%, 90% and 80% of bacteria introduced to wells have been killed respectively.

## RESULTS AND DISCUSSION

### Chemical analysis

Results of chemical analysis are shown in Tables 2. and 3.

Substance	Epicatchine	Proto catechin	Catechin	Rutin	Myricetin	Luteolin	Quercetin	Isorhamnetin	Kaempferol	Anthocyanins
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	Acid									
Extract	13.53±0.35	80.83±2.45	51.82±1.91	27.46±0.09	11.97±0.12	0.58±0.00	3.85±0.13	2.08±0.01	20.85±1.76	533.79±22.95
Juice	61.58±2.09	11.24±0.26	32.63±1.55	-	-	-	-	-	108.11±2.12	92.07±3.03

Table 2. Polyphenolic compounds in cherry extract and juice (mg/100 g dry matter)

Substance	Ferulic acid	Sinapinic acid	p-hydroxybenzoic acid	Vanillic acid	Caffeinc acid	Ellagic acid	Chlorogenic acid	Coumaric acid	Gentisic acid	Vitamin C	Galic acid
Extract	3.04±0.12	3.71±0.13	12.18±0.49	0.99±0.04	5.12±0.18	1.79±0.09	293.57±11.78	16.56±0.62	93.82±3.19	6.85±0.25	21.76±0.73
Juice	1.95±0.08	-	11.12±0.52	1.07±0.05	5.64±0.22	0.46±0.03	250.11±8.55	25.81±1.09	46.68±1.83	7.18±0.29	32.88±1.24

Table 3. Organic acids in cherry extract and juice (mg/100 g dry matter)

Our results, showed tree times more anthocyanins (625.86 mg/100g extract plus juice) than Majiene et al. (156-220 mg/100g fruits) (13).

Kołodziejczyk et al. investigated three fractions of sour cherry extract, obtained in successive extraction steps, and named them extract first, second and third. Our content of chlorogenic acid, quercetin and kaempferol is higher than in the first extract gained by Kołodziejczyk et al., but lower than the second and the third. The amount of isorhamnetin in our extract is higher, but the amount of the total anthocyanins is lower than in each of their three extracts (14).

Most of determined compounds are proved as antibacterial agents (15-22). Compounds that are present in the biggest amount in investigated extract and juice are anthocyanins. Antibacterial activity of fruits with antocyanins is well described (23-25). Also, Leitao et al., Lacombe et al. and Caillet et al. described antibacterial activity of anthocyanin fractions from cranberry juice (26-28). Thus, we believe they are responsible for antibacterial effect of a sour cherry.

#### Antimicrobial activity

The results are shown in Figures 1. and 2. The lowest value of extract that exhibited MIC80 effect was 0.00005 mg/ml (*A. haemolyticum*), and the highest value for the break point effect was 21.13 mg/ml (*S. Typhymurium*). The results obtained for juice are more heterogenic, 0.000121 mg/ml (*S. aureus* ATCC 11632) MIC 80 effect, and 25.91 mg/ml (*Enterococcus* sp.) for the break point. Sour cherry extract had a stronger effect than juice against all tested bacteria, except *S. aureus*, *S. aureus* ATCC 11632, *P. aeruginosa*, *S. Typhymurium* and *A. lwoffii* (Figures 1. and 2.). Extract had a stronger activity than streptomycin against *A. haemolyticum*, *R. equi* ATCC 6939, *Enterococcus* sp. and *S. agalactiae*. Both extract and juice were superior to streptomycin against *T. pyogenes* and *R. equi* (Figure 3.). The stronger activity of streptomycin against other tested bacteria is understandable, considering it is a conventional antibiotic. Antibiotics as well as synthetic substances exhibit a much higher antibacterial effect than natural products, but they also exhibit side effects (29). No antifungal and anti-algal activity was found. The similar results were obtained in our previous studies on raspberry (30) and blackberry (31).

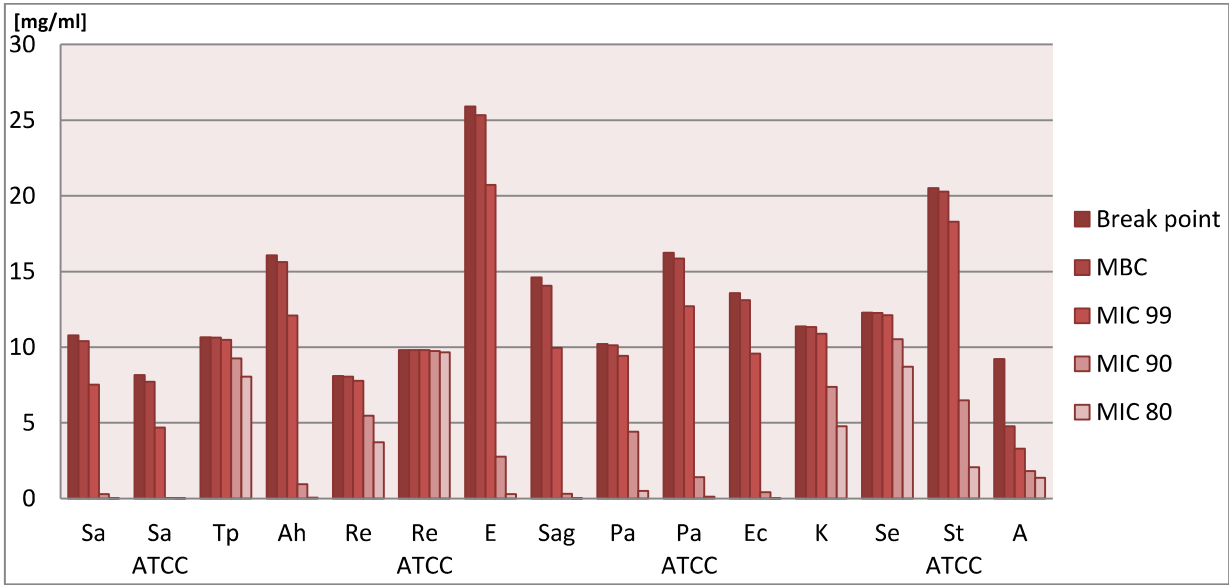


Figure 1. Antimicrobial activity of cherry juice <sup>1</sup>

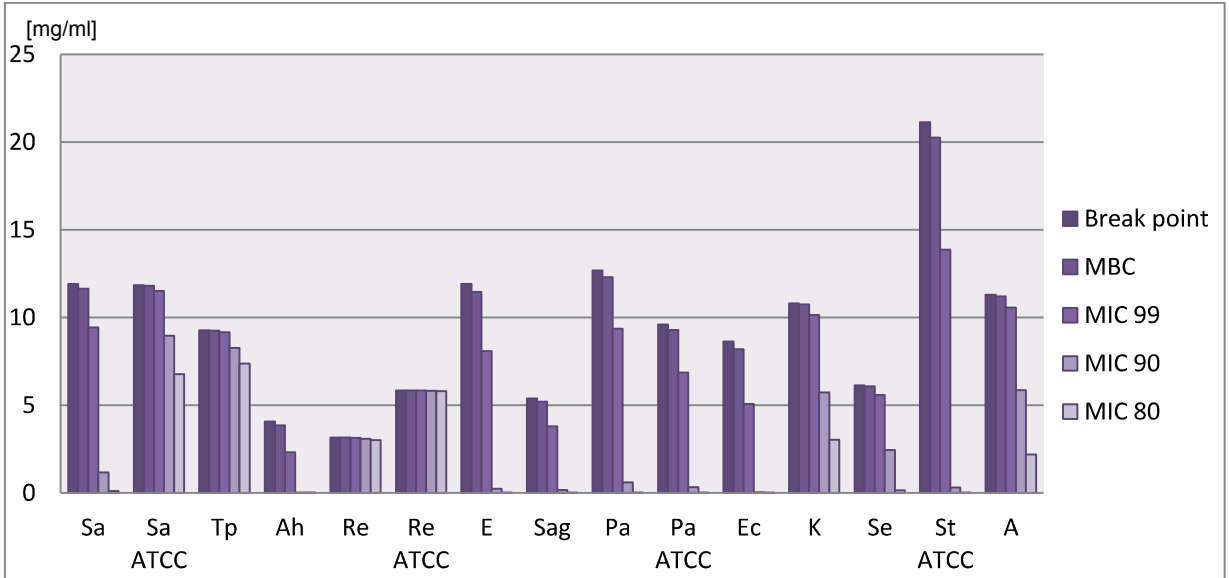


Figure 2. Antimicrobial activity of cherry extract

<sup>1</sup> Break point is the lowest concentration of investigated substance that kills all microorganisms inoculated in well. MBC is the lowest concentration of investigated substance that kills 99.9% of microorganisms inoculated in well. MIC 99, 90 and 80 are the lowest concentrations of investigated substance that kill 99%, 90% and 80% of microorganisms inoculated in well, respectively.

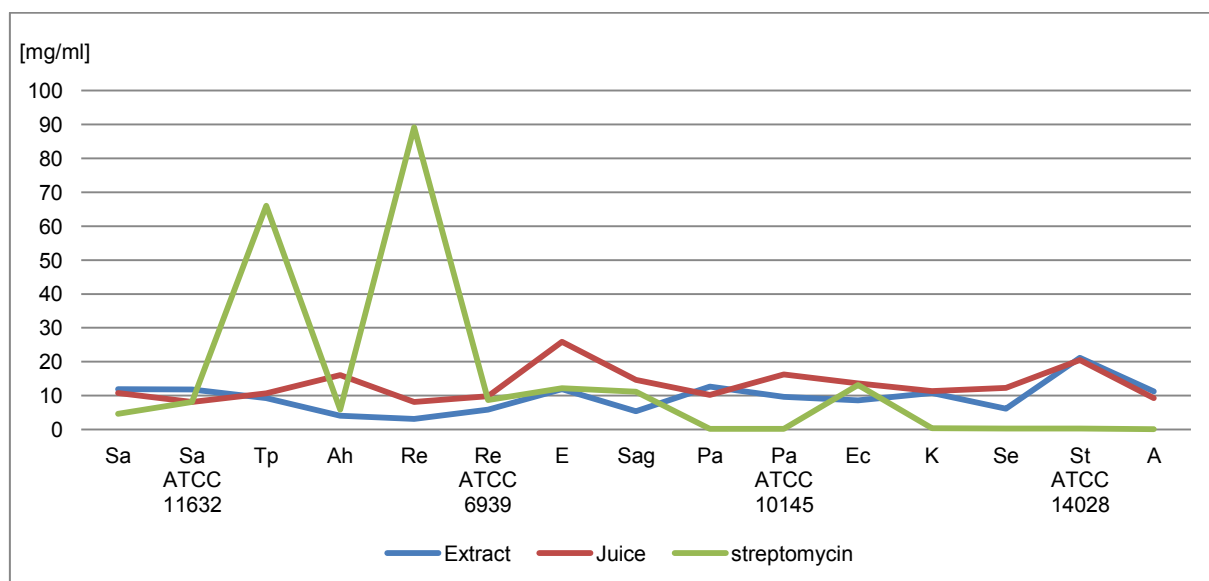


Figure 3. Break points of extract and juice in comparison to streptomycin

Coccia et al. assumed that the bactericidal effect was expressed only at concentrations higher than double MICs: 8.4 mg/ml for *S. aureus*, 8.8 mg/ml for *Acinetobacter* sp. and *P. aeruginosa* and 13.2 mg/ml for *E. coli* and *Enterobacter* sp. The results obtained for MBC values are in accordance with their assumptions for *Acinetobacter* sp. 11.21 mg/ml, *P. aeruginosa* 12.30 mg/ml and *S. aureus* 11.64 mg/ml and even better than their assumptions for *E. coli* 8.19 mg/ml and *Enterobacter* sp. 11.46 mg/ml. We also found that some concentrations of extract and juice exhibit a beneficial effect on bacterial growth. Our study as well as theirs showed no antifungal activity (32).

The results in presented study show that cherry extract caused MBC, MIC99, MIC90 and MIC80 effect in lower concentrations than juice on *E. coli* and *S. Typhymurium*. This coincides with the findings of Kirsch et al., but they used sour cherry methanol extract and water extract. The findings obtained for fungal strain (*C. albicans*) are in accordance with the study performed by Kirsch et al. who also did not find antifungal activity (33).

Kolodziejczyk et al. investigated the activity of cherry pomace ethanol extracts against *Salmonella Choleraesuis* and *Escherichia coli* O157:H7 and showed reduction of bacteria number at doses higher than 2,500 µg/ml. They noticed that extracts showed the bactericidal activity at concentrations higher than 10,000 µg/ml (14). The results obtained in our research are better, owing that MIC99 concentrations for *S. Enteritidis* and *E. coli* were 5.60 mg/ml and 5.08, while MBC values was 6.07 mg/ml and 8.19 mg/ml, respectively.

## CONCLUSIONS

The lowest values that exhibited MIC80 effect were 0.00005 mg/ml (*A. haemolyticum*) for extract and 0.000121 mg/ml (*S. aureus* ATCC 11632) for juice. *Rhodococcus equi* was the most susceptible strain in terms of break point against both extract (3.15 mg/ml) and juice (8.08 mg/ml). Based on obtained results, it can be reported that cherry fruits investigated in this paper, showed antibacterial activity to a wide range of relevant pathogens.

## ACKNOWLEDGEMENTS

Supported by the Ministry of Education and Science, Republic of Serbia, Grants 46012 and 41012.

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