

BIOACTIVE POTENTIAL OF SPENT *Rosa damascena* Mill. PETALS EXTRACT: ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES

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Abstract

The rose petal from *Rosa damascena* Mill. are often associated as a source of compounds with antioxidant activity and antimicrobial properties. The rose oil-bearing industry is generating enormous quantities of by-products with a potential to become part of the circular economy. Therefore, we aimed to produce an extract from the spent rose petals (SRPE) by-water: ethanol (70:30) extraction, followed by concentration and freeze drying. The phenolic profile evaluated by HPLC showed a presence of Gallic, Protocatechuic, p-Coumaric acids and Rutin. Also, the organic acid such as Ascorbic, Malic, Tartaric and Oxalic were found. The obtained extract exhibited a strong antioxidant activity evaluated by the radical scavenging activity against DPPH and ABTS radicals, metal chelating capacity against transition metal and oxygen and hydroxyl radical absorbance capacity (ORAC and HORAC). In addition to antioxidant activity, the extract manifested an antimicrobial activity against most of the pathogens responsible for food poisoning. The minimum inhibitory concentrations of rose extract for yoghurt and probiotic starter cultures were evaluated.

Key words: by-product, rose extract, antioxidant activity, MIC yoghurt starter culture, MIC probiotic starter culture.

INTRODUCTION

The growing consumer demand for natural bioactive compounds in food and pharmaceuticals leads to an increased research into plant-derived antioxidants and antimicrobials. *Rosa damascena* Mill., commonly known as the Damask rose, is widely cultivated for its essential oils and medicinal properties (Mahboubi, 2016). Beyond its traditional uses, *Rosa damascena* is a rich source of phenolic compounds, flavonoids, and organic acids, which have demonstrated significant antioxidant, anti-inflammatory, and antimicrobial activities (Dimitrova et al., 2017; Dinkova et al., 2022; Petkova et al., 2020).

Despite the high-value essential oil extracted from the petals, the rose oil industry generates large quantities of spent rose petals (SRP) as by-products, which remain largely underutilized (Boroski et al., 2021). Given the presence of bioactive compounds in SRP, there is a strong incentive to re-use these by-products in the circular economy by extracting their valuable secondary metabolites for potential food, pharmaceutical, and nutraceutical applications (Mohammadpour et al., 2023). Recent studies

confirmed that *R. damascena* petals contain a variety of bioactive compounds, including phenolic acids (Gallic acid, Protocatechuic acid, p-Coumaric acid), flavonoids (Rutin, Quercetin), and organic acids (ascorbic acid, malic acid, tartaric acid, oxalic acid) (Charoimek et al., 2024; Dragoev et al., 2021). These compounds are known for their antioxidant properties, which are primarily attributed to their radical-scavenging capacity, metal ion chelation, and inhibition of lipid peroxidation (Bora et al., 2020).

The antioxidant activity of *R. damascena* extracts has been previously evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), oxygen radical absorbance capacity (ORAC), and hydroxyl radical absorbance capacity (HORAC) assays, confirming its potential as a natural antioxidant in food preservation (Shang et al., 2019).

In addition to antioxidant activity, *R. damascena* extracts exhibit broad-spectrum antimicrobial properties against Gram-positive and Gram-negative bacteria, as well as fungi (Dimitrova & Ivanov, 2020; Mahmood et al., 2020). The antimicrobial activity is primarily attributed to

phenolic acids and flavonoids, which can disrupt microbial membranes, inhibit enzymatic activity, and interfere with bacterial quorum sensing (Almasoud et al., 2023).

Several studies reported the antibacterial efficacy of *R. damascena* against foodborne pathogens, including *Staphylococcus aureus* (MIC = 0.28 mg/mL), *Listeria monocytogenes* (MIC = 0.37 mg/mL) and *Escherichia coli* (MIC = 0.42 mg/mL) (Sadeghi et al., 2021).

However, limited research explored its effects on beneficial lactic acid bacteria (LAB), which are essential for yoghurt and probiotic dairy fermentations. It is crucial to determine the minimum inhibitory concentration (MIC) of *R. damascena* extracts on LAB such as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, ensuring that its antimicrobial activity does not compromise yoghurt fermentation and probiotic viability (Dimitrova et al., 2019; Ahmad et al., 2022).

Given the increasing interest in natural preservatives and functional ingredients, this study aims to characterize bioactive compounds from spent rose petals (SRP), to evaluate the antioxidant activity, to investigate the antimicrobial property of the extract against common foodborne pathogens (*S. aureus*, *L. monocytogenes*, *E. coli*) and determine the minimum inhibitory concentration (MIC) of the extract on yoghurt and probiotic starter cultures. The findings from this research will provide valuable insights into the potential of *R. damascena* petal extracts as natural preservatives, supporting the development of functional dairy products with enhanced shelf life and health benefits.

MATERIALS AND METHODS

Materials

The lyophilized spent *Rosa damascena* Mill. petals extract (SRPE) was prepared by the procedure described by Vlahova-Vangelova et al. (2025). Reconstituted Dry Skim Milk (RDSM), with 9-10% dry matter and 0.1% fat content, was prepared and sterilised by heating at 121°C for 10 min.

Starter cultures

Direct Vat Set (DVS) yoghurt starter culture YF-3331 (Chr. Hansen) containing

Lactobacillus delbrueckii subsp. *bulgaricus* and *Streptococcus thermophilus* strains, and probiotic yoghurt culture ABY-3 (Chr. Hansen) containing *Bifidobacterium* species, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* strains were used.

Test microorganisms

Fifteen microorganism strains, including five Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus cereus* NCTC 11145, *Staphylococcus aureus* ATCC 6538P, *Listeria monocytogenes* NBIMCC 8632, *Micrococcus luteus* 2YC_YT), five Gram-negative bacteria (*Salmonella enteritidis* ATCC 13076, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380, *Pseudomonas aeruginosa* ATCC 9027), yeasts *Candida albicans* NBIMCC 74, and four fungi (*Aspergillus niger* ATCC 1015, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Fusarium moniliforme* ATCC 38932) from the collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria, were selected for the antimicrobial activity test.

Culture media

Luria-Bertani Agar Medium with Glucose (LBG Agar). LBG agar was used for cultivation of the test bacteria. A quantity of 50 g of LBG-solid substance mixture (containing 10 g tryptone, 5 g yeast extract, 10 g NaCl, 10 g glucose and 15 g agar) was dissolved in 1 L of deionized water, pH 7.5±0.2.

Malt Extract Agar (MEA). MEA was used for cultivation of the test yeasts and fungi. A quantity of 50 g of the MEA-solid substance mixture (containing 30 g malt extract, 5 g mycological peptone and 15 g agar) was dissolved in 1 L of deionized water, pH 5.4±0.2. The culture media were prepared according to the manufacturer's instructions (Scharlab SL, Barcelona, Spain) and autoclaved at 121°C for 20 min before use.

Methods

Sample preparation

The SRPE is dissolved in distilled water in proper concentration according to each of the following assays.

Total Polyphenol Content (TPC)

Total polyphenols were determined according to the method of Singleton and Rossi (1965) with

some modifications from Dinkova et al. (2022) using the Folin-Ciocalteu's reagent. Gallic acid was employed as a calibration standard and the results were expressed in mg Gallic acid equivalents (GAE) per 100 g.

HPLC Determination of Phenolic Compounds

HPLC analyses were performed on an UHPLC system Nexera-i LC2040C Plus (Shimadzu Corporation, Kyoto, Japan) with a UV-VIS detector and a binary pump. The column was Poroshell 120 EC-C18 (3 mm×100 mm, 2.7 µm), thermostated at 26°C. The flow rate was 0.3 mL/min and the injection volume was 5 µL. The derivatives were detected at $\lambda = 280$ nm. The mobile phase consisted of A: 0.5% acetic acid and B: 100% acetonitrile. The gradient condition started with 14% (B), between 6 and 30 min, linearly increased to 25% (B), and then to 50% (B) at 40 min. The identification of compounds was confirmed by a comparison of retention times utilizing standard solutions and standard calibration curves of different phenolic compounds.

HPLC Determination of Free Sugars

The analysis of free sugars was performed on a ZORBAX Carbohydrate (5 µm, 4.6 × 150 mm, Agilent) and a ZORBAX Reliance Cartridge guard column. An UHPLC system Nexera-i LC2040C Plus (Shimadzu Corporation, Kyoto, Japan) with a binary pump and a 20 Å refractive index detector. Ten microliters of the extract were eluted at a flow rate of 0.6 mL/min at 30°C with a mobile phase composed of a mixture of acetonitrile and water (80:20 v/v). The concentration of sugars, detected by their retention time, in the sample was obtained using a calibration curve built by plotting the peak area against the concentrations of each standard.

HPLC Determination of Organic Acids

HPLC determination of organic acids was performed on an UHPLC system Nexera-i LC2040C Plus (Shimadzu Corporation, Kyoto, Japan) with a UV-VIS detector and a binary pump. Separation was performed on a Shimadzu Shim-pack GIST C18 (5 µm, 4.6×250 mm), Japan at 25°C. Twenty microliters of the extract, were injected and eluted (1.0 mL/min) isocratically with a 25 mM solution of K₂HPO₄ in water, whose pH was finely adjusted to 2.4 with H₃PO₄. The UV detector was set at 210 nm. The concentration of each organic acid in the

sample was calculated using a calibration curve obtained by using five different concentrations for each acid. The peak corresponding to different acids was confirmed by a comparison of the retention time with that of the standards.

Antioxidant Activity

Determination of radical scavenging capacity.

The 1,1-diphenyl-2-picrylhydrazil radical (DPPH•) was used according to the method of Brand-Williams et al. (1995), with modifications from Dinkova et al. (2014). The radical scavenging capacity against the 2,2-azinobis 3-ethylbenzthiazoline-6-sulfonic acid radical (ABTS•⁺) was evaluated following the method of Shopska et al. (2022). Both radical scavenging capacities are presented as percent of inhibition by the SRPE solution - 100 mg/L.

Determination of transition metals-chelating activity.

The ferric (Fe³⁺) reduction ability of plasma - FRAP assay was performed following the method of Benzie and Strain (1996) with modifications from Dinkova et al. (2014).

The cupric (Cu²⁺) reducing antioxidant capacity - CUPRAC test is done by method of Shopska et al. (2022).

Oxygen radical absorbance capacity (ORAC)

activity was measured on a microplate reader FLUOstar OPTIMA (BMG Labtech, Ortenberg, Germany) with excitation at $\lambda = 485$ nm and emission at $\lambda = 520$ nm, according to the method of Ou et al. (2001) with some modifications by Denev et al. (2010). Trolox was used for building the standard curve and results were expressed in micromole Trolox equivalents (µmol TE) per gram.

Hydroxyl radical averting capacity

(HORAC) activity was determined with excitation at $\lambda = 485$ nm and emission at $\lambda = 520$ nm, according to Ou et al. (2002). Gallic acid was used for the standard curve and results were expressed in micromole gallic acid equivalents (µmol GAE) per gram.

Antimicrobial activity assay and Minimum inhibitory concentration (MIC)

The antimicrobial activity and minimum inhibitory concentration (MIC) of SRPE determined according to the methods previously described by Tumbarski et al. (2024).

Determination of minimum inhibitory concentration (MIC) and maximum concentration without inhibitory effect (MCWI)

The MIC and MCWI of the polyphenol extract on a DVS starter culture were determined by inoculating and coagulation of reconstituted dry milk (RDSM). The test samples were divided into two batches. The first batch was heated to 43°C and inoculated with YF-3331 starter culture according to the manufacturer’s instructions (Chr. Hansen). The inoculated milk was distributed into eight test tubes. A solution of the polyphenol extract was prepared by dissolving lyophilized polyphenol extract in 10 mL of RDSM to obtain a final extract concentration of 10 mg/mL in milk (Tube 1). Serial dilutions were prepared from this stock solution as follows: Tube 1 was stirred and 5 ml were withdrawn and transferred to Tube 2. This serial transference was repeated until Tube 7. Tube 8 (containing only milk + starter culture) was used as a control. All tubes were then incubated at 43°C until coagulation of milk.

The second batch was heated to 37°C and inoculated with ABY-3 starter culture according to the manufacturer’s instructions (Chr. Hansen). The procedure was the same as for the first batch. All tubes were incubated at 37°C until milk coagulation.

The maximum concentration of polyphenols without inhibitory effect (MCWI) was determined as the highest concentration of extract in which the growth of the lactic acid bacteria was not inhibited (i.e., milk coagulation still occurred).

Statistical analysis

Descriptive statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) software (IMB, version 29.0, 2022).

RESULTS AND DISCUSSIONS

The concentration of total phenolic compounds (TPC) and the individual compounds are presented in Table 1. Our results for TPC confirm previously reported (Slavov et al., 2020; Vlahova-Vangelova et al., 2025). As previously reported by Slavov et al. (2020), Dragoev et al. (2021) and Charoimek et al. (2024) we also evaluated the presence of Gallic, Protocatechuic, p-Coumaric acids and Rutin.

Table 1. Phenolic composition of the SRPE

	TPC, mg GAE/g	Gallic acid, mg/g	Protocatechuic acid, mg/g	p-Coumaric acid, mg/g	Rutin, mg/g
SRPE	292.28 ±9.92	9.49 ±0.25	1.68 ±0.19	0.05 ±0.01	1.44 ±0.01

The SRPE contained small quantities of arabinose and fructose as presence of the natural sugars (Table 2). Slavov et al. (2017) reported a different quantity of both of the sugar depending on the extraction method.

Table 2. Neutral sugars of the SRPE

	Arabinose, g/100 g	Fructose, g/100 g
SRPE	4.7±0.11	5.0±0.15

In the solution of SRPE were found different organic acids such as oxalic, tartaric, malic and ascorbic (Table 3). Those results confirm what was reported by Halawani (2014).

Table 3. Organic acids composition of the SRPE

	Oxalic acid, mg/g	Tartaric acid, mg/g	Malic acid, mg/g	Ascorbic acid, mg/g
SRPE	4.43 ±0.04	51.40 ±1.73	24.63 ±0.06	11.54 ±0.10

The SRPE solution exhibited a strong radical scavenging activity against the DPPH• and ABTS•⁺ (Figure 1).

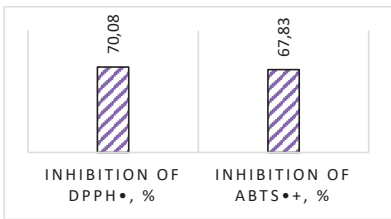


Figure 1. Radical scavenging activity of SRPE

The solution - 100 mg/L reduced up to 70% of the DPPH• radicals. Dina et al. (2021) also studied different extracts from spent *Rosa damascena* petals and their inhibitory activity was similar in the exact same concentration. Meanwhile, the inhibition of the ABTS•⁺ radical was around 68%. Similar lower radical scavenging activity against the ABTS•⁺ compared to the DPPH• was reported by others (Dudonne et al., 2009; Gulcin and Alwasel,

2023; Vlahova-Vangelova et al., 2025). In the meantime, Charoimek et al. (2024) report a strong inhibitory potential against both DPPH• and ABTS•⁺ free radicals. Furthermore, measuring at different wavelengths can either introduce or eliminate colour interference from the sample (Aleixandre-Tudo et al., 2017). Our findings support previous assertions that relying on a single methodology to assess antioxidant activity can sometimes lead to misleading results (Gulcin and Alwasel, 2023; Önder, 2023).

Table 4. Antioxidant activity of the SRPE

	FRAP, mM TE/g	CUPRAC, mM TE/g	ORAC, mM TE/g	HORAC, mM GAE/g
SRPE	4022.66 ±0.98	1858.33 ±3.41	4583.20 ±2.83	2479.40 ±3.22

The solution of SRPE exhibited strong iron-reducing potential - FRAP (Table 4). Our results are similar to reported by Slavov et al. (2020), Vlahova-Vangelova et al. (2025) and Dragoev et al. (2021).

The evaluated cupric (Cu²⁺) reducing antioxidant capacity - CUPRAC of SRPE was significantly lower compared to the FRAP, yet still sufficient. Others are reporting that the extract from spent rose petals posses a strong reduction ability against cupric ions (Petkova et al., 2020; Vlahova-Vangelova et al., 2025). In the meantime, FRAP and CUPRAC values were higher that those reported by Petkova et al. (2021) for microwave-assisted extraction.

Oxygen radical absorbance capacity (ORAC) of SRPE was significant (Table 4) and comparable to the presented by others (Dudonne et al., 2009; Slavov et al., 2017 and 2020). Meanwhile the hydroxyl radical averting capacity (HORAC) was 2479.40±3.22 mM GAE/g. Our results are comparable with the reported antioxidant activity of ethanolic extracts of waste rose flowers by Slavov et al. (2017) and the microwave-assisted extracts of Petkova et al. (2021).

All of the found phenolic compounds are well-known for their antioxidant activity against free radicals like DPPH• and ABTS•⁺ (Figure 1), also chelation of transition metals as ferric and cupric ions (Table 4), confirmed by our results. The results from the antimicrobial activity screening (Table 5) demonstrated that the SRPE

possessed the most significant inhibitory effect on *M. luteus* 2YC_YT and *C. albicans* NBIMCC 74 (MIC = 0.313 mg/mL), followed by *L. monocytogenes* NBIMCC 8632 and *P. vulgaris* ATCC 6380 (MIC = 0.625 mg/mL). The inhibitory activity against *B. subtilis* ATCC 6633, *B. cereus* NCTC 11145, *S. aureus* ATCC 6538P, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9027, and fungi *F. oxysporum* and *P. chrysogenum* was moderate to low, with MIC values > 1.25 mg/mL. The SRPE did not inhibit the growth of test microorganisms *S. enteritidis* ATCC 13076, *K. pneumoniae* ATCC 13883, and fungi *A. niger* ATCC 1015 and *F. moniliforme* ATCC 38932. The obtained results revealed the promising antimicrobial potential of the studied rose extract for further application as a valuable ingredient in food products and cosmetic formulations.

Table 5. Antimicrobial activity and MIC values of SRPE

Test microorganisms	MIC, mg/mL
<i>B. subtilis</i> ATCC 6633	5
<i>B. cereus</i> NCTC 11145	5
<i>S. aureus</i> ATCC 6538P	10
<i>L. monocytogenes</i> NBIMCC 8632	0.625
<i>M. luteus</i> 2YC_YT	0.313
<i>E. coli</i> ATCC 25922	5
<i>P. vulgaris</i> ATCC 6380	0.625
<i>P. aeruginosa</i> ATCC 9027	5
<i>C. albicans</i> NBIMCC 74	0.313
<i>F. oxysporum</i>	1.25
<i>P. chrysogenum</i>	2.5

As sources of biologically active compounds with antimicrobial effect, *R. damascena* extracts have been investigated in numerous studies. Chroho et al. (2022) examined the biological activity of *R. damascena* from Morocco and stated that the hydroethanolic flower extract exhibited an antimicrobial effect on *E. coli* (MIC = 20.83 mg/mL), *S. typhimurium* (MIC = 41.66 mg/mL), *S. aureus* (MIC = 20.83 mg/mL), and *L. monocytogenes* (MIC = 20.83 mg/mL), witch values were lower as compared to ours. These results confirmed that the *R. damascena* flowers might be used as natural antimicrobial agents in the cosmetic and pharmaceutical sectors. In a study conducted by Trendafilova et al. (2023), the extract from *R. damascena* flowers from Bulgaria, obtained by aqueous-ethanolic

extraction and its phenolic-enriched fraction, obtained by re-extraction with ethyl acetate, exhibited significant inhibitory activity against *Propionibacterium acnes*, *Staphylococcus epidermidis* ATCC 12228 and *S. aureus* ATCC 25923, and lower inhibitory activity against *B. cereus* ATCC 11778, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27583; however in contrast to our results, both extracts did not inhibit the yeasts *C. albicans* ATCC10231. Jafari-Sales et al. (2020) investigated a methanolic extract obtained from petals of *R. damascena* from Iran. The authors observed that the extract was more effective against Gram-positive bacteria than Gram-negative ones, with a diameter of the inhibition zones against *S. aureus* of 26.2 mm and *B. cereus* of 24.6 mm at the extract's concentration of 400 mg/mL. MIC values of the methanolic extract were 6.25 mg/mL (*S. aureus*), 12.5 mg/mL (*B. cereus*), 50 mg/mL (*E. coli*), and 100 mg/mL (*P. aeruginosa*), respectively. Considering the antimicrobial effect of the studied *R. damascena* extract, the authors concluded that it can be used as an alternative to conventional chemical drugs in the treatment of the infections caused by these microorganisms.

The antimicrobial potential of spent *Rosa damascena* petals extract (SRPE) was evaluated against Direct Vat Set (DVS) starter cultures YF-3331 and ABY-3 (Table 6), which are commonly used in yoghurt production. The minimum inhibitory concentration (MIC) and maximum concentration of extract without inhibitory effect (MCWI) were determined to assess the suitability of incorporating SRPE into yoghurt formulations without negative impact on fermentation.

Table 6. Antimicrobial activity of SRPE on DVS starter culture

SRPE, mg/mL	Starter culture	
	YF-3331	ABY-3
10	-	-
5	-	-
2.5	-	-
1.25	-	-
0.625	-	-
0.313	+	-
0.156	+	+
0 (control)	+	+

- Non coagulation; + Coagulation.

The MIC values represent the lowest concentration of SRPE that completely inhibits the growth of the starter cultures. In this study, the MIC for YF-3331 was found to be 0.625 mg/mL, while for ABY-3, the MIC was 0.313 mg/mL (Table 7). This suggests that ABY-3 was more sensitive to the antimicrobial effect of SRPE, which could be attributed to the higher polyphenolic content in the extract (Table 1), particularly compounds such as Gallic acid, Protocatechuic acid, and p-Coumaric acid (Shang et al., 2019). These compounds are known to interfere with bacterial cell membranes and inhibit enzyme functions, leading to antimicrobial activity (Bora et al., 2020).

Table 7. Minimum inhibitory concentration (MIC) and maximum concentration of polyphenols without inhibitory effect (MCWI)

Starter culture	MIC	MCWI
YF-3331	0.625 mg/mL	0.313 mg/mL
ABY-3	0.313 mg/mL	0.156 mg/mL

The MCWI refers to the highest concentration of SRPE that can be included in the fermentation process without inhibiting the growth of LAB. For the YF-3331 culture, the MCWI was found to be 0.313 mg/mL, and for ABY-3, it was 0.156 mg/mL. These data are in agreement with the results obtained from other authors (Dimitrova et al., 2019). The findings in the present study indicate that higher concentrations of SRPE could inhibit the starter cultures and affect yoghurt fermentation, particularly with the ABY-3 culture. As polyphenols are known for their antioxidant activity, it is possible that their oxidative stress on microbial cells could contribute to the observed antimicrobial effects (Mahmood et al., 2020).

CONCLUSIONS

The spent *Rosa damascena* petals extract (SRPE) exhibited strong antioxidant activity, effectively scavenging DPPH• and ABTS•+ radicals, with notable FRAP and CUPRAC values. The presence of phenolic compounds like Gallic acid and Rutin, along with organic acids such as ascorbic acid, contributed to its antioxidant and metal-chelating properties. These findings confirm SRPE as a potent natural antioxidant, with potential applications in foods.

The antimicrobial activity of SRPE is a promising characteristic for its use as a natural preservative in yogurt production, helping to extend shelf life and control pathogen contamination. However, for successful incorporation, SRPE must be carefully dosed to ensure probiotic viability and maintain fermentation efficiency. These findings confirm SRPE as a potent natural antioxidant, with potential applications in foods.

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