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EFFECT OF SELECTED PLANT SPECIES ON THE ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF JANJ CHEESE

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ABSTRACT

This study examined the effect of adding selected plant species on the antioxidant and antimicrobial properties of Janj cheese. Twelve cheese samples were prepared in two batches, each including one control sample and five samples enriched with oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), parsley (*Petroselinum crispum*), rosemary *(Rosmarinus officinalis*), and chives (*Allium schoenoprasum*). The first batch contained 0.50%, while the second batch had 1.00% concentrations of the plants. Total phenols, flavonoids, and non-flavonoid compounds were measured. Antioxidant activity was assessed through FRAP, DPPH, and ABTS assays. Antimicrobial activity was evaluated using the agar dilution method against four bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus*) and two *Candida albicans* strains. The results revealed a statistically significant increase ($p<0.05$) in antioxidant activity in the plant-enriched cheese samples, emphasizing their potential as natural sources of antioxidants. However, none of the cheeses showed antimicrobial activity under the tested conditions. In conclusion, the incorporation of these selected plant species improves the biological and functional properties of Janj cheese, supporting its potential as a nutritionally enhanced product.

Keywords: plant species, Janj cheese, antioxidant properties, antimicrobial properties.

INTRODUCTION

Cheese is a dairy product obtained by coagulating milk and separating whey. It can be made from various types of milk or dairy ingredients and is a rich source of high-quality proteins, essential amino acids, calcium, and fat-soluble vitamins [1], [2], [3], [4].

In both the dairy industry and gastronomy, traditional cheeses are valued for their use of local raw materials and artisanal production methods passed down through generations. These products preserve authentic sensory characteristics and microbial diversity, thereby supporting rural communities and local economies [5]. Typically produced from raw milk and natural starters, traditional cheeses develop complex microbial communities that differ markedly from those found in industrially produced cheeses [6]. Beyond their sensory appeal, they may provide functional benefits due to the presence of beneficial microbes and bioactive compounds with antioxidant and antimicrobial activities [5], [7]. Owing to their authenticity and geographical origin, traditional cheeses are often protected by geographical indications and quality labels, which enhance their market visibility and promote sustainable production practices [5], [8].

Among the traditional cheeses of Bosnia and Herzegovina, *Janj cheese* is particularly notable for its distinctive flavor and traditional handmade production. This cheese originates from the mountainous region along the Janj River, in western Bosnia (Šipovo municipality, Republika Srpska). It is primarily made from cow's milk, either raw or mildly heat-treated, without the addition of commercial starter cultures, allowing the development of a natural microflora that contributes to the unique sensory attributes of the cheese [9].

Many plants are rich in secondary metabolites such as phenolic compounds, flavonoids, terpenoids, alkaloids, and essential oils, which exhibit significant biological activities and have wide-ranging applications in nutrition and health. Phenolic compounds, in particular, are recognized for their strong antioxidant potential [10], while terpenoids and essential oils function as natural antimicrobial and antioxidant agents [11], [12]. Alkaloids also demonstrate antimicrobial activity, especially against antibiotic-resistant bacteria [13]. These bioactive compounds are increasingly used as natural preservatives in the food, cosmetic, and pharmaceutical industries, offering safer alternatives to synthetic additives [14], [15], [16], [17].

Enriching cheese with selected plants can enhance its antioxidant and antimicrobial properties, thereby extending shelf life and improving sensory characteristics [18], [19]. Plants such as oregano, rosemary, and garlic act as natural preservatives, reducing the need for synthetic additives [11], [14], [20]. Such functionally enriched cheeses preserve the authenticity of traditional products while providing added health benefits to consumers [2], [3], [5]. Antioxidants neutralize free radicals and reactive oxygen species, thereby preventing oxidative stress linked to chronic diseases [21]. Plants such as rosemary, oregano, thyme, and basil are rich in phenolic compounds that confer potent antioxidant protection [12]. Rosemary extract, for instance, exhibits high free radical scavenging capacity [22], while oregano and thyme, and basil are effective in preventing lipid oxidation in foods [18], [23].

Additionally, plant extracts exert antimicrobial effects through active compounds such as carvacrol and thymol from oregano and thyme, which disrupt bacterial membranes and cause cell death [11], [24]. Allicin, the major bioactive compound in garlic, exerts a bactericidal effect by inducing oxidative stress and interfering with protein synthesis in bacterial cells [20]. Owing to these properties, plant extracts are being extensively studied as natural preservatives and potential ingredients in functional foods and phytopharmaceuticals [11], [25], [26]. The aim of this study was to evaluate the influence of selected plant species on antioxidant and antimicrobial **properties** of Janj cheese.

MATERIAL AND METHODS

The samples of Janj cheese were produced at a rural household in Lipovača near Šipovo, with a long tradition in producing milk, Janj cream, and Janj braided cheese. Analyses were conducted at the Laboratory for Food Technologies and the Laboratory for Instrumental Analyses at the Faculty of Technology, University of Banja Luka. Twelve samples were produced for this study, divided into two batches of six each. Each batch included one control sample without plant addition. The remaining five samples in each batch contained oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), parsley (*Petroselinum crispum*), rosemary (*Rosmarinus officinalis*), and chives (*Allium schoenoprasum*), added at concentrations of 0.50% in the first batch and 1.00% in the second (Table 1). The production process was partially modified, the cheese was shaped not into the traditional braid form but into rolled forms. For each batch, 30 L of milk was used. After the evening milking, the milk was strained, covered with a cloth, and left at room temperature for 14 hours. The readiness of the milk for coagulation was verified by heating 100 mL of milk until curdling and whey separation occurred. The milk was then heated to 38 °C, and 60 mL of rennet (2.00% of the milk volume) was added. After incubation at this temperature for 20 minutes, the temperature was raised to 50 °C. The curd was gently stirred with a spoon for 10 minutes to facilitate cutting, then manually pressed against the sides of the container for an additional 10 minutes to expel the whey. At this stage, the whey temperature was 48 °C. The cheese mass was subsequently separated, weighed to 500 g, and stretched to a thickness of 1 cm. The surface was sprinkled with 1.50% salt and plant species at concentrations of 0.50% for the first batch and 1.00% for the second batch. The finished cheeses were placed on an inclined surface for 30 minutes to allow excess whey to drain, after which they were wrapped in plastic foil and stored at +4 °C until analysis.

Table 1. Plant species and their concentrations (%) in Janj cheese samples

Batch	Control	*Origanum vulgare* (%)	*Ocimum basilicum* (%)	*Petroselinum crispum* (%)	*Rosmarinus officinalis* (%)	*Allium schoenoprasum* (%)
1	-	0.50	0.50	0.50	0.50	0.50
2	-	1.00	1.00	1.00	1.00	1.00

Phenolic content and antioxidant activity analyses were performed exclusively on the cheese samples, while antimicrobial activity was evaluated on both the cheese samples and the plant species used during production. For analysis, 80% ethanol extracts of plant species and cheeses were prepared.

The total phenolic content (TPC) was determined spectrophotometrically using a modified Folin-Ciocalteu method [27], based on the colorimetric reaction of phenolic compounds with the Folin-Ciocalteu reagent and the measurement of the resulting color intensity at 765 nm. The non-flavonoid content (TNF) was determined using the formaldehyde precipitation method [28]. The flavonoid content (TF) was calculated as the difference between the total phenolic and non-flavonoid content. Results were expressed as micrograms of gallic acid equivalents per gram of sample (μg GAE/g). Antioxidant activity was evaluated using DPPH, $ABTS^+$, and FRAP assays. The DPPH assay measures the reduction of the violet DPPH radical (λ = 517 nm) to the yellow DPPH-H by antioxidants [29]. The $ABTS^+$ assay involves decolorization of the $ABTS^+$ radical (λ = 734 nm) generated by potassium persulfate [30]. Trolox was used as a standard, and results are expressed as micromoles of Trolox equivalents per gram (μmol TE/g). The FRAP assay [31] is based on the reduction of the Fe^{3+}–TPTZ complex to Fe^{2+}–TPTZ, measured by the intensity of the blue color formed under acidic conditions. All data were analyzed using Microsoft Excel 2013 and IBM SPSS Statistics 22.0. Results are presented as mean ± SD from three samples. Statistical differences were evaluated by one-way ANOVA with Tukey's HSD post-hoc test. Significance was set at $p < 0.05$.

To determine the antibacterial and antifungal activity of the plant species and cheese extracts, the agar dilution method was used to obtain the minimum inhibitory concentrations (MIC) and minimum bactericidal (MBC) or fungicidal (MFC) concentrations [32], [33], [34]. The final concentrations of the extracts in the medium were: 5, 2.5, 1.25, 0.625, and 0.213 mg/mL. The extracts were tested against four bacterial strains: *Escherichia coli* WDCM 00013, *Pseudomonas aeruginosa* WDCM 00024, *Staphylococcus aureus* WDCM 00034, *Bacillus cereus* WDCM 00151, and two fungal strains: *Candida albicans* WDCM 00054 and a *Candida albicans* clinical isolate.

RESULTS AND DISCUSSION

The analysis of total phenol content (TPC), non-flavonoid compounds (TNF), and flavonoids (TF) in Janj cheese samples enriched with different plant species revealed significant differences depending on the type and concentration of the added plant species ($p<0.05$) (Table 2).

In both test **batches** (0.50% and 1.00%), the control samples, without any plant species, had the lowest values of total phenols. In the first **batch**, TPC amounted to 485.22 µg GAE/g, and in the second, 511.69 µg GAE/g, which corresponds to the baseline phenolic profile of cheese without the influence of plant species. Josipović et al. [35] reported that the total phenol content in fresh cheese (cottage) is approximately 21 µg/g, but increases significantly with the addition of plant *species* up to 284 µg/g with 0.50% dried parsley and 345 µg/g with 1% dried rosemary.

The highest total phenol content in the tested samples of Janj cheese was recorded in samples with the addition of oregano (*Origanum vulgare*), significantly exceeding ($p<0.05$) the values observed for other plant species, with 990.89 µg GAE/g at 0.50% and 1303.17 µg GAE/g at 1.00% addition. This finding confirms numerous previous studies that have shown oregano to possess a remarkably high content of phenolic compounds, particularly thymol and carvacrol, which exhibit strong antioxidant and antimicrobial properties [36], [37], [38], [39]. Similar studies have demonstrated that oregano not only enhances the antioxidant capacity of foods but may also prolong shelf life due to its antimicrobial activity [14]. The phenol content in samples with rosemary (*Rosmarinus officinalis*) was also considerable, with values of 662.85 µg GAE/g (0.50%) and 1003.28 µg GAE/g (1.00%). This is consistent with the findings of Petersen and Simmonds [40], who identified rosmarinic and carnosic acids as the main phenolic components in rosemary with pronounced antioxidant properties. Rosemary is known for protecting lipids in foods from oxidation [22], [41], which is an important factor in the dairy industry where lipid oxidation is a frequent issue [42]. Samples with parsley (*Petroselinum crispum*) and basil (*Ocimum basilicum*) also showed a notable increase in phenolic compounds compared to the control, although with lower values than oregano and rosemary. According to studies by El-Sayed et al. [43], parsley contains numerous phenolic compounds that contribute to antioxidant activity, while Qamar et al. [44] noted that basil contains significant amounts of flavonoids and other phenols that influence overall antioxidant capacity. Chives (*Allium schoenoprasum*) had the lowest phenol content compared to the other tested plants, which is consistent with the data presented by Todorović et al. [37], who reported the lowest phenolic values for chives.

The content of non-flavonoid phenols (TNF) in samples with plant additions was significantly increased ($p<0.05$) compared to the control, indicating the presence of various phenolic acids, tannins, and other polyphenols that, although not flavonoids, play an important role in antioxidant mechanisms [45].

Flavonoids (TF) also represent a significant portion of total phenols, with the highest values ($p<0.05$) found in samples with oregano, which aligns with findings by Heim et al. [46], who emphasized the importance of flavonoids as some of the most potent natural antioxidants.

The increase in phenol concentration, and consequently enhanced antioxidant properties in cheese with the addition of plant species, indicates the potential of these natural additives to improve the quality of dairy products. Such

enrichment not only enhances the health-related aspects of cheese but can also contribute to extending its freshness and safety, as antioxidants slow down lipid oxidation and the development of microorganisms [26], [38].

The statistically significant differences between samples in all parameters ($p<0.05$) confirm the relevance of the type of plant and the concentration of its addition in influencing the phenolic composition of cheese. Furthermore, the increase in phenol content was proportional to the amount of additive, indicating the possibility of controlling antioxidant levels in products as needed. Enriching Janj cheese with oregano, rosemary, parsley, basil, and chives represents an effective approach to enhancing its biological properties and functional capacity, in line with the global trend of using natural additives in the food industry.

Table 2. Content of total phenols (TPC), non-flavonoids (TNF), flavonoids (TF) in the tested cheese samples

Plant species	Batch 1 (0.50%)			Batch 2 (1.00%)		
	TPC (µg GAE/g)	TNF (µg GAE/g)	TF (µg GAE/g)	TPC (µg GAE/g)	TNF (µg GAE/g)	TF (µg GAE/g)
-	485.22^{a} ±0.28	342.93^{a} ±3.01	142.29^{b} ±3.29	511.69^{a} ±8.68	359.29^{a} ±4.00	152.40^{a} ±4.68
Origanum vulgare	990.89^{e} ±8.99	714.54^{e} ±5.68	276.36^{e} ±8.97	1303.17^{d} ±9.28	994.49^{f} ±6.05	308.68^{e} ±3.24
Ocimum basilicum	593.50^{c} ±3.41	399.67^{b} ±2.59	193.83^{d} ±0.82	886.08^{b} ±9.98	689.78^{c} ±63.46	196.30^{c} ±3.51
Petroselinum crispum	591.22^{c} ±6.72	429.07^{c} ±2.17	162.15^{c} ±4.55	992.73^{c} ±4.09	761.04^{d} ±6.27	231.70^{d} ±2.18
Rosmarinus officinalis	662.85^{d} ±0.98	472.31^{d} ±6.80	190.53^{d} ±5.82	1003.28^{c} ±9.06	838.60^{e} ±5.64	164.68^{b} ±3.42
Allium schoenoprasum	555.64^{b} ±7.14	427.31^{c} ±4.43	128.33^{a} ±3.09	874.66^{b} ±7.91	672.60^{b} ±5.72	202.06^{c} ±2.19

a-e mean values with different letters in the same column are statistically significantly different with 95% probability ($p<0.05$)

The results presented in Table 3 indicate that the addition of selected plant species significantly influences the antioxidant activity of Janj cheese compared to the control sample ($p<0.05$). A trend of increased activity was also observed with the rise in plant concentration from 0.50% to 1.00%. Control samples exhibited low but detectable antioxidant activity, likely due to natural milk components such as proteins (e.g., casein) and vitamins A and E, which possess free radical scavenging abilities [47]. The control sample, without the addition of plant species, showed the lowest values across all tested methods (FRAP: 0.36–0.31 µmolFe^{2+}/g; DPPH: 70.92–77.44 µMTE/g; ABTS: 185.53–193.40 µMTE/g), confirming the limited natural antioxidant capacity of the cheese itself. Cheese enriched with oregano (*Origanum vulgare*) exhibited the highest antioxidant activity in all evaluated parameters (FRAP, DPPH, and ABTS). FRAP values increased from 2.10 µmolFe^{2+}/g in batch 1 to 2.96 µmolFe^{2+}/g in batch 2. In parallel, DPPH and ABTS values significantly increased, reaching 703.59 µMTE/g and 1704.98 µMTE/g, respectively, in batch 2. These results demonstrate the exceptionally strong radical-scavenging capacity of oregano, which is consistent with previous findings confirming its high content of phenolic compounds, especially carvacrol and thymol [48], [49], [50]. Their presence in oregano-enriched cheese contributes significantly to the overall increase in antioxidant activity. Rosemary (*Rosmarinus officinalis*) followed oregano in antioxidant potential. In cheese samples containing 1.00% rosemary, the FRAP value reached 1.94 µmolFe^{2+}/g, while DPPH and ABTS activities were 553.98 µMTE/g and 988.52 µMTE/g, respectively. Rosemary is rich in rosmarinic acid, carnosol, and carnosic acid, which are potent natural antioxidants commonly used to protect fats in food products [22]. The addition of basil (*Ocimum basilicum*) and parsley (*Petroselinum crispum*) also resulted in an increase in antioxidant activity compared to the control sample, although to a lesser extent than oregano-enriched cheese. In batch 2, basil-enriched samples had a FRAP value of 1.45 µmolFe^{2+}/g, while DPPH and ABTS values were 275.53 µMTE/g and 577.12 µMTE/g, respectively. Parsley showed slightly lower values but still significantly improved the antioxidant profile of the cheese compared to the control. These plants are rich in flavonoids such as apigenin and luteolin, which contribute to their antioxidant activity [51], [52]. The lowest values for most tested antioxidant activity parameters were recorded in samples enriched with chives (*Allium* *schoenoprasum*), although statistically significant differences ($p<0.05$) were still observed in comparison to the control. Increasing the concentration from 0.50% to 1.00% resulted in a slight increase in activity, indicating an effect of the higher additive amount. Although chives are known to contain numerous bioactive compounds, including phenolic and sulfur-containing compounds [53], [54], their overall radical-neutralizing capacity may be reduced in complex matrices such as plant-enriched cheese.

A strong correlation between total polyphenol content and antioxidant activity of plant extracts and additives has been previously confirmed [31], [55]. Djeridane et al. [56] demonstrated that extracts from phenolic-rich plants exhibit significant antioxidant activity as measured by DPPH and FRAP assays, highlighting the key role of polyphenols in neutralizing free radicals. Prior et al. [55] emphasized the importance of standardized methods for evaluating this relationship in foods and supplements, while Benzie and Strain [31] developed the FRAP methodology, which is widely accepted for assessing antioxidant power and showed that total phenols contribute significantly to such activity. This relationship is also confirmed in the present study, where the increase in total phenols in cheese enriched with plant species was followed by a clear rise in antioxidant activity. This further underscores the importance of phenolic compounds in enhancing the biological properties of food, particularly in products such as cheese, where oxidative processes can negatively impact quality, safety, and shelf life.

Table 3. Antioxidant activity (FRAP, DPPH, ABTS) of Janj cheese samples with added plant species at concentrations of 0.50% and 1.00%

Plant species	Batch 1 (0.50%)			Batch 2 (1.00%)		
	FRAP (μmolFe^{2+}/g)	DPPH (μMTE/g)	ABTS (μMTE/g)	FRAP (μmolFe^{2+}/g)	DPPH (μMTE/g)	ABTS (μMTE/g)
-	0.36^{a} ±0.00	70.92^{a} ±5.20	185.53^{a} ±9.62	0.31^{a} ±0.01	77.44^{a} ±4.41	193.40^{a} ±9.17
Origanum vulgare	2.10^{e} ±0.02	436.19^{e} ±8.94	1181.03^{e} ±5.60	2.96^{e} ±0.04	703.59^{f} ±7.78	1704.98^{e} ±9.02
Ocimum basilicum	0.65^{c} ±0.02	101.31^{c} ±5.83	451.56^{c} ±5.56	1.45^{c} ±0.01	275.53^{d} ±6.77	577.12^{c} ±8.60
Petroselinum crispum	0.52^{b} ±0.01	94.83$^{b.c}$ ±2.90	411.65^{b} ±8.39	0.95^{b} ±0.08	245.85^{c} ±7.34	525.98^{b} ±9.74
Rosmarinus officinalis	1.51^{d} ±0.06	373.63^{d} ±8.74	708.93^{d} ±3.45	1.94^{d} ±0.08	553.98^{e} ±2.81	988.52^{d} ±6.58
Allium schoenoprasum	0.48^{b} ±0.08	83.62^{b} ±2.23	441.50^{c} ±3.34	0.96^{b} ±0.08	224.41^{b} ±2.03	534.78^{b} ±5.89

a-e mean values with different letters in the same column are statistically significantly different with 95% probability ($p<0.05$)

The analysis of the obtained results clearly indicates that the addition of selected plant species significantly contributes to the increase in the antioxidant activity of the examined Janj cheese samples. Cheese samples with a higher percentage of plant species exhibited greater antioxidant capacity compared to those with lower concentrations of the same plants, suggesting a dose-dependent effect. The control samples, without the presence of plants, demonstrated the lowest antioxidant activity values, thereby confirming the essential role of plant addition in enhancing the biochemical properties of cheese. In addition to improving antioxidant potential, the spices could have a positive impact on the sensory characteristics of the product [18], [35], further supporting their application in the production process. Based on these findings, the use of plants as natural additives is recommended for improving the functional and sensory properties of cheese, as well as enhancing the overall quality of the product.

Extracts from cheeses enriched with 1% plant species did not exhibit antimicrobial activity against the tested microorganisms under the applied experimental conditions.

Although previous studies, such as that by Zhong et al. [57], indicated a possible synergistic effect of low concentrations of bioactive compounds, especially when compounds of similar polar nature are present simultaneously, the results in this study suggest that the concentrations of active substances in the cheese did not reach the threshold required to express inhibitory effects. The absence of antimicrobial activity may be due to the diluting effect of the complex cheese matrix, which reduces the availability and stability of active components, as well as possible changes in their bioactivity during production and maturation processes. Therefore, additional analyses were conducted on the plant species themselves, independently of the cheese matrix, to determine their inherent antimicrobial activity. This approach aimed to clarify whether the plants, in the absence of complex interactions within the dairy product, possess the potential to inhibit the growth of selected microorganisms. These findings highlight the need for further research focused on optimizing formulations, such as the application of higher concentrations of plant species, microencapsulation, or the use of carriers, in order to increase the efficacy of bioactive compounds in dairy products.

The best antibacterial activity was demonstrated by oregano and rosemary extracts, which showed the lowest minimum inhibitory concentrations (MIC) against Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus* (Table 4). These results indicate the potential of these plants as natural antibacterial agents, highlighting their possible application in food preservation and safety.

Table 4. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of ethanol extracts of selected plant species

Plant species	***E. coli* WDCM 00013**		***P. aerug.* WDCM 00024**		***B. cer.* WDCM 00151**		***S. aur.* WDCM 00034**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
	mg/mL	**mg/mL**	**mg/mL**	**mg/mL**	**mg/mL**	**mg/mL**	**mg/mL**	**mg/mL**
Origanum vulgare	5	>5	2.5	5	0.625	0.625	0.625	0.625
Ocimum basilicum	>5	>5	5	>5	>5	>5	5	5
Petroselinum crispum	>5	>5	>5	>5	>5	>5	>5	>5
Rosmarinus officinalis	>5	>5	2.5	5	1.25	5	0.625	0.625
Allium schoenoprasum	>5	>5	5	>5	>5	>5	>5	>5

Oregano and rosemary extracts also inhibited the growth of the Gram-negative bacterium *Pseudomonas aeruginosa*, with MIC values of 2.5 mg/mL and minimum bactericidal concentrations (MBC) of 5 mg/mL, but exhibited very weak activity against *Escherichia coli*, with higher MIC and MBC values. Other extracts at tested concentrations showed no effect, except basil and chives extracts on *P. aeruginosa*. The antibacterial action of plants from the Lamiaceae family has been confirmed in several studies [58], [59]. Ličina et al. [60] found that the antibacterial activity of ethanol extract of *Origanum vulgare*, growing wild in Serbia, against Gram-positive bacteria *S. aureus* ATCC 25923 and *B. cereus* (isolate) was somewhat stronger, with lower MIC values than in our study, while MIC values for Gram-negative *E. coli* 25922 and *P. aeruginosa* ATCC 27853 were two and four times higher, respectively [60].

In this study rosemary extract showed significant inhibitory effects on *S. aureus*, *B. cereus*, and *P. aeruginosa*. However, the MIC and MBC concentrations were considerably lower than those reported by Golshani and Sharifzadeh [61] and Manilal et al. [62]. Zhong et al. [57] demonstrated that different components of the polar fraction of rosemary extract exhibit varying inhibitory activities against different bacterial species. In this study Gram-positive bacteria were more sensitive to the extracts than Gram-negative ones, with *P. aeruginosa* showing higher susceptibility than *E. coli*. The greater efficacy of plant species against Gram-positive bacteria has been confirmed in many studies and is generally attributed to differences in cell wall structure.

The antifungal activity of the ethanol extracts of selected plant species was weakly expressed, except for oregano and basil extracts, which exhibited a MIC of 5 mg/mL against both *Candida* species (Table 5). Ličina et al. [60] reported the same MIC value for *Candida albicans* ATCC 10231, although in their experiment the isolate showed slightly higher resistance.

Table 5. Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of ethanol extracts of selected plant species

Plant species	***C. albicans* WDCM 00054**		***C. albicans* izolat**	
	MIC	**MFC**	**MIC**	**MFC**
	mg/mL	**mg/mL**	**mg/mL**	**mg/mL**
Origanum vulgare	5	>5	5	>5
Ocimum basilicum	5	>5	5	>5
Petroselinum crispum	>5	>5	>5	>5
Rosmarinus officinalis	>5	>5	>5	>5
Allium schoenoprasum	>5	>5	>5	>5

CONCLUSIONS

The addition of selected plant species had a significant effect on the biological properties of Janj cheese, primarily through the enhancement of its antioxidant capacity. The highest antioxidant activity was observed in cheese enriched with oregano, followed by the sample with rosemary. Basil and parsley showed a moderate effect, while chives had the least impact. Increasing the concentration of plant species from 0.50% to 1.00% resulted in a proportional increase in total phenolic content and antioxidant activity, indicating a dose-dependent relationship and enabling precise control over the product's functional properties.

Although oregano and rosemary extracts exhibited pronounced antibacterial activity in independent tests, extracts obtained from the enriched Janj cheese did not demonstrate significant antimicrobial effects. This discrepancy may be attributed to the reduced concentration and bioavailability of active compounds within the complex cheese matrix, as well as possible interactions during the production process that may diminish their efficacy. These findings highlight the challenges of transferring the full antimicrobial potential of plant species into dairy products and underscore the need for further research focused on optimizing formulations, delivery methods, or synergistic combinations to enhance bioactivity.

Incorporating selected plant species, especially oregano and rosemary, represents a promising and natural approach to improving the biological and functional qualities of Janj cheese. Moreover, this strategy aligns with the growing consumer demand for natural, clean-label, and functional foods that offer additional health benefits. The results support the potential application of aromatic herbs in traditional Janj cheese production to develop value-added products that foster innovation while preserving cultural heritage and responding to contemporary health trends.

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DECLARATIONS OF INTEREST STATEMENT

The authors affirm that there are no conflicts of interest to declare in relation to the research presented in this paper.

LITERATURE

1. Official Gazette of Bosnia and Herzegovina. (2011). Regulation on milk and dairy products. No 21.
2. Walther, B., Schmid, A., Sieber, R., & Wehrmüller, K. (2008). *Cheese in nutrition and health. Dairy Science & Technology, 88*(4–5), 389–405. https://doi.org/10.1051/dst:2008012
3. Jeronimo, E., & Malcata, F. X. (2016). Cheese: Composition and health effects. In *Encyclopedia of Food and Health* (pp. 741-747). https://doi.org/10.1016/B978-0-12-384947-2.00137-9
4. Rizzoli, R. (2022). Dairy products and bone health. *Aging Clinical and Experimental Research*, *34*, 9–24. https://doi.org/10.1007/s40520-021-01970-4
5. Montel, M-C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D. A., Desmasures, N., & Berthier, F. (2014). Traditional cheeses: Rich and diverse microbiota with associated benefits. *International Journal of Food Microbiology*, *177*, 136–154. https://doi.org/10.1016/j.ijfoodmicro.2014.02.019
6. Wolfe, B. E., Button, J. E., Santarelli, M., & Dutton, R. J. (2014). Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. *Cell*, *158*(2), 422–433. doi: 10.1016/j.cell.2014.05.041
7. Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology*, *15*(2), 67–78. https://doi.org/10.1016/j.tifs.2003.09.004
8. Vujko, A., Arsić, M., & Bojović, R. (2025). From local product to destination identity: Leveraging cave-aged cheese for sustainable rural tourism development. *Agriculture*, *15*(11), 1137. https://doi.org/10.3390/agriculture15111137
9. Mujić, S. (2022). *Domaćice u Janju "upliću" sir – jedine ga tako prave u svijetu.* Agro Klub. https://www.agroklub.ba/prehrambena-industrija/domacice-u-janju-uplcu-sir-jedine-ga-tako-prave-u-svijetu/76466/
10. Pérez-Jiménez, J., Neveu, V., Vos, F., & Scalbert, A. (2010). Identification of the 100 richest dietary sources of polyphenols: An application of the Phenol-Explorer database. *European Journal of Clinical Nutrition*, *64*(S3), S112–S120. DOI: 10.1038/ejcn.2010.221
11. Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, *94*(3), 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022
12. Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology*, *46*(2), 446–475. https://doi.org/10.1016/j.fct.2007.09.106
13. Cushnie, T. P. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, *44*(5), 377-386. https://doi.org/10.1016/j.ijantimicag.2014.06.001
14. Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, *88*(2), 308-316. https://doi.org/10.1046/j.1365-2672.2000.00969.x
15. Tajkarimi, M. M., Ibrahim, S. A., & Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. *Food Control*, *21*(9), 1199-1218. https://doi.org/10.1016/j.foodcont.2010.02.003
16. Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. *Food Control*, *46*, 412-429. https://doi.org/10.1016/j.foodcont.2014.05.047
17. Raut, J. S., & Karuppayil, S. M. (2014). A status review on the medicinal properties of essential oils. *Industrial Crops and Products*, *62*, 250-264.

https://doi.org/10.1016/j.indcrop.2014.05.055

18. Josipović, R., Markov, K., Frece, J., Stanzer, D., Cvitković, A., Mrvčić, J. (2016). Upotreba začina u proizvodnji tradicionalnih sireva, *Mljekarstvo, 66*(1), 12-25. https://doi.org/10.15567/mljekarstvo.2016.0102
19. Gad, A., & Sayd, A. (2015). Antioxidant properties of rosemary and its potential uses as natural antioxidant in dairy products—A review. *Food and Nutrition Sciences*, *6*, 179-193. DOI: 10.4236/fns.2015.61019
20. Ankri, S., & Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. *Microbes and Infection*, *1*(2), 125-129. https://doi.org/10.1016/S1286-4579(99)80003-3
21. Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*, *4*(2), 89-96. PMCID: PMC3614697
22. Nieto, G., Ros, G., & Castillo, J. (2018). Antioxidant and Antimicrobial Properties of Rosemary (*Rosmarinus officinalis,* L.): A Review. *Medicines*, *5*(3), 98. https://doi.org/10.3390/medicines5030098
23. Gülçin, İ. (2012). Antioxidant activity of food constituents: An overview. *Archives of Toxicology*, *86*(3), 345–391. https://doi.org/10.1007/s00204-011-0774-2
24. Ultee, A., Kets, E. P. W., & Smid, E. J. (1999). Mechanisms of Action of Carvacrol on the Food-Borne Pathogen *Bacillus cereus*. *Applied and Environmental Microbiology*, *65*(10), 4606-4610. https://doi.org/10.1128/AEM.65.10.4606-4610.1999
25. Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, *6*(12), 1451-1474. https://doi.org/10.3390/ph6121451
26. Muntean, D., & Vulpie, S. (2023). Antioxidant and antibacterial activity of plant extracts. *Antibiotics*, *12*(7), 1176. https://doi.org/10.3390/antibiotics12071176
27. Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant Activity of Apple Peels. *Journal of Agricultural and Food Chemistry, 51*(3), 609–614. https://doi.org/10.1021/jf020782a
28. Alberto, M. R., Rinsdahl Canavosio, M. A., & Manca de Nadra, M. C. (2006). Antimicrobial effect of polyphenols from apple skins on human bacterial pathogens. *Electronic Journal of Biotechnology, 9*(3). DOI: 10.2225/vol9-issue3-fulltext-1
29. Liyana-Pathirana, C. M., & Shahidi, F. (2005). Antioxidant Activity of Commercial Soft and Hard Wheat (*Triticum aestivum* L.) as Affected by Gastric pH Conditions. *Journal of Agricultural and Food Chemistry, 53*(7), 2433–2440. https://doi.org/10.1021/jf049320i
30. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, *26*(9–10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3
31. Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, *239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292
32. European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). (n.d.). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. *Clinical Microbiology and Infection*, *6*(9), 509-515.
33. Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution method to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, *3*(2), 163-175. https://doi.org/10.1038/nprot.2007.521
34. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal*

of Pharmaceutical Analysis, *6*, 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

35. Josipović, R., Knežević Medverec, Z., Frece, J., Markov, K., Kazaić, S., & Mrvčić, J. (2015). Improved properties and microbiological safety of novel cottage cheese containing spices. *Food Technology and Biotechnology*, *53*(4), 454-462. DOI: 10.17113/ftb.53.04.15.4029
36. Zhang, X. L., Guo, Y. S., Wang, C. H., Li, G. Q., Xu, J. J., Chung, H. Y., Ye, W. C., Li, Y. L., & Wang, G. C. (2014). Phenolic compounds from *Origanum vulgare* and their antioxidant and antiviral activities. *Food Chemistry*, *152*, 300–306. https://doi.org/10.1016/j.foodchem.2013.11.153
37. Todorović, V., Dančetović, A., Dabetić, N., Šobajić, S., & Vidović, B. (2018). Antioksidativna aktivnost odabranih začina sa tržišta Srbije. *Hrana i Ishrana*, *59*(2), 74-79. DOI: 10.5937/HraIsh1802074T
38. Simirgiotis, M. J., Burton, D., Parra, F., López, J., Muñoz, P., Escobar, H., & Parra, C. (2020). Antioxidant and antibacterial capacities of *Origanum vulgare* L. essential oil from the arid Andean region of Chile and its chemical characterization by GC-MS. *Metabolites*, *10*(10), 414. https://doi.org/10.3390/metabo10100414
39. Khorsand, G. J., Morshedloo, M. R., Mumivand, H. et al. (2022). Natural diversity in phenolic components and antioxidant properties of oregano (*Origanum vulgare* L.) accessions, grown under the same conditions. *Scientific Reports*, *12*, 5813. https://doi.org/10.1038/s41598-022-09742-4
40. Petersen, M., & Simmonds, M. S. J. (2003). Rosmarinic acid. *Phytochemistry*, *62*(2), 121–125. https://doi.org/10.1016/S0031-9422(02)00513-7
41. Guo, M., Yang, L., Li, X., Tang, H., Li, X., Xue, Y., & Duan, Z. (2023). Antioxidant efficacy of rosemary extract in improving the oxidative stability of rapeseed oil during storage. *Foods*, *12*(19), 3583. https://doi.org/10.3390/foods12193583
42. Clarke, H. J., McCarthy, W. P., O'Sullivan, M. G., Kerry, J. P., & Kilcawley, K. N. (2021). Oxidative Quality of Dairy Powders: Influencing Factors and Analysis. *Foods (Basel, Switzerland)*, *10*(10), 2315. https://doi.org/10.3390/foods10102315
43. El-Sayed, M. M., Metwally, N. H., Ibrahim, I. A., Abdel-Hady, H., & Abdel-Wahab, B. S. A. (2018). Antioxidant activity, total phenolic and flavonoid contents of *Petroselinum crispum* Mill. *Journal of Applied Life Sciences International*, *19*(2), 1–7. DOI: 10.9734/JALSI/2018/45113
44. Qamar, F., Sana, A., Naveed, S., & Faizi, S. (2023). Phytochemical characterization, antioxidant activity and antihypertensive evaluation of *Ocimum basilicum* L. in l-NAME induced hypertensive rats and its correlation analysis. *Heliyon*, *9*(4), e14644. DOI: 10.1016/j.heliyon.2023.e14644
45. Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, *20*(7), 933-956. https://doi.org/10.1016/0891-5849(95)02227-9
46. Heim, K.E., Tagliaferro, A.R., & Bobilya, D.J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry*, *13*(10), 572-584. https://doi.org/10.1016/S0955-2863(02)00208-5
47. Pihlanto, A. (2006). Antioxidative peptides derived from milk proteins. *International Dairy Journal*, *16*(11), 1306–1314. https://doi.org/10.1016/j.idairyj.2006.06.005
48. Stanojević, L. P., Stanojević, J. S., Cvetković, D. J., & Ilić, D. P. (2016). Antioxidant activity of oregano essential oil (*Origanum vulgare* L.). *Biologica Nyssana*, *7*(2), 131–139. DOI: 10.5281/zenodo.200410
49. Han, F., Ma, G. Q., Yang, M., Yan, L., Xiong, W., Shu, J. C., Zhao, Z. D., & Xu, H. L. (2017). Chemical composition and antioxidant activities of essential oils from different parts of the oregano. *Journal of*

Zhejiang University Science B, *18*(1), 79–84. https://doi.org/10.1631/jzus.B1600377

50. Dragan, F., Moisa, C. F., Teodorescu, A., Burlou-Nagy, C., Fodor, K. I., Marcu, F., Popa, D. E., & Teaha, D. I. M. (2022). Evaluating in vitro antibacterial and antioxidant properties of *Origanum vulgare* volatile oil. *Farmacia*, *70*(6), 1114-1122. https://doi.org/10.31925/farmacia.2022.6.15
51. Stan, M., Soran, M. L., Varodi, C., & Lung, I. (2012). Extraction and identification of flavonoids from parsley extracts by HPLC analysis. *AIP Conference Proceedings*, *1425*(1), 50–52. https://doi.org/10.1063/1.3681964
52. Ghasemzadeh, A., Ashkani, S., Baghdadi, A., Pazoki, A., Jaafar, H. Z., & Rahmat, A. (2016). Improvement in flavonoids and phenolic acids production and pharmaceutical quality of sweet basil (*Ocimum basilicum* L.) by ultraviolet-B irradiation. *Molecules*, *21*(9), 1203. https://doi.org/10.3390/molecules21091203
53. Lenková, M., Bystrická, J., Tóth T., Hrstková, M. (2016). Evaluation and comparison of the content of total polyphenols and antioxidant activity of selected species of the genus Allium. *Journal of Central European Agriculture*, *17*(4), 1119-1133. https://doi.org/10.5513/JCEA01/17.4.1820
54. Vuković, S., Popović-Đorđević, J. B., Kostić, A. Ž., Pantelić, N. D., Srećković, N., Akram, M., Laila, U., & Katanić Stanković, J. S. (2023). *Allium* species in the Balkan region—Major metabolites, antioxidant and antimicrobial properties. *Horticulturae*, *9*(3), 408. https://doi.org/10.3390/horticulturae9030408
55. Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*(10), 4290–4302. DOI: 10.1021/jf0502698
56. Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, *97*(4), 654–660. https://doi.org/10.1016/j.foodchem.2005.04.028
57. Zhong, X., Wang, X., Zhou, N., Li, J., Liu, J., Yue, J., Hao, X., Gan, M., Lin, P., & Shang, X. (2021). Chemical characterization of the polar antibacterial fraction of the ethanol extract from *Rosmarinus officinalis*. *Food Chemistry*, *344*, 128674. https://doi.org/10.1016/j.foodchem.2020.128674
58. Btissam, R., Fatima, E. M., Kamal, E., Hassane G., & Mohamed, N. (2018). Composition and antibacterial activity of hydro-alcohol and aqueous extracts obtained from the Lamiaceae family. *Pharmacognosy Journal*, *10*(1), 81-91. DOI:10.5530/pj.2018.1.16
59. György, E., Laslo, E., & Salamon, B. (2023). Antimicrobial impacts of selected Lamiaceae plants on bacteria isolated from vegetables and their application in edible films. *Food Bioscience*, *51*. https://doi.org/10.1016/j.fbio.2022.102280
60. Ličina, B. L., Stefanović, O. D., Vasić, S. M., Radojević, I. D., Dekić, M. S., & Čomić, L. J. R. (2013). Biological activities of the extracts from wild growing *Origanum vulgare* L. *Food Control*, *33*(2), 498-504. https://doi.org/10.1016/j.foodcont.2013.03.020
61. Golshani, Z., & Sharifzadeh, A. (2014). Evaluation of antibacterial activity of alcoholic extract of rosemary leaves against pathogenic strains. *Zahedan Journal of Research in Medical Sciences*, *16*(3), 12-15.
62. Manilal, A., Sabu, K. R., Woldemariam, M., Aklilu, A., Biresaw, G., Yohanes, T., Seid, M., & Merdekios, B. (2021). Antibacterial activity of *Rosmarinus officinalis* against multidrug-resistant clinical isolates and meat-borne pathogens. *Evidence-Based Complementary and Alternative Medicine*, Article 6677420. https://doi.org/10.1155/2021/6677420