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ANTI-QUORUM SENSING AND ANTITUMOR ACTIVITY OF *PRUNELLA VULGARIS, SAMBUCUS NIGRA, CALENDULA OFFICINALIS*: potential use in food industry

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ABSTRACT

With the emergence of health related side effects of synthetic substances, the trend towards natural products has increased and has directed researchers to determine their pharmacological properties. At the same time, the resistance of the microorganisms to the antibiotics used in the treatment revealed that they should be controlled without allowing them to gain resistance. In this study, the total phenolic content, volatile composition and antioxidant, antimicrobial, anti-quorum sensing and antitumor activities of *Prunella vulgaris*, *Sambucus nigra* and *Calendula officinalis* extracts were determined. The antioxidant capacity of the extracts was determined by the Trolox Equivalent Antioxidant Capacity (TEAC) method, volatile component analyses were determined by GC-MS, and antimicrobial activity was determined by the disc diffusion method. *Chromobacterium violaceum* 026 and *Agrobacterium tumefaciens* A136 were used to determine the anti-quorum sensing activity. Additionally, the antitumor potential of the extracts was determined by the potato disc method. *Prunella vulgaris* was the plant with the highest antioxidant capacity, while the extract with the highest antimicrobial activity was determined to be *Sambucus nigra* against *Staphylococcus aureus* ATCC 25923. The results showed that all extracts have anti-quorum sensing properties. *Prunella vulgaris* was the plant with the highest anti-quorum sensing properties. There was a correlation between the extract concentration and tumor inhibition. The *Prunella vulgaris* extract was found to have the highest antitumor activity. As a result, it was determined that the plants used in the study have the potential to be used in alternative medicine treatment and can be utilized for the control of microorganisms.

Keywords: *Prunella vulgaris*, *Sambucus nigra*, *Calendula officinalis*, Anti-quorum sensing, Antitumor activity, GC-MS

INTRODUCTION

The treatment of infectious diseases is based on compounds intended to inhibit or kill microbial growth. Resistance to antibiotics is a severe problem for public health **(Bouyahya *et al.*, 2017)**. Medicinal and aromatic plants have become an essential part of traditional health systems. Phenolic compounds are secondary metabolites with many biological effects, including antioxidant and antimicrobial properties. Studies have shown that these compounds also inhibit bacterial communication. Biofilm formation, bacteriocin production, conjugation, virulence gene expression, pigment production and bioluminescence formation are believed to be regulated by the intercellular communication mechanism known as quorum sensing (QS) in bacteria **(Liu *et al.*, 2017)**. Many types of bacteria use these intercellular signaling mechanisms for a variety of factors, including bacterial pathogenicity and food degradation. Developing new antibacterial drugs based on QS is an attractive strategy to inhibit bacterial growth **(Gopu *et al.*, 2015)**. Plant secondary metabolites and their semisynthetic derivatives play an important role in anticancer drug treatment **(Pan *et al.*, 2010)**. Crown gall is a neoplastic disease caused by *Agrobacterium tumefaciens* in plants. The Ti plasmid causes the proliferation of plant cells without passing through apoptosis, and the histology is similar to that of human and animal cancers and the nucleic acid content is similar in tumor formation **(Islam *et al.*, 2009)**. That's why researchers use this microorganisms for controlling antitumor mechanisms **(Ramezani *et al.*, 2016).**

Prunella vulgaris L. (Labiatae) is used in the treatment of sore throat, fever, and wounds in European and Chinese alternative medicine. It is also kown as 'self - heal'. **(Rasool *et al.*, 2010)**. *Prunella* species contain triterpenoids and their saponins, phenolic acids, sterols, and the associated glycosides, flavonoids, organic acids, volatile oils, and saccharides. The active components related to these functions are mainly triterpenoids, phenolic acids, flavonoids, and polysaccharides **(Bai *et al.*, 2016)**. Pharmacological studies have revealed that *Prunella* plants possess antibacterial **(Mahboubi *et al.*, 2015)**, antioxidative **(Zhang *et al.*, 2011)**, and antitumor **(Hwang *et al.*, 2013)** activities.

Sambucus nigra, also called elderberry, is a common species that grows in Europe, Asia, North Africa and America **(Veberic *et al.*, 2009)**. Phenolic groups commonly found in elderberry are anthocyanins, flavonol glycosides, hydroxycinnamic acids, and flavonols **(Mikulic-Petkovsek *et al.*, 2015)**. Anthocyanins and other flavonoids (e.g., quercetin) show antioxidant, anticarcinogenic, immunostimulatory, antibacterial, antiallergic, and antiviral activities. Due to these properties, their consumption can contribute to the prevention of several degenerative diseases, such as cardiovascular disease, cancer, inflammatory diseases, and diabetes **(Veberic *et al.*, 2009)**. Several bioactivities of *S. nigra* have been reported, including antimicrobial **(Mohammadsadeghi *et al.*, 2013; Hearts *et al.*, 2010; Hleba *et al.*, 2013)**, antioxidant **(Passos da Silva *et al.*, 2017)**, and antitumor **(Thole *et al.*, 2006)** activities.

Calendula officinalis belongs to the Asteraceae family **(Chaleshtori *et al.*, 2016)**, commonly known as the pot marigold, and it is widely cultivated outdoors in warm temperate regions of the world **(Larcin *et al.*, 2016)**. The traditional medical use of marigold is related to bioactive phytochemicals such as terpenoids, sterols, saponins, carotenoids, and phenolics in the flower extracts **(Martin *et al.*, 2016)**. Many reports have stated that *C. officinalis* extracts have antimicrobial **(Cetin *et al.*, 2017)** and antioxidant **(Mubashar Sabır *et al.*, 2015)** activities.

This study aimed to determine the total phenolic content, volatile composition and antioxidant, antimicrobial, anti-quorum sensing and antitumor activities of various extracts obtained from different parts of *Prunella vulgaris*, *Sambucus nigra* and *Calendula officinalis*.

MATERIAL AND METHODS

Bacterial strains

Staphylococcus aureus ATCC 25923, *Staphylococcus aureus* RSSK 1009, *Bacillus cereus* NCTC 7464, *Bacillus subtilis* ATCC 6633, *Salmonella* Typhimurium ATCC 14028, *Salmonella* Enteritidis ATCC 13076, *Escherichia*

coli ATCC 25922, and *Escherichia coli* ATCC 8739 were supplied by Canakkale Onsekiz Mart University, Faculty of Engineering, Microbiology Laboratory. *Agrobacterium tumefaciens* A136 and *Chromobacterium violaceum* 026 were supplied by Prof. Dr. Ji Hyang Kweon, from Konkuk University, Environmental Engineering Faculty.

Plant material

Prunella vulgaris was collected in June 2015, whereas *Sambucus nigra* and *Calendula officinalis* were collected in September 2015. All plants were obtained from Kütahya Municipality Hekim Sinan Medical Plants Research Center. The altitude of this center is 900 meters. *Prunella vulgaris* and *Calendula officinalis* were dried at room temperature whereas *Sambucus nigra* was not dried. *Prunella vulgaris* was used as a whole plant and milled (Retsch GM 300, Germany) for 30 seconds at 10-second intervals. The branches and fruit parts of *Sambucus nigra* were used, and only the flowers of *Calendula officinalis* were used.

Obtaining the crude extracts

The previously dried and fruit branched plants were extracted with ethanol and methanol (1:10, w/v) at 25°C in a shaking incubator (Jeio Tech, IS-971R, Seoul, Korea) at 250 rpm for 8 hours. Extractions were carried out in parallel, and after this time, the liquid parts of the extracts were passed through 0.45 µm syringe filters (Sartorius Stedim) and stored at -18°C until analysis.

Trolox equivalent antioxidant capacity (TEAC Assay)

The antioxidant capacity of extracts obtained with different solvents was determined by TEAC assay **(Aydeniz and Yılmaz, 2012)**. The ABTS radical (0.0384 g) was dissolved in distilled water in a flask. Two milliliters of 12.25 mM potassium persulfate (Sigma Aldrich) was added to the dissolved $ABTS^{+}$ radical (Sigma Aldrich), and the mixture was diluted to 10 mL with distilled water. The radical solution (1 mL) and different concentrations of the extracts were mixed, and the absorbance of each extract was determined at 734 nm (Shimadzu, UV- 1800, Japan). The antioxidant capacities of the extracts were determined using the Trolox equation calibration curve.

Determination of the total phenolic content

The total phenolic content of the extracts was determined using the Folin-Ciocalteu reagent **(Spanos and Wrolstad, 1990)**. One hundred microliters of extract, 900 µL of distilled water, and 5 mL of 0,2 N Folin-Ciocalteu reagent (Sigma Aldrich) were vortexed for 30 seconds. Then, 4 mL of Na_2CO_3 (75 g/L) was added to the mixture. The absorbance of the solutions was measured at 750 nm after 2 hours. Quantitation was expressed as gallic acid equivalents based on the calibration curve.

Determination of volatile compounds by GC-MS

The volatile components of the extracts were determined by GC-MS (Wilmington DE). The solid-phase microextraction (SPME) technique was used for the isolation of volatile components from the extracts **(Nalbant, 2017)**. Five milliliters of extract and 1 g of NaCl were added to a 40 mL volume vial and incubated at 40°C in a water bath for 20 minutes to allow the volatiles in the headspace to equilibrate. The SPME fiber (2 cm-50/30 µm DVB) was immersed in the vial for 20 minutes under the same conditions. At the end of the extraction period, the fiber was removed from the vial and inserted into the injection port of a GC-MS. GC-MS was performed with an HP-5 capillary column (30 m ×0,25 mm i.d.; film thickness 0,25 um). The oven temperature was kept at 40°C for 2 minutes, increased from 40°C to 120°C at a rate of 4°C/min, held at 120°C for 2 minutes, increased from 120-250°C at a rate of 8°C/min, and held at 250°C for 5 minutes. Helium was applied as the carrier gas with a flow rate of 1.50 mL/min. Identification of the volatile components was performed using the Wiley Registry of Mass Spectral Data **(Wiley, 2005)** and the National Institute of Standards and Technology **(NIST, 2008)**.

Antibacterial activity assay

The antibacterial activities of the extracts were determined by the disc diffusion method **(Burt and Reinders, 2003)**. 20 µL of plant extracts were loaded onto sterile filter paper discs (6 mm in diameter, Oxoid), air dried in a laminar flow hood on sterile petri plates, and placed onto Mueller Hinton Agar (MHA, Biolife) plates seeded overnight with 0.5 McFarland cultures of 4 Gram-positive bacteria (*S. aureus* ATCC 25923, *S. aureus* RSSK 1009, *B. cereus* NCTC 7464, and *B. subtilis* ATCC 6633) and 4 Gram-negative bacteria (*S.* Typhimurium ATCC 14028, *S.* Enteritidis ATCC 13076, *E. coli* ATCC 25922, and *E. coli* ATCC 8739). Ceftriaxon (30 µg/disc, Oxoid) was used as a positive control, and ethanol and methanol, the extraction solvents, were used as negative controls. The plates were incubated at 37°C. After 24 hours, the inhibition zones around the discs were measured and compared with the control groups.

Determination of the minimum inhibitory concentration

Minimum inhibitory concentrations of the extracts were determined by the microdilution method **(Burt and Reinders, 2003)**. 50 µL of Mueller Hinton Broth (Biolife), 50 µL of extracts at various concentrations, and 50 µL of culture (*S. aureus* ATCC 25923, *S. aureus* RSSK 1009, *B. cereus* NCTC 7464, and *B. subtilis* ATCC 6633, *S.* Typhimurium ATCC 14028, *S.* Enteritidis ATCC 13076, *E. coli* ATCC 25922, and *E. coli* ATCC 8739) were loaded into 96-well plates. After 18-24 hours of incubation at 37°C, the absorbance was measured at 450 nm by a spectrophotometer (Thermo scientific, Multiscan FC, USA). The maximum concentration observed was calculated according to the OD values.

Anti-quorum sensing activity

The anti-quorum sensing activities of the extracts were determined by the disc diffusion method **(McClean *et al.*, 1997; Chenia, 2013)**. *Chromobacterium violaceum* 026 (CV026) was used for the determination of short AHLs and inoculated into Luria Bertani Broth containing 50 µL of kanamycin (20 mg/mL, Sigma Aldrich) and incubated at 30°C for 24-48 hours. *Agrobacterium tumefaciens* A136 was used for the determination of long AHLs and inoculated into Luria Bertani Broth containing 250 µL of spectinomycin (10 mg/mL, Sigma Aldrich) and 50 µL of tetracycline (4,5 mg/mL, Sigma Aldrich) and incubated at 30°C for 24-48 hours. A total of 125 µL of overnight culture (CV026) and 20 µL of C_6HSL signal (Sigma Aldrich) were inoculated into Luria Bertani Agar plates. 20 µL of X-gal (Acros), 125 µL of overnight culture (*A. tumefaciens* A136), and 20 µL of C_8HSL signal (Sigma Aldrich) were inoculated into Luria Bertani agar plates. 20 µL of extracts penetrated paper discs placed in plates. After 24 hours of incubation at 30°C, zone-forming extracts were detected.

Determination of antitumor activity

The antitumor activity of the extracts was evaluated using the potato disc assay **(Ramezani *et al.*, 2016)**. Potatoes (*Solanum tuberosum* L.) were peeled and immersed for 30 minutes in a 1% hypochlorite solution, cut with a cork borer to a thickness of 0.5 cm and a diameter of 13 mm. Sliced potato discs were placed on agar plates (1.5 g/100 mL). *Agrobacterium tumefaciens* A136 was incubated for 24-48 hours at 28°C in Luria Bertani Broth containing 50 µL of tetracycline and 250 µL of spectinomycin. The culture (2 mL) was mixed with 2 mL each of 10, 100 and 1000 ppm extract. 20 µL of each mixture was added to the surface of the potato discs. After 21 days of incubation at 28°C, all the discs were stained with Lugol's solution for 20 minutes, and the tumors were counted with a stereomicroscope. The tumor inhibition percentages of the extracts were calculated according to the following formula.

Percent inhibition: 100- $\frac{\text{The average number of tumors in the sample}}{\text{The average number of tumors in the control}}$ **(Ramezani *et al.*, 2016)**

Statistical analysis

Statistical calculations were carried out with the Minitab 17.0 software package. Descriptive statistics were applied to the findings, and it was determined whether the difference in the averages was significant ($P < 0.05$), and a correlation test was applied to determine the relationship between the findings.

RESULTS AND DISCUSSION

Antioxidant capacity and volatile compounds

In the TEAC assay, the highest antioxidant capacity belonged to the methanol extract of *Prunella vulgaris* (26.80±3.70 mmol Trolox/g extract), while the lowest activity belonged to the ethanol extract of the branch of *Sambucus nigra* (2.24±0.60 mmol Trolox/g extract) (Table **1**). The total phenolic content was the highest in the ethanol (404.60±15.50 mg GA/g extract) and methanol extracts (395.10 ±13.20 mg GA/g extract) of *S. nigra*, whereas the lowest was in the methanol (163.42±6.08 mg GA/g extract) and ethanol extracts (118.88±5.81 mg GA/g extract) of *Calendula officinalis* flowers (Table 1). There was no significant difference between the extracts of the fruit of *S. nigra* in terms of antioxidant and total phenolic contents. In addition, there was no significant difference between the phenolic composition of the extracts, and the major components were benzeneacetaldehyde and palmitic acid. **Passos da Silva *et al.* (2017)** stated that the berry extracts of *S. nigra* had a higher total phenolic content than the branch extract. Also in this study, antioxidant activity and total phenolic content were higher in fruits compared to branches. In another study, the total phenolic content of *S. nigra* was higher than the result obtained in this study **(Akhtar and Mirza, 2018)**. The phenolic composition of *P. vulgaris*, which has the highest antioxidant capacity among the studied plants, was defined as propanamide, 2-hydroxy-N-ethyl-1,3-dithioisoindoline, dodecane, tetradecane, hexadecane, palmitic acid, ethyl palmitate, linoleic acid, and 7-pentadecyne. Unlike the ethanol extract, myristic acid, D-glycero-D-galacto-heptose, pentadecanoic acid, linolenyl alcohol, and stearic acid were identified in the

methanol extract. **Golembiovska *et al*. (2014)** determined dodecane, tetradecane, hexadecane, myristic acid, pentadecanoic acid, and palmitic acid to be similar volatile components to our study. The methanol extract of *C. officinalis* had the highest antioxidant capacity (16,9 mmol Trolox/g extract) after *P. vulgaris*. *C. officinalis* contains sesquiterpene glycosides, saponins, xanthophylls, triol triterpenes, flavonoids, volatiles, α-cadinene, α-cadinol, and α-muurolol, which show antioxidative and antimicrobial effects **(Chaleshtori *et al*., 2016)**. In this study, α-thujene and α-pinene, D-limonene, α-cubebene, α-copaene, (-)-isoledene, germacrene D, alloaromadendrene, trans-caryophyllene, naphthalene, α-humulene, (-)-β-selinene, (+)-ledene, dihydroactinidiolide, δ-cadinene, dodecanoic acid, oplopenone, T-muurolol, β-eudesmol, t-cadinol, calendin, tetradecanoic acid, linoleic acid, methyl linolenate, tricosane, and eicosane were identified in the ethanolic extract of *C. officinalis* flowers. Additionally, α-phellandrene, valencene, α-guaiene, α-muurolene, α-longipinene, elemol, myristic acid, pentadecanoic acid, palmitic acid, linolenic acid, and stearic acid were defined in methanolic extract differently from ethanolic extract. Similarly, **Caamal-Herrera *et al*. (2008)** identified the major volatile constituents of hydroalcoholic extracts of *C. officinalis* flowers as α-thujone and α-cadinol.

Table 1 Antioxidant capacity (TEAC) and total phenolic content (TPC) of the extracts

Plant	Plant part	Solvent	TEAC (mmol trolox/g extract)	TPC (mg GA/ g extract)
P. vulgaris	whole plant	MeOH	26.80 ± 3.70*	377.30 ± 12.70
S. nigra	branch		4.10 ± 2.20	332.80 ± 12.60
S. nigra	fruit		10.40 ± 6.40	395.10 ± 13.20
C. officinalis	flower		16.90 ± 4.80	163.42 ± 6.08
P. vulgaris	whole plant	EtOH	19.10 ± 5.20	336.45 ± 7.27
S. nigra	branch		2.24 ± 0.60	174.60 ± 21.80
S. nigra	fruit		10.90 ± 3.90	404.60 ± 15.50
C. officinalis	flower		12.30 ± 4.20	118.88 ± 5.81

* Values are expressed as the mean ± standard deviation of three parallel measurements. MeOH: Methanol EtOH: Ethanol

Anti-quorum sensing activity

Researchers are increasingly interested in medicinal herbal products to identify novel therapeutic and nonpathogenic agents that may act as nontoxic QS inhibitors to control infections without allowing the development of bacterial resistance **(Bouyahya *et al*., 2017)**. Studies have shown that the virulence expression of pathogenic bacteria is based on quorum sensing systems as central regulators **(Liu *et al*., 2017)**. It is known that various plants synthesize QS inhibitors by degrading QS signals or competing with signal receptors **(Kalia *et al*., 2018)**. The ability of the extracts to inhibit communication between microorganisms (anti-quorum sensing) is shown in Figure 1.

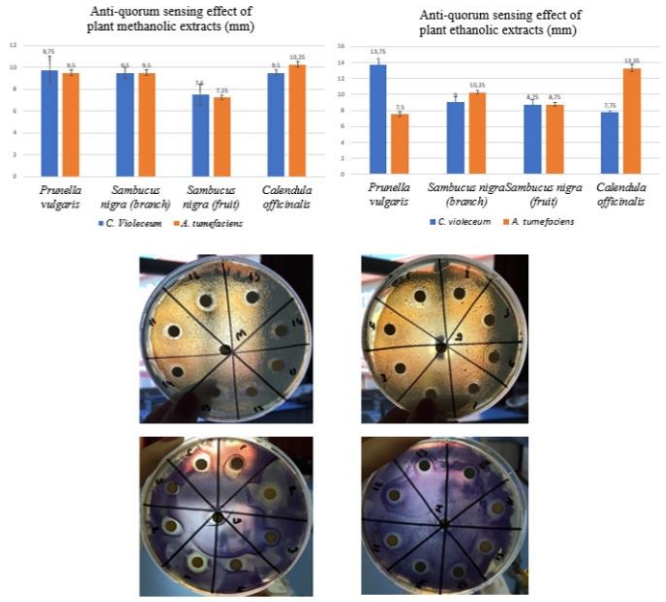

Figure 1 Anti-quorum sensing effect of the plant extracts

All plants in this study showed anti-QS activity. The methanolic extract of *P. vulgaris* exhibited 13.75 mm QS inhibition on CV026. Additionally, this inhibition zone was the highest observed zone among the studied plants. **Koh and Tham (2011)** determined the QS activity of the acetone-water extracts of 10 plants, including *P. vulgaris*. They reported that *P. vulgaris* formed a 15.5 mm QS inhibition zone, which is similar to our results. QS studies of other plants in our study have not been found in the literature. The lowest activity belongs to the methanolic extract of the fruit of *S. nigra*, and the inhibition zones for the other extracts varied between 8,75-13,75 mm. The anti-quorum sensing activity of *S. nigra* has not been found in the literature, but it has been reported that *Sambucus ebulus* exhibits intense anti-quorum sensing activity against *S. aureus* **(Quave *et al*., 2011)**. The most well-known mechanisms of plant extracts and phytochemicals are chemical structures similar to QS signals and their ability to damage signal receptors **(Truchado *et al*., 2015)**. There are plants in the literature with higher QS activity than the plants studied in this study **(Rubegeta *et al*, 2019; Liu *et al*., 2017**). This difference is due to the phytochemical contents of those plants.

Antibacterial activity

The antibacterial activities of the extracts determined by the disc diffusion method against pathogenic bacteria are shown in Table 2. The ethanol extract of the branch of *S. nigra* produced the highest inhibition zone (12.50 ± 0.86 mm) against *S. aureus* ATCC 25923. **Hearst *et al*. (2010)** observed that *S. nigra* leaves form a 6 mm zone against *Bacillus cereus*. In our study, the extracts formed zones ranging from 6.0-7.0 mm against *B. cereus* NCTC 7464. The reason for the low inhibition of the extract against *Bacillus cereus* may that these bacteria are spore-forming, so the antibacterial activity of the extract was insufficient. **Hleba *et al*. (2013)** found that the methanol extract of *S. nigra* forms a 13.5 mm zone against *E. coli*. In our study, the highest inhibition against *E. coli* ATCC 8739 was obtained from the ethanol extract of the fruit with an 8.5 mm zone.

Table 2 Inhibition zones of the plant extracts against test culture

Plant	Plant part	Solvent	*S. aureus* ATCC 25923	*S. aureus* RSSK 9001	*B.cereus* NCTC 7464	*B. subtilis* ATCC 6633	*S.* Typhimurium ATCC 14028	*S.* Enteritidis ATCC 13076	*E.coli* ATCC 25922	*E. coli* ATCC 8739
P.vulgaris	whole plant	MeOH	n.s	n.s	6.7 ± 0.75	7.5 ± 0.95	6.5 ± 0.50	n.s	n.s	8.2 ± 1.31
S.nigra	branch		8.5 ± 1.44*	7.7 ± 0.25	n.s	8.2 ± 1.44	8.5 ± 1.19	6.2 ± 0.25	6.5 ± 0.50	7.5 ± 0.86
S.nigra	fruit		9.5 ± 1.06	8.0 ± 0.40	6.7 ± 0.75	7.7 ± 1.03	8.2 ± 0.62	8.2 ± 0.25	7.7 ± 1.03	7.0 ± 0.40
C. officinalis	flower		10.7 ± 0.47	10.2±0.25	8.2 ± 1.31	7.2 ± 0.75	8.2 ± 0.47	7.7 ± 1.03	n.s	8.7 ± 0.47
P.vulgaris	whole plant	EtOH	8.0 ± 1.09	7.0 ± 1.60	7.7 ± 1.75	10.5 ± 1.72	8.2 ± 1.31	9.7 ± 1.17	10.2 ± 1.53	8.5 ± 1.50
S. nigra	branch		12.5 ± 0.86	8.5 ± 0.95	6.7 ± 0.75	9.2 ± 1.97	8.0 ± 0.40	8.0 ± 1.08	8.2 ± 1.31	7.5 ± 0.64
S. nigra	fruit		8.5 ± 1.44	6.2 ± 0.25	7.0 ± 1.00	n.s	8.2 ± 0.85	8.0 ± 0.81	7.0 ± 0.57	8.5 ± 0.50
C. officinalis	flower		8.7 ± 0.47	10.0±0.40	7.5 ± 0.86	6.7 ± 0.47	8.5 ± 0.28	7.7 ± 1.03	n.s	7.7 ± 1.06
ceftriaxone			25.7 ± 0.47	24.5 ± 1.50	20.5 ± 0.28	30.0 ± 0.40	29.5 ± 2.06	31.7 ± 1.49	30.7 ± 0.47	31.25 ± 1.49

***** Values are expressed as the mean ± standard deviation of three parallel measurements**.** n.s: not sensitive at the tested concentration
MeOH: Methanol EtOH: Ethanol, Ceftriaxone: positive control

However, the ethanol extract of *P. vulgaris* and the branch extract of *S. nigra* also showed a higher antibacterial effect on *B. subtilis* ATCC 6633, a spore-forming bacteria. The difference between the antibacterial activity of the extracts is due to the chemical structure or the antibacterial compounds having different concentrations in the content of the extracts and the differences between bacteria strains. **Chaleshtori *et al*. (2016)** found that the antimicrobial activity of *C. officinalis* essential oil was higher in Gram (-) bacteria than Gram (+) bacteria. In contrast to this study, *C. officinalis* extracts showed higher antibacterial activity against Gram (+) bacteria compared to Gram (-) bacteria in our study. This situation depends on several analytical, genetic, and environmental factors. The quantitative and qualitative values of secondary metabolites may show differences with genetic drift, physiological conditions, season, harvest time, as well as the method of analysis and sample preparation technique **(Chen *et al*., 2012)**. The MIC values of the extracts are shown in Table **3**. The lowest MIC value (3 mg/mL) was determined against *S. aureus* RSSK 9001 from the ethanol extract of *P. vulgaris*. **Mahboubi *et al*. (2015)** determined the MIC value of *P. vulgaris* extract with ethanol as 3.2 mg/mL against *S. aureus* and 6.4 mg/mL against *B. subtilis*. These authors also found for a result of 12.8 mg/mL against *E. coli*. Our results are in parallel with this study. The *S. nigra* extracts had a lower inhibition concentration on Gram (-) bacteria that ranged from 12.5-25 mg/mL. **Mohammadsadeghi *et al*. (2013)** determined the MIC values of the methanol extract of *S. nigra* as 5 mg/mL for *S. aureus* and *B. subtilis*, 2.7 mg/mL for *E. coli*, and 1.9 mg/mL for *S*. Typhimurium. There was no correlation between the antioxidant capacity and total phenolic content of extracts in our study (P>0.5). **Sengül *et al*. (2009)** reached similar results. They explained that the determined antioxidant capacity is not only caused by phenolic compounds but also by other phytochemical components and the Folin-Ciocalteu method, which is used to determine the total phenolic substances and is not an absolute measurement.

Table 3 Minimum inhibitory concentration of the plant extracts (mg/mL)

Plant	Plant part	Solvent	*S. aureus* ATCC 25923	*S. aureus* RSSK 9001	*B.cereus* NCTC 7464	*B. subtilis* ATCC 6633	*S.* Typhimurium ATCC 14028	S. Enteritidis ATCC13076	*E.coli* ATCC 25922	*E. coli* ATCC 8739
P.vulgaris	whole plant	MeOH	6.0±0.01	6.0±0.01	12.5±0.01	12.5±0.01	6.0±0.01	6.0±0.01	12.5±0.01	12.5±0.01
S.nigra	branch		12.5±0.01	12.5±0.01	25±0.01	12.5±0.01	25±0.01	25±0.01	12.5±0.01	12.5±0.01
S.nigra	fruit		12.5±0.01	6.0±0.01	25±0.01	25±0.01	12.5±0.01	12.5±0.01	25±0.01	25±0.01
C. officinalis	flower		12.5±0.01	6.0±0.01	25±0.01	25±0.01	25±0.01	12.5±0.01	25±0.01	25±0.01
P.vulgaris	whole plant	EtOH	12.5±0.01	3.0±0.01	6.0±0.01	6.0±0.01	12.5±0.01	25±0.01	6.0±0.01	6.0±0.01
S. nigra	branch		12.5±0.01	12.5±0.01	12.5±0.01	12.5±0.01	25±0.01	25±0.01	12.5±0.01	12.5±0.01
S. nigra	fruit		6.0±0.01	6.0±0.01	25±0.01	25±0.01	12.5±0.01	12.5±0.01	25±0.01	25±0.01
C. officinalis	flower		12.5±0.01	6.0±0.01	25±0.01	25±0.01	25±0.01	12.5±0.01	12.5±0.01	25±0.01

* Values are expressed as the mean ± standard deviation of three parallel measurements. MeOH: Methanol EtOH: Ethanol

Antitumor activity

Medicinal and aromatic plants have been used to treat diseases since ancient years. Studies have focused on the pharmacological properties of medicinal plants, including antibacterial, antioxidant, antitumor, and bioactive products, such as essential oils, polyphenols, and flavonoids. It has been found that many plants used traditionally inhibit many bacteria and reduce tumor cells in cell lines and free radicals in the human body **(Bouyahya *et al*., 2017)**. It has been discovered that secondary metabolites in plants such as terpenoids, phenolic acids, tannins, lignins, flavonoids, coumarins, and alkaloids show significant antioxidant activity and are essential in cancer treatment **(Tagne *et al*., 2014)**. The antitumor activities of the extracts are shown in Figure 2.

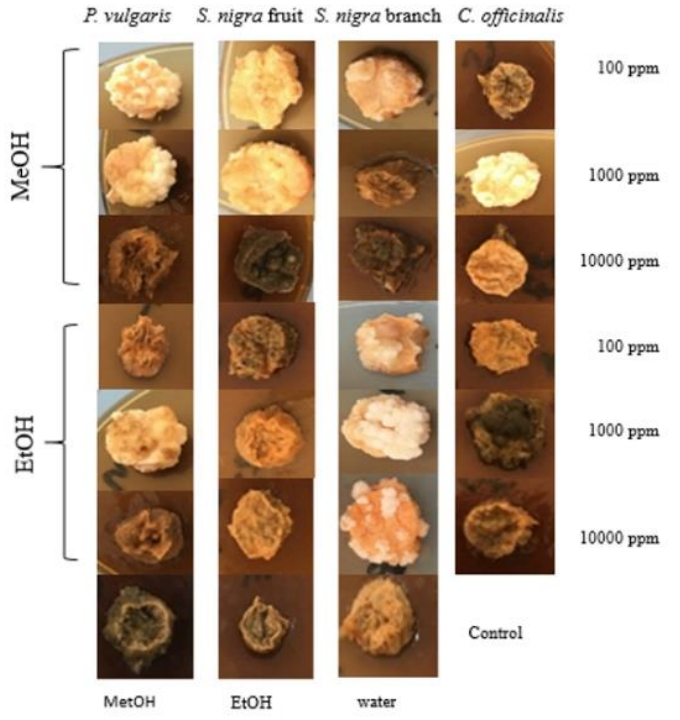

Figure 2 Antitumor activity of the plant extracts

When the antitumor activities of the extracts were evaluated, there was a positive correlation between increasing concentration and percent inhibition. The extract with the highest tumor inhibition (76.2%) at a concentration of 10000 ppm was the ethanol extract of *P. vulgaris*, and the extract with the lowest activity (44.7%) was the methanol extract of *C. officinalis*. In addition, the rankings of antioxidant capacity and total phenolic content were also the same for these plants. These results show that bioactive properties such as antitumor and antioxidant activity are derived from secondary metabolites in plant content.

Similarly, another study reported that a *Fagonia cretica* L. extract had a maximum tumor inhibition of 77.4%, and its inhibition increased with increasing concentration **(Hussain *et al*., 2007)**. In another study, *Rumex hastatus* D. Don chloroform and saponin fractions were reported to have 86.7% and 93.3% tumor inhibition **(Ahmad *et al*., 2016)**. The difference in the tumorigenic approach of the extracts might be related to the profile of bioactive compounds. A value of ≥20% tumor inhibition is considered as an essential value for plant extracts **(Ferrigni *et al*., 1982)**. As a result, the plants used in the study could be developed as a potential antitumor substance.

CONCLUSION

In this study, the usability of *Prunella vulgaris*, *Sambucus nigra* and *Calendula officinalis* as antimicrobial and antioxidant sources and their antitumor and anti-quorum sensing properties were investigated. It has been demonstrated that the plants investigated in this study have functional properties and could be used in various industries including food, pharmaceutical and cosmetic. The intense black-purple color and antimicrobial, antioxidant properties of *S. nigra* increase the potential of being used as a natural food additive Bioactive properties of extracts can be increased by using different solvents and different extraction techniques. The effects of extract concentrations on organoleptic properties of foods should be investigated by sensory tests.

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