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Biosynthesis of silver nanoparticles using lingonberry and cranberry juices and their antimicrobial activity

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ABSTRACT

In this study lingonberry and cranberry juices were used for silver nanoparticle synthesis. The berry juices were characterized by total phenolics, total anthocyanins and benzoic acid content, respectively 1.9–2.7 mg/ml, 55.2–83.4 mg/l and 590.8–889.2 mg/l. The synthesis of silver nanoparticles was performed at room temperature assisting in solutions irradiated by ultraviolet for 30 min. Ultraviolet–visible (UV–vis) spectroscopy and microscopy confirmed the formation of nanoparticles as well as the dark red color of colloid of silver samples showed the formation of stable nanoparticles. Broad localized surface plasmon resonance (LSPR) peaks in UV–vis spectra indicated the formation of polydispersive silver nanoparticles and LSPR was observed at 485 nm and 520 nm for the silver nanoparticles synthesis using lingonberry and cranberry juices, respectively. The antimicrobial activity of silver nanoparticles was determined against the reference strains of microorganisms that could be found in food products: *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 13076, *Listeria monocytogenes* ATCC 19111, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231 and foodborne *B. cereus* producing and non-producing enterotoxins. Silver nanoparticles showed a broad spectrum of antimicrobial activity and were most active against *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633 and *B. cereus* ATCC 11778 reference cultures, and less active against *C. albicans* ATCC 10231 and foodborne *B. cereus*. It can be concluded that lingonberry and cranberry juices could be used as bioreductants for silver ions.

1. Introduction

Nanoscience has flourished during the past twenty years. The progress in this area largely depends on the ability to synthesize nanoparticles from various materials in various sizes and shapes, as well as to their effective inclusion into complex structures. The chemical and physical technologies used for the synthesis of nanoparticles are fairly expensive and their by-products and wastes are toxic and harmful for the environment [1–3]. Scientists propose the synthesis of nanoparticles using a variety of biological systems, such as yeast, fungi, bacteria, fruit and plant extracts as an alternative to the chemical and physical technologies [4,5]. The usual method followed for metal nanoparticle synthesis is reduction [6]. In producing nanoparticles using plant extracts, the extract is simply mixed with a solution of the metal salt at room temperature and the reaction is complete within minutes [7]. The nature of the plant extract, its concentration, the concentration of the metal salt, the pH, temperature and contact time are known to affect the rate of production of the nanoparticles, their quantity and other characteristics [8]. A variety of plant and fruit extracts such as *Murraya koenigii* [9], mangosteen [10] and *Mangifera indica* [11] leaf, *Tansy* fruits [12], latex of *Jatropha curcas* [13], *Cinnamomum zeylanicum* leaf broth [14], *Camellia sinensis* extract [15], *Aloe vera* plant extract [16], mushroom extract [17], and even honey [18] have been used for the synthesis of silver nanoparticles. However, there is no data in the literature about the usage of cranberry and lingonberry juices for silver nanoparticles synthesis.

Cranberries and cranberry products (mash, depectinized mash, pomace, raw juice, clarified juice and juice concentrate) displayed good antioxidant capacity of antiradical, antiviral and antibacterial

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properties. Cranberries have been associated with several cardiovascular health benefits [19], and anti-carcinogenic properties [20]. Cranberry and lingonberry cold-compressed juices have anti-inflammatory and anti-atherothrombotic actions [21]. The cranberry phenols displayed good free radical-scavenging properties, but were less efficient at inhibiting and peroxidation of lipids [22]. The anti-adhesion mechanism of cranberry-proanthocyanins prevents docking of bacteria on host tissues [23]. Bacteria showed the greatest resistance toward the cranberry extracts obtained from the mash and the macerated and depectinized mash [24].

The lingonberries contain plentiful organic acids, vitamin C, provitamin A (as beta carotene), vitamins of group B (B_1, B_2, B_3), potassium, calcium, magnesium, and phosphorus. In addition to these nutrients, they also contain phytochemicals that are thought to counteract urinary-tract infections, and the seeds are rich in omega-3 fatty acids. These phenolics have been proposed to have beneficial effects on health as antioxidants and anticarcinogens. Lingonberries exhibited the highest antiproliferative activities among the berries [25]. Lingonberries are one of the richest sources of phenolic compounds while they also contain cyanidin glycosides and 30 compounds of hydroxycinnamic acids [26]. Lingonberries are rich in benzoic acid and are often used as antimicrobial agents in food preparations.

Biosynthesis of nanomaterials is more advantageous than the other methods of synthesis. Plant or berry extracts as biological materials have been successfully used to synthesize silver nanoparticles. In this research work, we report on the synthesis of silver nanoparticles at ambient conditions assisted by ultraviolet irradiation using lingonberry and cranberry juices. In addition, the silver nanoparticles were tested for its antimicrobial activity. It is known that silver nanoparticles cause irreparable damage to the cellular membrane [27,28], which enables the accumulation of nanoparticles in the cytoplasm [27]. It is suggested that the antimicrobial activity of silver nanoparticles arises due to this damage and not its toxicity [28]. It is therefore expected that small size nanoparticles are able to penetrate across membranes easily [28,29]. Similarly, nanocrystal antibacterial activity is found to have a dependence on crystal shape [28]. It is important to determine the antimicrobial activity of silver nanoparticles against various pathogens, especially those producing toxins.

2. Materials and methods

2.1. Preparation of juices

Lingonberry juice was prepared using freshly collected berries from the forest of South Lithuania. They were surface cleaned with distilled water, crushed and filtered through 8–12 μm of blacband filter (*Filtrak*, Germany) to obtain their juices. Cranberry 100% juice (*SIA "Aneva J"*, Latvia) without any additives was bought in a local supermarket. Both juices were stored at the 5 ± 1 °C in the dark and used for experiments.

2.2. Synthesis of silver nanoparticles

Different amounts of berry juices (Table 1) of 10% concentration and 25 μl of aqueous solution of 1 M $AgNO_3$ were added to the 13 ml of distilled water for synthesis of silver nanoparticles. Solutions were irradiated by 7 W Black light UV rays (360 nm) for 30 min. Lingonberry and cranberry juices of 10% concentrations without silver nitrate solutions were used as control samples.

The active acidity of the juices (control solutions) and the solutions with formed silver nanoparticles was measured by a pH meter "HI 98103Checker® pH Tester" (*Hanna instruments*, USA).

Table 1
Amounts of solutions used for silver nanoparticles biosynthesis.

Juice	Sample ID	Amount of juice (μl)
Lingonberry	LAg2	50
	LAg3	100
	LAg4	200
	LAg5	300
Cranberry	CAg2	50
	CAg3	100
	CAg4	200
	CAg5	300

2.3. Confirmation of the formed silver nanoparticles

Formation of silver nanoparticles was confirmed by UV–vis spectral analysis. The absorbance spectra were recorded by a UV–vis spectrometer (Ocean optics USB 4000) at the scanning region from 200 to 800 nm. The change in the maximum in the absorbance (A_{max}) at varying wavelengths (λ_{max}) presented the change in the color intensity and revealed a possible hyperchromic effect expressed as color retention percentage (CR% = Abs1 × 100/Abs1).

The formation of silver nanoparticles was confirmed by microscopic analysis. A few drops of Ag nanoparticle solutions were examined under a hyperspectral imaging dark field microscope CytoViva (*CytoViva*, UK) using a 10,000 magnification and TECNAI F20 electron transmission microscope (TEM) (*FEI*, USA) equipped with field emission electron gun. TEM accelerating voltage was 200 kV, while bright field images recorded on an Orius CCD (*GATAN*, USA) camera.

2.4. Determination of some chemical amounts in juices

The total anthocyanin content was determined using a pH differential method. The absorbance was measured using a Genesys 5 spectrophotometer (*Thermo Spectronic*, USA) at 510 nm and at 700 nm in buffers with pH levels of 1.0 and 4.5 according to Wrolstad [30].

Anthocyanin pigment content, expressed as cyanidin-3-galactoside mg/l, was calculated using the following equation:

$$\frac{(A_{510\ \mathrm{pH}1} - A_{700\ \mathrm{pH}1}) - (A_{510\,\mathrm{pH}4.5} - A_{700\ \mathrm{pH}4.5})}{\varepsilon \times L} \times MW \times DF \times 10^3$$

where MW is the molecular weight, for cyanidin-3-galactoside 445.2 g/mol, DF the dilution factor, ε the molar extinction coefficient, for cyanidin-3-galactoside 41700, L the pathlength in cm.

The amount of total phenolics was determined with the Folin–Ciocalteu reagent according to the literature [31] using gallic acid as a standard. The reagent was prepared by diluting a stock solution (*Sigma–Aldrich Chemie GmbH*, Germany) with distilled water (1:10, v/v). Samples (1.0 ml, two replicates diluted with water 1:10) were introduced into test cuvettes, then 5.0 ml of Folin–Ciocalteu's phenol reagent and 4.0 ml of Na_2CO_3 (7.5%) were added. The absorbance of all samples was measured at 765 nm using the Genesys 5 spectrophotometer (*Thermo Spectronic*, USA) after incubating at 20 °C for 30 min. Results were expressed as milligrams of gallic acid equivalent in ml.

The benzoic acid content was determined according to ISO 22855:2008.[1] Samples (10 ml) were diluted with 75 ml of extraction solution (60 volume parts ammonium acetate/acetic acid buffer solution and 40 volume parts of methanol). The flasks were inserted into an ultrasonic bath for 10 min. Then the samples were

[1] Fruit and vegetable products – determination of benzoic acid and sorbic acid concentrations – high-performance liquid chromatography method.