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Antioxidant capacity of orange and lemon peel extracts and their use in biosynthesis of silver nanoparticles

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Abstract

Fruit peels are generally considered as environmental burden and waste. In this paper were studied the antioxidant capacity of orange and lemon fruit peel, as well as their application in biosynthesis of silver nanoparticles (AgNPs). The fruit peels were collected, air dried at room temperature for seven days and homogenized by grinding. The homogenization of the sample was done for 2 hours at 200 rpm in the shaker in 80% methanol. The antioxidant capacities of the obtained fruit peel extracts were quantified by FRAP and ABTS methods. The results showed that the antioxidant capacity obtained for lemon peel by FRAP (mmol Fe^{2+}/100g DW) was 6.31±0.14 and by ABTS (mg TE/100g DW) was 1.50±0.06, while for orange peel was 5.65±0.12 and 1.36±0.09, respectively. AgNPs were synthesized by using aqueous extracts of orange and lemon peels, as a reducing agent, and silver nitrate salts as a source of silver ions. Positive inhibitory effect on the growth of new *Escherichia coli* colonies have shown AgNPs synthesized at a basic pH value and at a 0.1 mM $AgNO_3$ using orange or lemon peel extract, while for a 0.5 mM $AgNO_3$ using lemon peel extract.

MATERIALS AND METHODS

Plant material

Fig. 1. Air dried and grinded samples of orange and lemon peel.

Extraction

0.5 g of the sample were weight on an analytical balance (± 0.0001 g) and homogenized with 25 mL of extraction solvent (80% methanol) in a 100 mL Erlenmeyer flask. The homogeneous mixture was extracted on orbital shaker for 2 hours at 200 rpm. The extract was filtered and stored at -18 ° C until analysis. Before analysis extracts were diluted two times.

Determination of antioxidative capacity by FRAP method

This method is based on the reduction of the colourless iron (III)-tripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous form (Fe^{2+}) of intense blue color. The antioxidant capacity of the tested samples was determined spectrophotometrically by measuring the absorbance at 593 nm. Briefly, FRAP reagent was prepared with 200 mL sodium acetate buffer solution (300 mmol L^{-1}, pH 3.6), a 20 ml tripyridyltriazine (TPTZ) solution (10 mmol L^{-1} in 40 mmol L^{-1} HCl), 20mL $FeCl_3$ solution (20 mmol L^{-1}) and 24 mL distilled water. 0.200 ml of fruit peels extracts prepared in methanol was added to 3.8 mL of FRAP reagent. The mixture was incubated at room temperature for 4 minutes and its absorbance was measured at 593nm. The control solution was made of 0.20 mL distilled water and 3.8ml FRAP reagent. The antioxidant capacity was determined using the calibration curve and represented as mmol $FeSO_4$ equivalents per 100 g^{-1} of sample in dry weight.

Determination of antioxidant capacity by ABTS method

This method is based on the "quenching" of the blue-green radical cation of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS radical cation). The addition of antioxidants results in the reduction of the previously generated ABTS radical which is measured by monitoring the decrease in the absorption of ABTS radicals and is compared with the decrease in absorbance caused by the addition of a certain amount of 6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water-soluble vitamin E analogue, under the same conditions.

Antioxidant capacity of analysed fruit peels determined by FRAP and ABTS method

Sample	FRAP (mmol Fe^{2+}/100g DW)	ABTS (mg TE/100g DW)
Orange peel	5.65±0.12	1.36±0.09
Lemon peel	6.31±0.14	1.50±0.06

Reductive capacity of methanol fruit peel extracts determined by the FRAP method is in the range of 6.31±0.14 mmol Fe^{2+}/100g DW which is detected for lemon peel and 5.65 ±0.12 mmol Fe^{2+}/100g DW for orange peel.
From results obtained by ABTS assay it is possible to notice that lemon has the highest antioxidant capacity, 1.50 mg TE/100 g DW, followed by orange with 1.36 mg TE/100 g DW.

Green Synthesis of Silver Nanoparticles (AgNPs)

Orange and lemon peels extracts 5% w/v were used for the synthesis of silver nanoparticles. The experiment was conducted at real pH = 4.7 (orange) and 3.8 (lemon), at neutral pH = 7 and at base-adjusted pH = 10. The source of silver was $AgNO_3$ solution.
Silver nanoparticles synthesized in this way were characterized on a UV-Vis spectrophotometer (Perkin Elmer Lambda XLS).

Antibacterial activity of AgNPs against *Escherichia coli*

In order to demonstrate the **antibacterial activity** of the obtained solutions, the certified reference material E. coli was used for the experiment, which was kept at a specially prescribed temperature of −80˚C in a specially designed chamber (Arctiko) that was calibrated by an authorized institution.
E. coli was seeded on a substrate (Condalab) that favors the growth and development of bacterial colonies of E. coli (TBX). Complete preparation was performed in a laminar chamber (Faster) to ensure completely sterile conditions.
Petri plates with agar and certified reference material E. coli were placed in an incubator (Memmert) at 44˚C (a temperature that favors the development of E. coli) for 24 h. Experiment has been done according to the BAS EN ISO 17025 standard. After the development of bacterial colonies on the plates, previously synthesized solutions of silver nanoparticles were applied to check the antibacterial activity. After applying the solution, the plates were returned to the incubator at 44˚C for 24 h, and the growth inhibition or destruction of individual bacterial colonies was monitored the day after.

Conclusion

Fruit peels, generated as agricultural co-products, can be considered valuable natural source of antioxidants. The results of this research show that orange and lemon peel contain bioactive compounds with antioxidant capacity.
This paper presents the green synthesis of silver nanoparticles with the help of orange peel as an environmentally friendly way of synthesizing nanoparticles. In addition to having an ecological aspect, this synthesis also has an economic aspect. This synthesis shows the sustainable development of organic waste recycling. UV-Vis spectra showed the formation of silver nanoparticles with an absorption maximum at 450 nm. The experimental results showed a very promising green way of synthesizing silver nanoparticles with minimal impact on the environment. Inhibitory action on the growth of new *E. coli* colonies was shown by silver nanoparticles synthesized at a base medium of 0.1mM $AgNO_3$ using orange or lemon peel extract, and at 0.5mM $AgNO_3$using lemon peel extract.

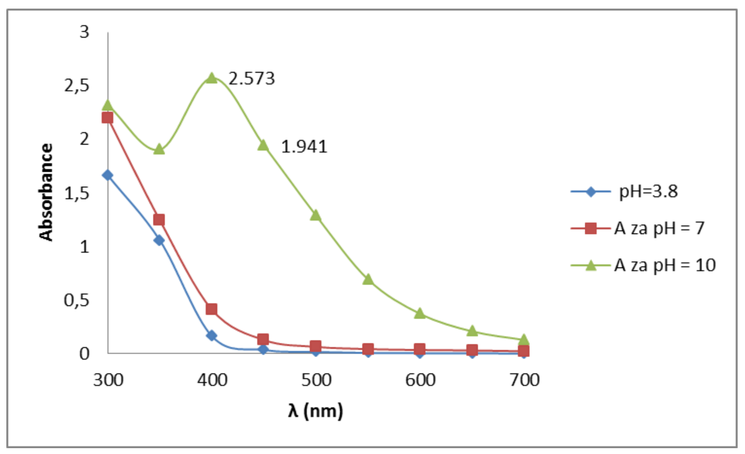
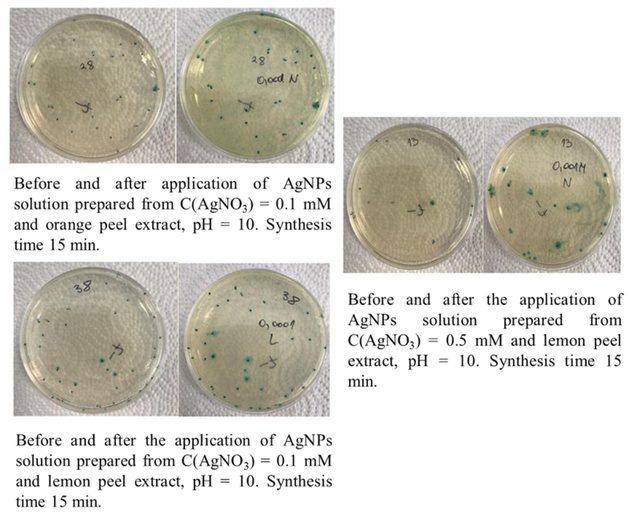


Fig. 2 UV-Vis spectrum for different solutions of silver nanoparticles synthesized from lemon peel extract as a function of pH value at a concentration of 0.5 mM $AgNO_3$.



Before and after application of AgNPs solution prepared from $C(AgNO_3)$ = 0.1 mM and orange peel extract, pH = 10. Synthesis time 15 min.

Before and after the application of AgNPs solution prepared from $C(AgNO_3)$ = 0.1 mM and lemon peel extract, pH = 10. Synthesis time 15 min.

Before and after the application of AgNPs solution prepared from $C(AgNO_3)$ = 0.5 mM and lemon peel extract, pH = 10. Synthesis time 15 min.

Fig. 3 Antibacterial activity of AgNPs against *E. coli.*

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