Green synthesis of silver nanoparticles and their antibacterial activity against udder pathogens

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The study presents a novel process for synthesizing silver nanoparticles using dry lavender leaves (Lav-AgNPs) as both a reducing agent and stabilizer, and evaluates their inhibitory effect on udder pathogens of dairy cows.

Material and methods:

For the practical purposes of the study, two dairy farms were monitored, with a total of 480 cows milked twice daily.

Before milk sampling, cows were subjected to clinical examination and udder palpation. After sensory evaluation, the anterior milk strips from each quarter were evaluated using CMT.

Tests for catalase activity, hemolysis, pigment formation, coagulase, and gram staining were performed using the methods published by Malinowski et al. (2006). Species identification was performed using biochemical tests, including STAPHYtest 24, STREPTOtest 24, or ENTEROtest 24, and evaluated with the TNW Pro-Auto 7.0 software (Erba-Lachema, Brno, CZ).

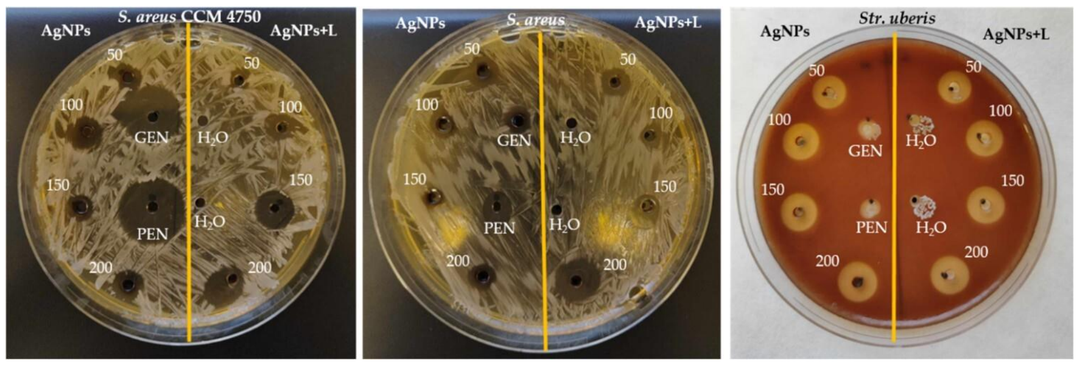
Using green synthesis, silver nanoparticles were obtained from the mixing of silver nitrate solution with reducing substances extracted from levander leaves in five different concentrations: 50 µg/l, 100 µg/l, 150 µg/l and 200 µg/l.

Pure silver nanoparticles (Ag) were used as a control group at the same concentrations as Lav-AgNPs and penicillin (PEN) at a concentration of 10 µg/ml.

Pathogens resistant to ß-lactam antibiotics that were isolated from mastitis cows (*Staphylococcus aureus* and *Streptococcus uberis*) were tested for inhibitory activity by the disk diffusion method.

Results:

Figure 1: In-vitro bactericidal potential of Lav-AgNPs and pure AgNPs against selected resistant udder pathogens (µg/mL

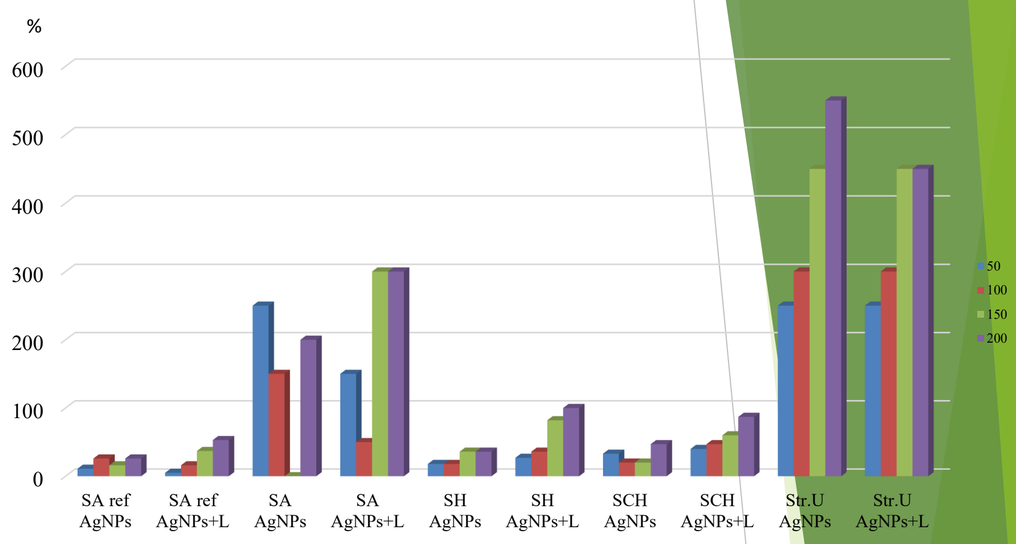


Note: PEN (penicillin; 10 µg); GEN (gentamicin; 10 µg); Lav-AgNPs – silver nanoparticles derived from dry lavender leaves; AgNPs – pure silver nanoparticles.

As the concentration of Lav-AgNPs increased, the bactericidal ability also increased, resulting in a larger diameter of the antibacterial ring.

Analysis of the obtained results shows that Lav-AgNPs at concentrations of 150 µg/l and 200 µg/l had an effect on the inhibition zone for resistant *Staphylococcus aureus* (9-13 mm) as well as for *Streptococcus uberis* (12-14 mm). For penicillin, the inhibition zone was 5 mm for both pathogens. In conclusion, green synthesis provides an anti-bacterial product that represents an alternative to antibiotic substances.

Figure 2: Relative efficiency of AgNPs and Lav-AgNPs with respect to penicillin (%).



Note: SA ref – reference strain S. *aureus* CCM 4750; SA – S. *aureus* isolated from mastitic samples; SH – S. haemolyticus isolated from mastitic samples; SCH – S. *chromogenes* isolated from mastitic samples; Str. U – *Str. uberis* isolated from mastitic samples; AgNPs – pure colloidal silver nanoparticles; AgNPs+L colloidal solution of silver nanoparticles prepared using an extract from dried lavender leaves.

Figure 2 shows the calculation of the relative antibacterial activity of AgNP and Lav-AgNP with respect to the tested antimicrobial agents. The inhibition zones were calculated for penicillin and their percentage effect at different concentrations of AgNP and Lav-AgNP against all tested pathogens. The calculation showed that Lav-AgNP concentrations of 150 and 200 µg/ml showed 100% or higher effect against S. *aureus* and *Str. uberis*. For the other tested NAS, the relative efficacy was lower than 100%.

Table 1: Inhibition zone of AgNPs and Lav-AgNPs against udder pathogens causing bovine mastitis (mean ± SD).

Concentration	Zone of inhibition ((M ± SD, mm)					
	S. aureus CCM 4750		*S. aureus*		*Str. uberis*	
PEN	30±0.91		5±0.30		5±0.45	
	AgNPs	AgNPs+Lav	AgNPs	AgNPs+Lav	AgNPs	AgNPs+Lav
50 µg/ml	5±0.40^{A}	4±0.54^{a}	8±070^{B}	6±0.72^{b}	8±0.84^{B}	8±0.75^{c}
100 µgm/l	8±0.46	6±0.36^{a}	6±0.55	4±0.41	9±0.60	9±0.58^{b}
150 µg/ml	6±0.35^{A}	10±0.46^{a}	2±0.48^{B}	9±0.66	12±0.54^{C}	12±0.60^{b}
200 µgm/l	8±0.65^{A}	13±0.73^{a}	7±0.74	13±0.48	14±0.80^{B}	14±0.56

Note: Values in rows labeled with different letters (Aa,Bb,Cc,) exhibit statistical significance ($p < 0.05$) according to Tukey's test of ANOVA; PEN 10 µg/mL; AgNPs - pure colloidal silver nanoparticles; AgNPs+Lav - colloidal solution of silver nanoparticles prepared using an extract from dried lavender leaves.

Table 1 shows that pure colloidal particles of AgNPs did not have such an inhibitory effect as the colloidal solution of Lav-AgNPs prepared using an extract from dried lavender leaves in the same concentrations. As the concentration of Lav-AgNPs increased, the bactericidal ability also increased, resulting in a larger diameter of the antibacterial ring. The maximum inhibition zone of Lav-AgNPs was 13 ± 0.48 mm for S. *aureus* and 14±0.56 mm for *Str. uberis*.

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