

Supplementary material

Biosynthesis and characterization of silver nanoparticles synthesized using extracts of *Agrimonia eupatoria* L. and *in vitro* and *in vivo* studies of potential medicinal applications

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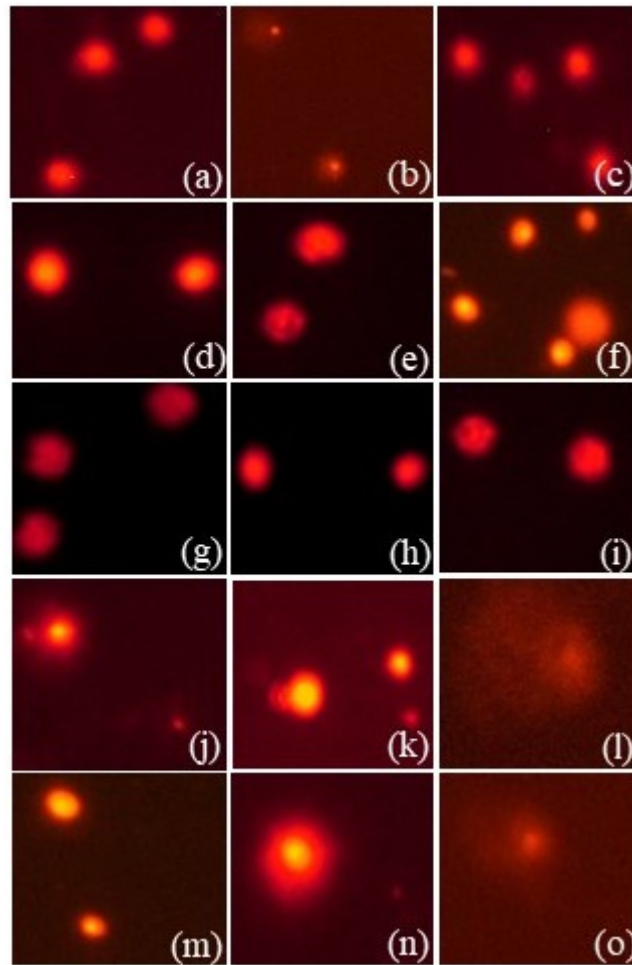


Figure S1. Representative alkaline comet assay images showing the DNA damage in the third instar larvae of *D. melanogaster* treated with different concentrations of the *Agrimonia eupatoria* water and acetone extract. Negative control (a), EMS (b), silver nitrate (c), water extract of *Agrimonia eupatoria* 0.5 mg/mL (d), water extract of *Agrimonia eupatoria* 1 mg/mL (e), water extract of *Agrimonia eupatoria* 2 mg/mL (f), acetone extract of *Agrimonia eupatoria* 0.5 mg/mL (g), acetone extract of *Agrimonia eupatoria* 1 mg/mL (h), acetone extract of *Agrimonia eupatoria* 2 mg/mL (i), water extract of *Agrimonia eupatoria* 0.5 mg/mL + 1 mM EMS (j), water extract of *Agrimonia eupatoria* 1 mg/mL + 1 mM EMS (k), water extract of *Agrimonia eupatoria* 2 mg/mL + 1 mM EMS (l), acetone extract of *Agrimonia eupatoria* 0.5 mg/mL + 1 mM EMS (m), acetone extract of *Agrimonia eupatoria* 1 mg/mL + 1 mM EMS (n) and acetone extract of *Agrimonia eupatoria* 2 mg/mL + 1 mM EMS (o).

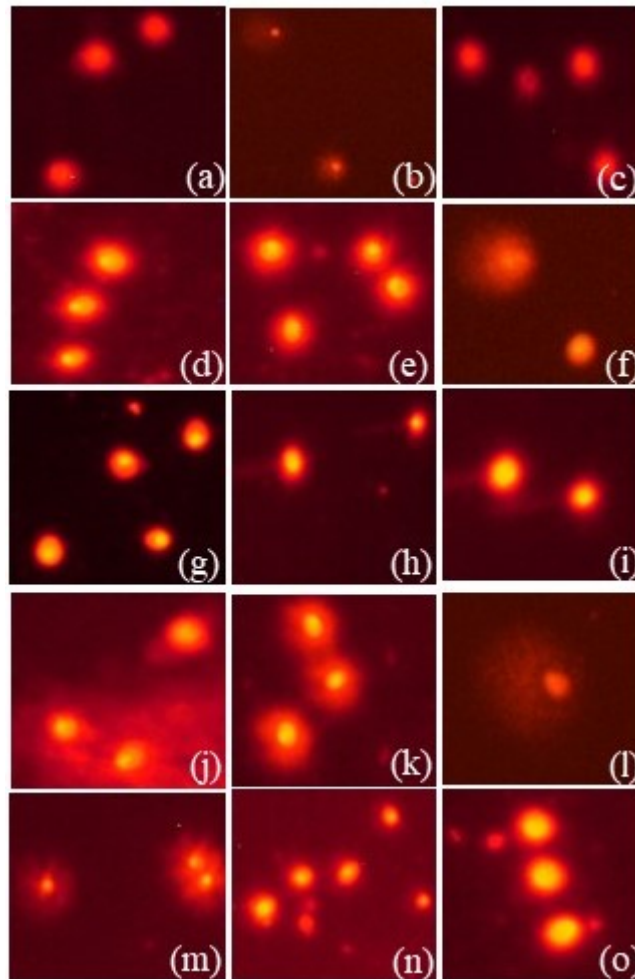


Figure S2. Representative alkaline comet assay images showing the DNA damage in the third instar larvae of *D. melanogaster* treated with different concentrations of the silver nanoparticles biosynthesized from water and acetone extracts of *Agrimonia eupatoria*. Negative control (a), EMS (b), silver nitrate (c), AgNPs-H₂O 0.5 mg/mL (d), AgNPs-H₂O 1 mg/mL (e), AgNPs-H₂O 2 mg/mL (f), AgNPs-acetone 0.5 mg/mL (g), AgNPs-acetone 1 mg/mL (h), AgNPs-acetone 2 mg/mL (i), AgNPs-H₂O 0.5 mg/mL + 1 mM EMS (j), AgNPs-H₂O 1 mg/mL + 1 mM EMS (k), AgNPs-H₂O 2 mg/mL + 1 mM EMS (l), AgNPs-acetone 0.5 mg/mL + 1 mM EMS (m), AgNPs-acetone 1 mg/mL + 1 mM EMS (n) and AgNPs-acetone 2 mg/mL + 1 mM EMS (o).

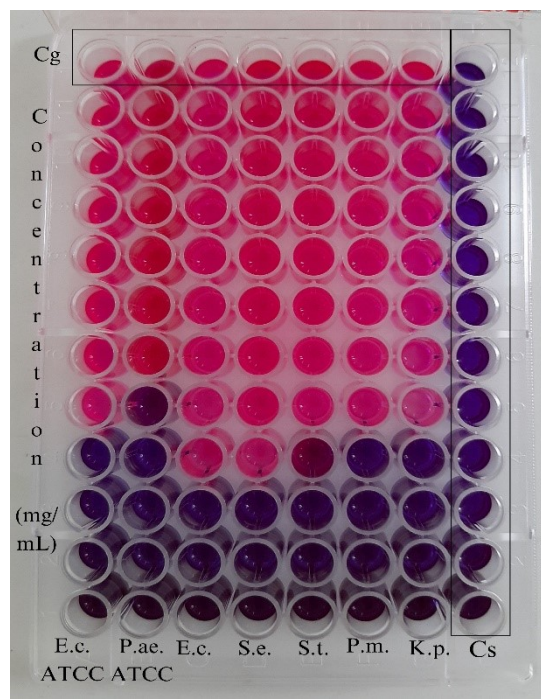


Figure S3. Effect of AgNPs – acetone on Gram negative bacteria (MIC values)

E.c. ATCC – *Escherichia coli* ATCC 25922; P.ae ATCC – *Pseudomonas aeruginosa* ATCC 27853; E.c. – *Escherichia coli*; S.e. – *Salmonella enterica*; S.t. – *Salmonella typhimurium*; P.m. – *Proteus mirabilis*; K.p. – *Klebsiella pneumoniae*; Cs – Sterility control; Cg – Growth control; Concentration range 5 – 0.0098 mg/ml

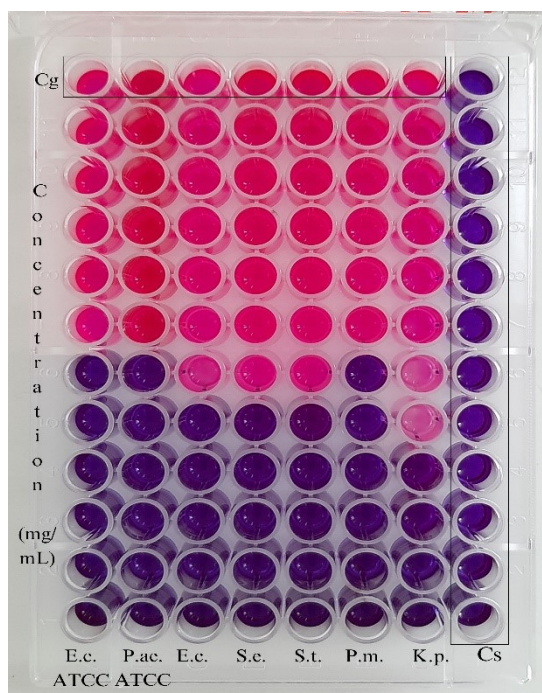


Figure S4. Effect of AgNPs – H₂O on Gram negative bacteria (MIC values)

E.c. ATCC – *Escherichia coli* ATCC 25922; P.ae ATCC – *Pseudomonas aeruginosa* ATCC 27853; E.c. – *Escherichia coli*; S.e. – *Salmonella enterica*; S.t. – *Salmonella typhimurium*; P.m. – *Proteus mirabilis*; K.p. – *Klebsiella pneumoniae*; Cs – Sterility control; Cg – Growth control; Concentration range 5 – 0.0098 mg/ml

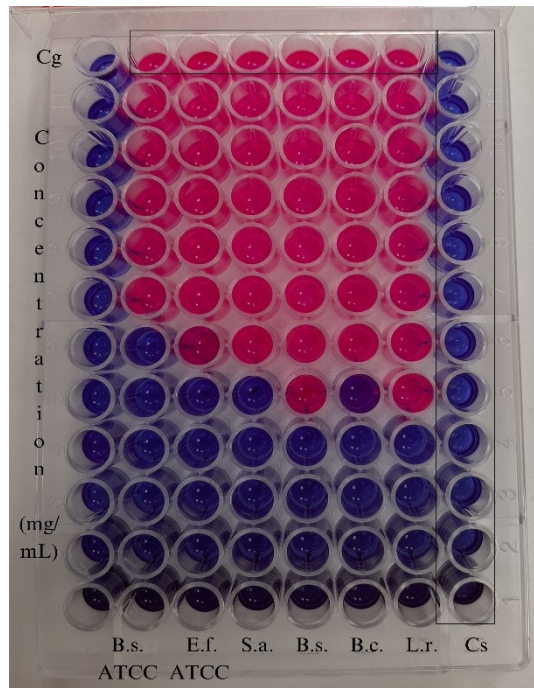


Figure S5. Effect of AgNPs – acetone on Gram positive bacteria (MIC values)
 B.s ATCC – *Bacillus subtilis* ATCC 6633; E.f. ATCC – *Enterococcus faecalis* ATCC 29212;
 S.a. – *Staphylococcus aureus*; B.s. – *Bacillus subtilis*; B.c. – *Bacillus cereus*; L.r. –
Lactobacillus rhamnosus; Cs – Sterility control; Cg – Growth control; Concentration range 5
 – 0.0098 mg/ml

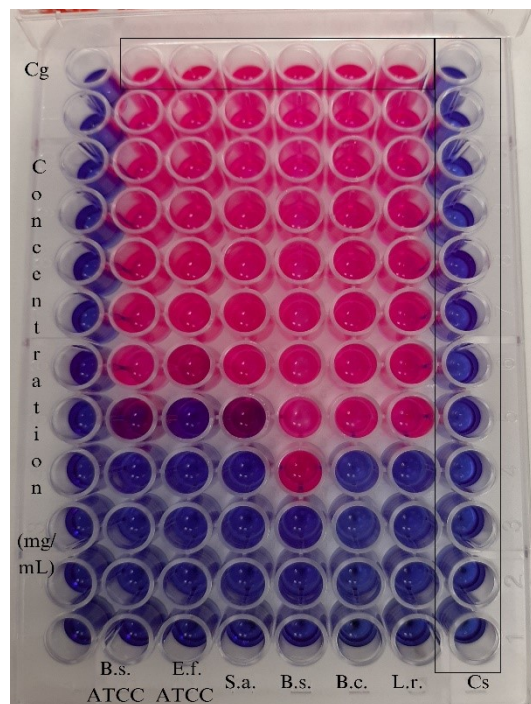


Figure S6. Effect of AgNPs – H₂O on Gram positive bacteria (MIC values)
 B.s ATCC – *Bacillus subtilis* ATCC 6633; E.f. ATCC – *Enterococcus faecalis* ATCC 29212;
 S.a. – *Staphylococcus aureus*; B.s. – *Bacillus subtilis*; B.c. – *Bacillus cereus*; L.r. –
Lactobacillus rhamnosus; Cs – Sterility control; Cg – Growth control; Concentration range 5
 – 0.0098 mg/ml

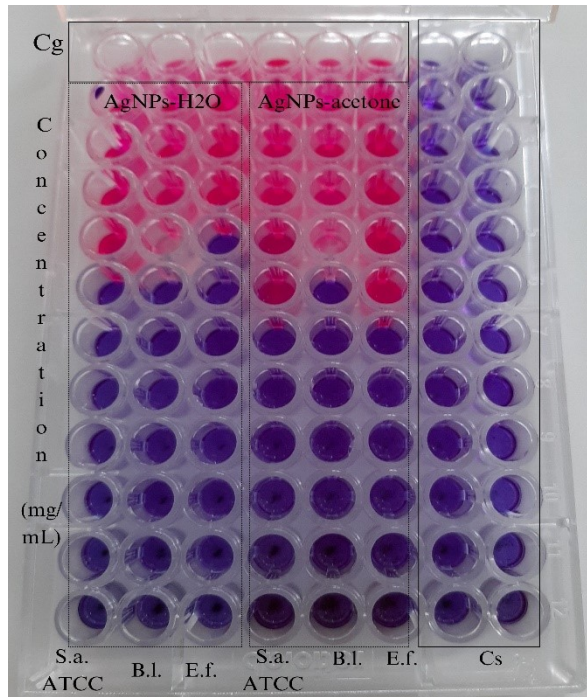


Figure S7. Effect of AgNPs – H₂O and AgNPs – acetone on Gram positive bacteria (MIC values)

S.a. ATCC – *Staphylococcus aureus* ATCC 25923; B.f. – *Bifidobacterium animalis* subsp. *lactis*; E.f. *Enterococcus faecalis*; Cs – Sterility control; Cg – Growth control; Concentration range 5 – 0.0098 mg/ml

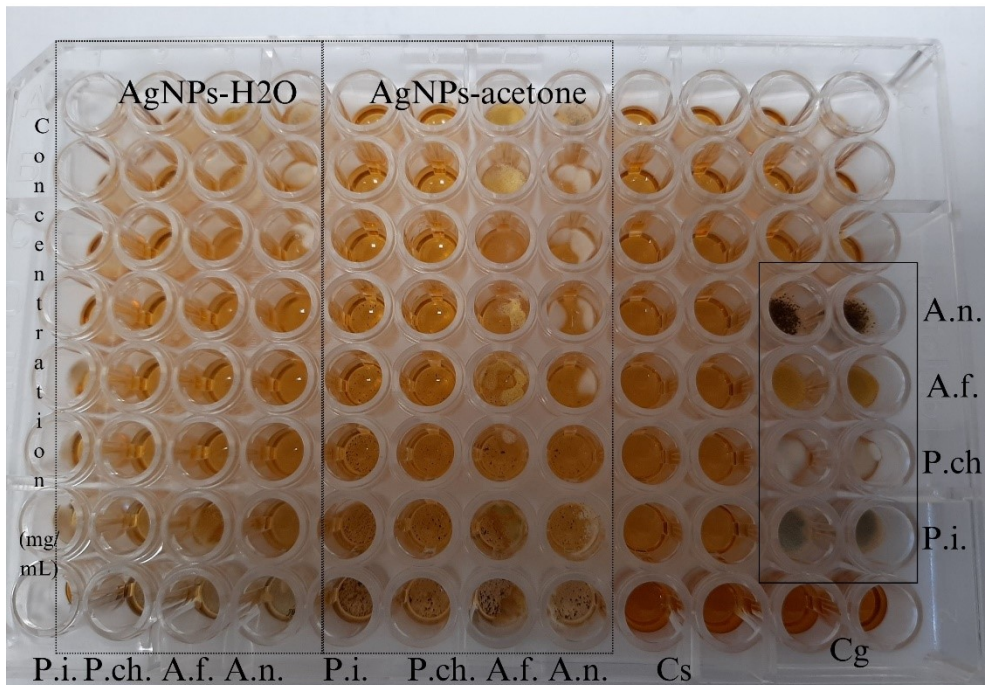


Figure S8. Effect of AgNPs – H₂O and AgNPs – acetone on filamentous fungi (MIC values)
P.i. – *Penicillium italicum*; P.ch. – *Penicillium chrisogenum*; A.f. – *Aspergillus flavus*; A.n. – *Aspergillus niger*; Cs – Sterility control; Cg – Growth control; Concentration range 5 – 0.078 mg/ml

List of Supplementary figures

Figure S1. Representative alkaline comet assay images showing the DNA damage in the third instar larvae of *D. melanogaster* treated with different concentrations of the *Agrimonia eupatoria* water and acetone extract.

Negative control (a), EMS (b), silver nitrate (c), water extract of *Agrimonia eupatoria* 0.5 mg/mL (d), water extract of *Agrimonia eupatoria* 1 mg/mL (e), water extract of *Agrimonia eupatoria* 2 mg/mL (f), acetone extract of *Agrimonia eupatoria* 0.5 mg/mL (g), acetone extract of *Agrimonia eupatoria* 1 mg/mL (h), acetone extract of *Agrimonia eupatoria* 2 mg/mL (i), water extract of *Agrimonia eupatoria* 0.5 mg/mL + 1 mM EMS (j), water extract of *Agrimonia eupatoria* 1 mg/mL + 1 mM EMS (k), water extract of *Agrimonia eupatoria* 2 mg/mL + 1 mM EMS (l), acetone extract of *Agrimonia eupatoria* 0.5 mg/mL + 1 mM EMS (m), acetone extract of *Agrimonia eupatoria* 1 mg/mL + 1 mM EMS (n) and acetone extract of *Agrimonia eupatoria* 2 mg/mL + 1 mM EMS (o).

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Negative control (a), EMS (b), silver nitrate (c), AgNPs-H₂O 0.5 mg/mL (d), AgNPs-H₂O 1 mg/mL (e), AgNPs-H₂O 2 mg/mL (f), AgNPs-acetone 0.5 mg/mL (g), AgNPs-acetone 1 mg/mL (h), AgNPs-acetone 2 mg/mL (i), AgNPs-H₂O 0.5 mg/mL + 1 mM EMS (j), AgNPs-H₂O 1 mg/mL + 1 mM EMS (k), AgNPs-H₂O 2 mg/mL + 1 mM EMS (l), AgNPs-acetone 0.5 mg/mL + 1 mM EMS (m), AgNPs-acetone 1 mg/mL + 1 mM EMS (n) and AgNPs-acetone 2 mg/mL + 1 mM EMS (o).

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Figure S6. Effect of AgNPs – H₂O on Gram positive bacteria (MIC values)

B.s ATCC – *Bacillus subtilis* ATCC 6633; E.f. ATCC – *Enterococcus faecalis* ATCC 29212; S.a. – *Staphylococcus aureus*; B.s. – *Bacillus subtilis*; B.c. – *Bacillus cereus*; E.f. – *Enterococcus faecalis*; L.r. – *Lactobacillus rhamnosus*; Cs – Sterility control; Cg – Growth control; Concentration range 5 – 0.0098 mg/ml

Figure S7. Effect of AgNPs – H₂O and AgNPs – acetone on Gram positive bacteria (MIC values)

S.a. ATCC – *Staphylococcus aureus* ATCC 25923; E.f. *Enterococcus faecalis*; B.f. – *Bifidobacterium animalis* subsp. *lactis*; Cs – Sterility control; Cg – Growth control; Concentration range 5 – 0.0098 mg/ml

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P.i. – *Penicillium italicum*; P.ch. – *Penicillium chrisogenum*; A.f. – *Aspergillus flavus*; A.n. – *Aspergillus niger*; Cs – Sterility control; Cg – Growth control; Concentration range 5 – 0.039 mg/ml