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Antiangiogenic research using AgNPs-FD

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ABSTRACT

Background: *Ficus deltoidea* L. Jack is a folk medicinal plant known for its pharmacological properties, including anti-inflammatory, anticancer, and anti-angiogenic. This study aimed to evaluate the anti-angiogenic properties of silver nanoparticles biosynthesized using *F. deltoidea* leaf extract (AgNPs-Fd).

Methods: The AgNPs-Fd were synthesized by mixing 100 mL 1 mM aqueous silver nitrate ($AgNO_3$) and 100 mL 0.1% *F. deltoidea* ethanolic leaf extract. The resulting AgNPs-Fd were observed for color change and Tyndall effects. Reaction mixture color change from pale brown to reddish brown was observed at 48 h at 37°C^{o}C. The characterization of AgNPs-Fd was completed with UV–Vis spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy. For quantitative analysis of the vascular network in the *chorio allantoic membrane* (CAM) assay, AngioTool open-source software was used.

Results:The plasmon resonance peak for AgNPs-Fd at 430 nm was visible in the UV-Visible spectrum, indicating the formation of AgNPs-Fd. The *F. deltoidea* extract and nanoparticles interacted well according to FTIR analysis. The AgNPs-Fd morphology of 20 nm particle sizes was observed using TEM. The chromatographic analysis of AgNPs-Fd identified potential anti-angiogenic compounds, such as phytol, stigmasterol, lupeol, and sitosterol. The angiogenic inhibition properties of AgNPs-Fd were tested using the CAM assay. The 90mg dose AgNPs-Fd treatment in CAMs demonstrated significant anti-angiogenesis, indicating effectiveness in controlling vessel formation.
Conclusion: The present study suggests that eco-friendly work and the “green” process of AgNPs-Fd is potentially applicable for nanobiotechnology in anti-angiogenic fields.

Keywords: anti-angiogenic; *Ficus deltoidea*; green synthesize; nanomaterials

Biosynthesis of AgNPs-Fd

1 Aqueous silver nitrate ($AgNO_3$) (100 mL, 1 mM) was combined with 100 mL 0.1%*F. deltoidea* leaf extract.

2 The pH of the solution was adjusted to 7.0, and it was subsequently incubated on a rotary shaker at 150 rpm for 48 h at 28°C

Characterization of AgNPs-Fd

3 The resulting AgNPs-Fd were confirmed using UV-Vis spectrophotometry (Shimadzu, UV-1280, Japan) in the range of 350–750 nm

4 The TEM (MIRA3 model, Czech Republic) was operated to display the surface morphology of the AgNPs-Fd. XRD was applied to evaluate the chemical characterization of AgNPs-Fd

5 To determine phytochemicals surrounding the AgNPs-Fd, FTIR spectroscopy (Agilent, Cary 630 model, US) was performed

Phytochemical analysis

6 Phytochemical analysis of the AgNPs-Fd was performed using GC–MS (HP-5MS UI, Agilent, USA) to evaluate the chemical compounds that potentially serve as anti-angiogenic.

7 For this, samples of AgNPs-Fd were dissolved in ethanol in a microtube, vortexed, and centrifuged for three minutes at 9500 rpm

8 The resulting supernatant was used for identification and injected into the GC–MS apparatus.

9 The condition of the GC–MS is as follows: column: HP-5MS UI, gas carrier: helium UHP (He), injector temperature: 290°C, split flow: 10 ml/min, split ratio: 10; front inlet flow: 1.00 ml/min, MS transfer line temp: 230°C, ion source temp: 200°C, mass list range (amu): 40–500, purge flow: 3 ml/min, gas saver flow: 5 ml/min, and gas saver time: five minutes

CAM Assay

10 To analyze AgNPs-Fd for anti-angiogenic properties, a CAM assay was performed following previous methods byRibatti [24], Camposano and Torre [28], and Gamallo and Espere [29]

11 In total, 24 chicken eggs were collected in preparing for the CAM analysis and were dosed with *F. deltoidea* extract (Fd) or AgNPs-Fd. The doses used in the paper disk for the CAM assay were as follows: negative control (30 ng basic fibroblast growth factor (bFGF); positive control (30 µg cortisone acetate with 30 ng bFGF); treatment groups (30 ng bFGF with AgNPs-Fd at 45, 60, 75 and 90mg, respectively)

12 A basic fibroblast growth factor (bFGF-Thermo Fisher Scientific, USA) is a group of proteins secreted by tissues to regulate cell metabolism, proliferation, differentiation, and survival. The bFGF was used to induce neovascularization[30]

13 Before incubation, eggshells were cleansed with 70% alcohol. The eggs were incubated in for six days at 37–39°C with 50–60% humidity

14 The boundaries of the air space, which indicated the location of the embryo, were marked on all eggshells with a pencil (1 × 1 cm)

15 Candling, with egg binoculars, was used to determine the location of the embryo. By using a povidone-iodine solution, the eggshell was cleaned at the pole containing the air space and the part above the embryo

16 Using a needle, a small hole was made in the air chamber and vacuumed until the air moved from

the pole to the top of the egg.

17 Next, a window measuring 1 × 1 cm was made on the marked area using a mini drill. Paper discs for each treatment were then embedded onto the CAM through this window

18 The holes in the polar regions and 1 × 1 cm windows were sealed with paraffin film after they had been planted according to the treatment

19 The eggs were then incubated for 48 h at 37–39°C with 50–60% humidity

20 After, the eggs were removed and subsequently killed by freezing for 24 h.

21 The eggs were then opened by cutting the eggshell into two parts, beginning with the part closest to the air cavity

22 The egg contents were slowly and carefully removed, keeping the CAM attached to the shell. Each CAM was photographed and analyzed using AngioTool 0.6 software

23 AngioTool is a small, easy-to-use application that allows for the quick, hands-free and reproducible measurement of vascular networks in micrographs

24 AngioTool calculates a variety of morphological and geographical data, such as the area covered by a vascular network, the number of vessels, vessel length, vascular density, and lacunarity.

25 AngioTool also computes the "branching index" (branch points/unit area), which quantifies specimen sprouting activity[26].