

Fern-synthesized nanoparticles in the fight against malaria: LC/MS analysis of *Pteridium aquilinum* leaf extract and biosynthesis of silver nanoparticles with high mosquitocidal and antiplasmodial activity

ABSTRACT

Malaria remains a major public health problem due to the emergence and spread of *Plasmodium falciparum* strains resistant to chloroquine. There is an urgent need to investigate new and effective sources of antimalarial drugs. This research proposed a novel method of fern-mediated synthesis of silver nanoparticles (AgNP) using a cheap plant extract of *Pteridium aquilinum*, acting as a reducing and capping agent. AgNP were characterized by UV-vis spectrophotometry, Fourier transform infrared (FTIR) spectroscopy, energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD). Phytochemical analysis of *P. aquilinum* leaf extract revealed the presence of phenols, alkaloids, tannins, flavonoids, proteins, carbohydrates, saponins, glycosides, steroids, and triterpenoids. LC/MS analysis identified at least 19 compounds, namely pterisin, hydroquinone, hydroxy-acetophenone, hydroxy-cinnamic acid, 5, 7-dihydroxy-4-methyl coumarin, trans-cinnamic acid, apiole, quercetin 3-glucoside, hydroxy-L-proline, hypaphorine, khellol glucoside, umbelliferose, violaxanthin, ergotamine tartrate, palmatine chloride, deacylgymnemic acid, methyl laurate, and palmitoyl acetate. In DPPH scavenging assays, the IC₅₀ value of the *P. aquilinum* leaf extract was 10.04 g/ml, while IC₅₀ of BHT and rutin were 7.93 and 6.35 g/ml. In mosquitocidal assays, LC₅₀ of *P. aquilinum* leaf extract against *Anopheles stephensi* larvae and pupae were 220.44 ppm (larva I), 254.12 ppm (II), 302.32 ppm (III), 395.12 ppm (IV), and 502.20 ppm (pupa). LC₅₀ of *P. aquilinum*-synthesized AgNP were 7.48 ppm (I), 10.68 ppm (II), 13.77 ppm (III), 18.45 ppm (IV), and 31.51 ppm (pupa). In the field, the application of *P. aquilinum* extract and AgNP (10 × LC₅₀) led to 100 % larval reduction after 72 h. Both the *P. aquilinum* extract and AgNP reduced longevity and fecundity of *An. stephensi* adults. Smoke toxicity experiments conducted against *An. stephensi* adults showed that *P. aquilinum* leaf-, stem-, and root-based coils evoked mortality rates comparable to the permethrin-based positive control (57, 50, 41, and 49 %, respectively). Furthermore, the antiplasmodial activity of *P. aquilinum* leaf extract and green-synthesized AgNP was evaluated against CQ-resistant (CQ-r) and CQ-sensitive (CQ-s) strains of *P. falciparum*. IC₅₀ of *P. aquilinum* were 62.04 g/ml (CQ-s) and 71.16 g/ml (CQ-r); *P. aquilinum*-synthesized AgNP achieved IC₅₀ of 78.12 g/ml (CQ-s) and 88.34 g/ml (CQ-r). Overall, our results highlighted that fern-synthesized AgNP could be candidated as a new tool against chloroquine-resistant *P. falciparum* and different developmental instars of its primary vector *An. stephensi*. Further research on nanosynthesis routed by the LC/MS-identified constituents is ongoing.

Keyword: Anophelinae; Antioxidant; Fecundity; Longevity; Mosquito-borne diseases; Nanosynthesis; Nanotechnology; Smoke toxicity