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Single-step biosynthesis and characterization of silver nanoparticles using *Zornia diphylla* leaves: A potent eco-friendly tool against malaria and arbovirus vectors

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ABSTRACT

Mosquitoes (Diptera: Culicidae) are vectors of important pathogens and parasites, including malaria, dengue, chikungunya, Japanese encephalitis, lymphatic filariasis and Zika virus. The application of synthetic insecticides causes development of resistance, biological magnification of toxic substances through the food chain, and adverse effects on the environment and human health. In this scenario, eco-friendly control tools of mosquito vectors are a priority. Here single-step fabrication of silver nanoparticles (AgNP) using a cheap aqueous leaf extract of *Zornia diphylla* as reducing and capping agent pf Ag^{+} ions has been carried out. Biosynthesized AgNP were characterized by UV–visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive spectroscopy (EDX) and X-ray diffraction analysis (XRD). The acute toxicity of *Z. diphylla* leaf extract and biosynthesized AgNP was evaluated against larvae of the malaria vector *Anopheles subpictus*, the dengue vector *Aedes albopictus* and the Japanese encephalitis vector *Culex tritaeniorhynchus*. Both the *Z. diphylla* leaf extract and Ag NP showed dose dependent larvicidal effect against all tested mosquito species. Compared to the leaf aqueous extract, biosynthesized Ag NP showed higher toxicity against *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus* with LC_{50} values of 12.53, 13.42 and 14.61 μg/ml, respectively. Biosynthesized Ag NP were found safer to non-target organisms *Chironomus circumdatus*, *Anisops bouvieri* and *Gambusia affinis*, with the respective LC_{50} values ranging from 613.11 to 6903.93 μg/ml, if compared to target mosquitoes. Overall, our results highlight that *Z. diphylla*-fabricated Ag NP are a promising and eco-friendly tool against larval populations of mosquito vectors of medical and veterinary importance, with negligible toxicity against other non-target organisms.

1. Introduction

Arthropods are dangerous vectors of important pathogens and parasites, which may hit as epidemics or pandemics in the increasing world population of humans and animals. Among them, mosquitoes (Diptera: Culicidae) represent a key threat for millions of organisms worldwide, since they act as vectors for important parasites and pathogens, including malaria, dengue, filariasis and Zika virus [1,2]. The control of Culicidae vectors is a crucial prevention tool. Mosquito larvae are usually targeted using organophosphate insecticides, insect growth regulators and microbial control agents. Indoor residual spraying and insecticide-treated bed nets are also employed to reduce transmission of mosquito-borne disease in tropical countries [3,2]. However, the use of synthetic pesticides often lead to high operational costs, negative impacts on human health and the environment, and genetic resistance in a number of vector species [4]. Concerning biological control, several fish species (e.g. guppy, *Poecilia reticulata*; mosquito fish, *Gambusia affinis*) have been used as effective predators of mosquito larvae [5]. However, introduced larvivorous fishes are often considered a threat to native aquatic fauna, including amphibians [6,7,8,9,10,11], highlighting the need to carefully consider the ecological cost of species introductions intended to contribute to mosquito control [12,13]. In this scenario, effective and eco-friendly control methods of mosquito vectors are urgently needed.

In latest years, major efforts have been made to evaluate plant-borne larvicidal, pupicidal, ovicidal, adulticidal, oviposition deterrent and adult repellent compounds. A number of botanicals have been reported

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as highly effective against different mosquito vectors, even when employed at low doses [14,15,16,17,18]. Furthermore, the plant-mediated biosynthesis (i.e., green synthesis) of nanoparticles offers significant advantages over chemical and physical methods, since it is cheap, needs only single steps, and does not require high grades of pressure, energy, and temperature or the use of highly toxic chemicals [19,20]. In latest years, a growing number of plants fungi have proposed for efficient and rapid extracellular synthesis of metal nanoparticles, which act as effective mosquitocidals [21,22,23], also in field conditions [24]. Notably, while extensive efforts have been conducted to investigate non-target effects of nanoparticles against aquatic organisms [25,26,27,28], moderate efforts have been done to shed light on the biotoxicity of green-synthesized nanoparticles against aquatic arthropods sharing the same ecological niche as mosquitoes [29,30,31].

Zornia diphylla (L.) Pers. (Fabales: Fabaceae) is a prostrate herbaceous flowering plant. Leaves are digitately 2-foliolate; leaflets up to 2.5 cm long, lanceolate, dotted with black glands. Flowers are yellow, enclosed in leafy bracts four in spikes. Pods are with three to six joints, densely prickly. This plant grows as a weed in South India. The ethnomedical literature shows that *Z. diphylla* reported that this species is widely used to treat dysentery, venereal diseases and also to induce sleep in children [32]. In this study, we reported a method to biosynthesize silver nanoparticles (Ag NP) using the aqueous leaf extract of the *Z. diphylla*, a cheap and eco-friendly material acting as reducing and stabilizing agent. Ag NP were characterized by UV–vis spectrophotometry, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray analysis (EDX) and transmission electron microscopy (TEM). The aqueous extract of *Z. diphylla* and the synthesized Ag NP were tested for their larvicidal potential against the malaria vector *Anopheles subpictus*, the dengue vector *Aedes albopictus* and the Japanese encephalitis vector *Culex tritaeniorhynchus*. Furthermore, we evaluated the biotoxicity of *Z. diphylla* aqueous extract and green-synthesized Ag NP on non-target aquatic organisms sharing the same ecological niche of *Anopheles* and *Aedes* mosquito vectors.

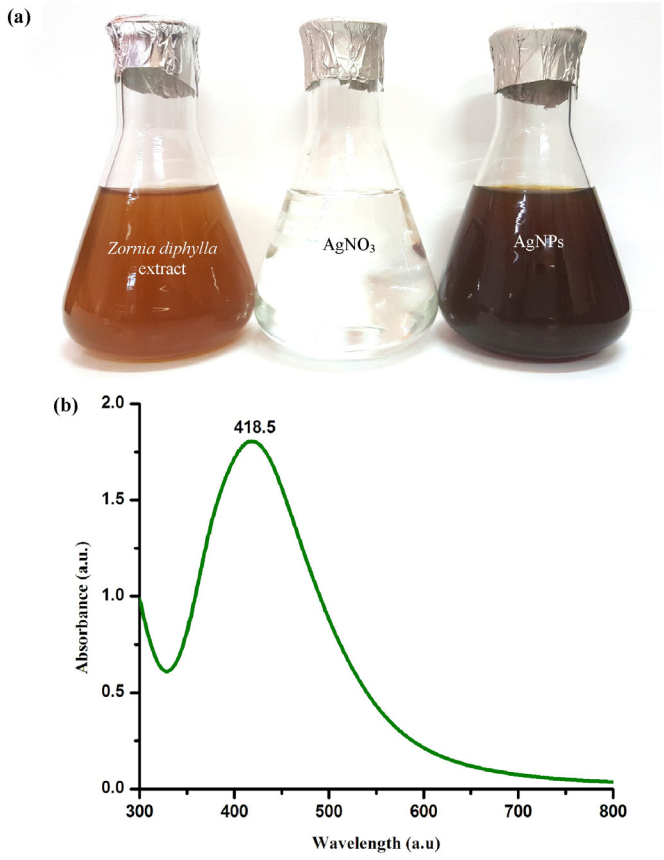

Fig. 1. (a) Color intensity of *Zornia diphylla* aqueous extract before and after the reduction of silver nitrate (1 mM). The color change indicates Ag^{+} reduction to elemental nanosilver. (b) UV–visible spectrum of silver nanoparticles after 180 min from the reaction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and Methods

2.1. Materials

Healthy and fresh leaves of *Z. diphylla* were collected from Nilgiris, Western Ghats (11° 10′N to 11° 45′ N latitude and 76° 14′E to 77° 2′ E longitude), Tamil Nadu State, India. The identity was confirmed at the Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu. Voucher specimens were numbered and kept in our research laboratory for further reference (AUDZ-210). Silver nitrate was purchased from Qualigens Fine Chemicals (Thermo Fisher Scientific, Mumbai, India).

2.2. Preparation of Plant Leaf Extract

The leaves of *Z. diphylla* were dried in the shade and ground to fine powder in an electric grinder. Aqueous extract was prepared by mixing 50 g of dried leaf powder with 500 ml of water (boiled and cooled distilled water) with constant stirring on a magnetic stirrer. The suspension of dried leaf powder in water was left for 3 h and filtered through Whatman no. 1 filter paper and the filtrate was stored in an amber-colored airtight bottle at 10 °C temperature until testing.

2.3. Synthesis of Silver Nanoparticles

The broth solution of fresh leaves was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300-ml Erlenmeyer flask along with 100 ml of sterilized double-distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered with Whatman filter paper no. 1, stored at −15 °C and tested within a week. The filtrate was treated with aqueous 1 mM $AgNO_3$ (21.2 mg of $AgNO_3$ in 125 ml of Milli-Q water) solution in an Erlenmeyer flask and incubated at room temperature. Eighty-eight milliliters of an aqueous solution of 1 mM silver nitrate was reduced using 12 ml of leaf extract at room temperature for 10 min, resulting in a brown–yellow solution indicating the formation of Ag NP.

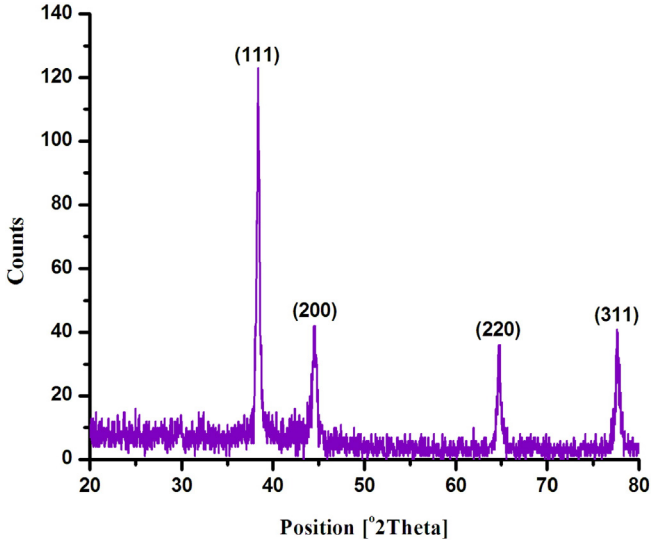

Fig. 2. XRD pattern of silver nanoparticles synthesized using *Zornia diphylla* aqueous extract. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)