

Curcuma aromatica mediated biosynthesis of silver nanoparticles and its larvicidal activity against Anopheles sp

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Abstract

Diseases transmitted by mosquitoes like malaria, dengue, and chikungunya remain significant public health threats, especially in the developing world. Not only do they impact human populations but also ecological systems. The widespread use of chemical insecticides has resulted in resistance among mosquito populations as well as environmental risks. Consequently, there is an increasing desire to use more environmentally friendly alternatives, particularly those that are plant-based phytochemicals. When combined with nanotechnology, phytochemicals become more biologically effective. Of all the nanomaterials, silver nanoparticles (AgNPs) possess high antimicrobial and insecticidal activities and are good candidates for use in controlling mosquitoes. AgNPs were synthesized in this research using aqueous *Curcuma aromatica* rhizome extract. AgNPs formation was attested by UV-Vis spectroscopy with a surface plasmon resonance peak at 417 nm. FT-IR revealed the roles of phytochemicals in reducing and stabilizing the nanoparticles. Elemental silver presence was attested by EDAX, while TEM and FESEM analysis indicated particle sizes of 30 to 70 nm with a predominantly spherical shape. Larvicidal activity against *Anopheles* larvae obtained from stagnant domestic wastewater was evaluated. The synthesized AgNPs were found to possess outstanding larvicidal activity with an LC_{50} value of 3.76 mg/L after 24 hours of exposure, which reflects strong dose-dependent toxicity. The research confirms that AgNPs synthesized through *Curcuma aromatica* offer a very good, eco-friendly, and low-cost alternative to synthetic insecticides. These nanoparticles hold vast potential in integrated mosquito control programs and disease prevention, sustainably.

1 Introduction

The mosquito family is the most significant class of insects in terms of its impact on overall health. Mosquito-borne diseases are widespread because of favorable ecological conditions. Mosquito-borne diseases have a financial burden, since they decrease commercial and labour productivity, especially in countries with tropical and subtropical climates [1]. The situation is getting worse further due to drug resistance, unregulated population growth, poverty, inadequate public health services, and weak health infrastructure. Just 10% of the estimated 4000 mosquito species that exist are believed to be capable of transmitting diseases that have a substantial direct and indirect impact on human welfare and health. A significant contributor to human mortality remains diseases spread by mosquitoes. Mosquitoes are known to carry a variety of diseases. There are many different types of habitats where mosquitoes can reproduce, including ponds, marshes, ditches, pools, sewers, and other similar water reservoirs. Many genera have shown specific breeding preferences. While *Culex* and *Mansonia* may also be found in polluted habitats, such as septic tanks, *Aedes* breeds in homes, peri-domestic areas, and other tiny water collections, such as desert coolers. Freshwater areas are associated with some *Anopheles* species. In terms of public health, mosquitoes are the most important group of insects because they transmit diseases such as malaria (*Anopheles*), filariasis (*Culex*, *Mansonia*), and dengue (*Aedes aegypti*), which cause millions of fatalities each year [2].

In recent years, synthetic insecticides have been widely employed to control mosquito populations, upsetting the natural biological controls and leading to resurgences in mosquito populations. Moreover, it has prompted the development of resistance in vector species [3]. Environmental and human health issues were sparked by biologically amplifying harmful compounds along the food chain, negative impacts on environmental quality, and undesired effects on creatures that weren't intended to be affected [4], which began exploring for other control strategies. As a result, research into more effective and sustainable mosquito control techniques seems highly promising. While it is slow, biological management is cheap, safe for ecosystems and living things, and may be successful for a very long period. It does not totally eradicate disease or infections, but rather returns them to their natural equilibrium [5]. Among the biological solutions, plants and plant extracts seem to be the best choice. The "Chemical factories" of nature are plants. The use of natural product chemistry with nanotechnology to lower mosquito populations during their larval stage can offer a variety of additional vector control effects. A range of plants, including herbs, shrubs, and large trees, were used to extract mosquito toxins. The plant families Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae, and Rutaceae all have a variety of larvicidal, adulticidal, or repellent qualities that they may utilize on different mosquito species. In recent years, in addition to their direct use as phytoextracts, nanotechnology have become more and more popular as biocontrol agents against insects and microbes. Plant-generated nanoparticles are more stable and synthesized more quickly than those made by microorganisms because of the variety of metabolites present in plant products and/or extracts [6, 7]. As a result, due to their distinct physical, chemical, and biological features, biologically synthesised AgNps are turning into one of the fastest-growing materials for improvised approaches [8].

More focus is now being placed on biological production of nanoparticles as a result of a growing need to develop environmentally acceptable materials for green synthesis. Several plant species have been employed in this strategy. Hence, utilising *Curcuma aromatica* rhizome-extract, the current study offers a natural approach for making stable, bioactive AgNPs that are effective against *Anopheline* mosquitoes collected and reared in household water.

2 Materials and methods

2.1 Preparation of aqueous extract from the rhizomes of *Curcuma aromatica*

For the preparation of the aqueous extract fresh rhizomes were collected directly from the farmers around Erode district, Tamil Nadu, India, and dried in light-free conditions. The dried rhizomes were cut into small pieces and ground to a coarse powder using a blunder. The powder was further boiled in tripe-distilled water at a ratio of 1:10 (w/v) under agitation at $60 \pm 2^\circ\text{C}$ for 45 min. The boiled residue was then left covered for 30 min and filtered using a muslin cloth. The filtered solution was further centrifuged (10,000 rpm, 30 min, 4°C) to remove any excess debris. Supernatants were then collected and filtered

through Whatman no.1 filter paper. The filtered solution was further lyophilized to remove water and a fine powder was obtained, which is the aqueous extract used for further experiments.

2.2 Biosynthesis of silver nanoparticles (AgNPs)

The aqueous extract of *C. aromatica* was used in the reduction of Ag^+ ions to Ag^0 . Different concentrations of AgNO_3 were diluted from $1 \times 10^{-3}\text{M}$ stock solution using triple distilled water at room temperature. Further, different concentrations of aqueous extract of *Curcuma aromatica* was added and incubated in dark conditions. The change in the colour intensity of the solution was noted at different time intervals. The change in the colour from light yellow to dark yellow indicated the formation of AgNPs⁹ and this change was observed at 12h. The reduced solution was centrifuged at 8000 rpm for 20 min and the supernatant was discarded. The final residue was collected in a separate tube containing Millipore water. The prepared samples were further used for characterization and larvicidal activity.

2.3 Characterization of biosynthesized silver nanoparticles

The absorption maxima of the biosynthesized AgNP was determined using UV-Vis spectrophotometry (UV-2450 (Elico)). Using an FTIR spectrometer, the chemical makeup of the produced AgNPs was investigated (perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75°C , and the dried powders were characterized in the $4000 - 400\text{ cm}^{-1}$ range using the KBr pellet method. The shape and assembly of the AgNPs were determined using electron microscopic studies (SEM, Quanta FEG 450 TEM, JEOL JEM 2010). Further, to confirm the purity of the AgNPs synthesized EDX analysis was carried out to determine the level of AgNPs present and other impurities if any.

2.4. Mosquito larvae collection

The samples of stagnant water were gathered from roadside ditches, and domestic wastewater drains near school and college canteen drains in and around Gandhi Nagar, Adyar, Chennai, using sterile wide-mouth containers.

2.5 Identification and classification of mosquito larvae

The malaria-transmitting *Anopheles* sp. mosquito larvae were removed from samples of still water and identified morphologically using the Atlas of Mosquito handbook. According to protocol, the newly found *Anopheles* sp. mosquito larvae were cared for and reared in a lab environment using tap water in plastic and enamel trays [11].

2.6 Mosquito Larvicidal Bioassay

The WHO13-recommended procedures for assessing the toxicity of biologically produced nanoparticles and the susceptibility of mosquito larvae to pesticides were followed. The larvicidal bioassays were conducted at room temperature. Fourth instar larvae were randomly inserted into 200 ml of sterilized double-distilled water and then placed in an environmental chamber at 27°C with a 16: 8 h light/dark cycle. All twenty-five 4th instar larvae were exposed to AgNPs over some time, and this allowed researchers to assess their efficiency as mosquito larvicides. The larvae were divided into four tiny

specimen bottles each holding 25ml of distilled water, and each of the extract concentrations was then applied to the larvae in a total volume of 200ml of distilled water, which was taken in 500ml bowls. Double-distilled water was used as a solvent to dilute the nanoparticle solutions to the necessary concentrations (2.0, 4.0, 5.0 mg/L). Four trials, each with four repetitions, were conducted at each concentration under test, and the anti-larval effects of the control (aqueous plant extracts) were also evaluated. The larval mortalities on *Anopheles* sp. larvae in the fourth instar were assessed at 3, 6, 12, and 24 hours after exposure to estimate the acute toxicities. The number of dead larvae was recorded beginning with the first hour of exposure, and the percentage of mortality was calculated using an average of four replicates. Using Abbott's method, the data on larval mortality were adjusted to account for control mortality (Fig. 1).

3 Results and Discussion

While medical science has made strides, mosquitoes continue to play a significant role in the transmission of some of the most serious and deadly illnesses, such as encephalitis, filariasis, chikungunya, yellow fever, and dengue fever. Malaria, yellow fever, dengue fever, chikungunya, and chikungunya are some of these illnesses [10]. In recent years, synthetic insecticides have been widely employed to control mosquito populations, upsetting the natural biological controls and leading to resurgences in mosquito populations. It has also caused concerns about the environment and human health, stimulated the development of resistance, and had negative effects on organisms who are not the target, which began exploring other control strategies [11]. The creation of human-use nanoparticles with adjustable size, shape, chemical composition, and dispersion is the fundamental aim of nanotechnology. Ag, Pt, Au, and Pd in particular are some of the noble metals used to make nanoparticles that are now the focus of the bulk of studies [12]. Silver nanoparticles, one of the four metals, are essential in biology and medicine. There is an increasing need to create safe, non-toxic, and environmentally friendly (green chemistry) processes for nanoparticle production and assembly. Silver nanoparticles may be produced by plants, bacteria, fungus, and yeast by reducing silver ions both extracellularly and intracellularly. Drugs attached to nanoparticles are said to offer benefits over traditional versions of the drugs [13]. Drugs that are attached to nanoparticles have longer half-life in vivo, longer circulation durations, and can deliver a powerful dose of a medicine where it is required [14]. To obtain the required delivery characteristics, the drug nanoparticle's size and surface properties can be changed [15]. A high-localized concentration may be achieved where it is needed and adverse effects are decreased since the medicine attached to the nanoparticles cannot circulate widely [16]. Because nanoparticles have a huge surface area per unit mass, drug loading may be quite high. Drugs that are attached to nanoparticles dissolve easily in liquids and can penetrate deeply into organs and tissues. Because silver nanoparticles are thought to be promising agents for a variety of biological functions, including antimicrobial ones, they were examined as a mosquito larvicidal agent [17].

3.1 Biosynthesis and Characterization of AgNPs

Curcuma aromatica aqueous rhizome extracts were effectively used to create silver nanoparticles. A sophisticated network of enzymes and antioxidant metabolites coexist in plants in significant amounts to protect cellular components from oxidative damage. Plant extracts may act as reducing and stabilizing agents in the synthesis of nanoparticles. It is well acknowledged that the source of the plant extract influences the characteristics of the nanoparticles [18]. This is because various extracts have various combinations and quantities of organic reducing agents [19].

In order to enhance bio-reduction, an aqueous extract of the plant is frequently mixed with an aqueous solution of the pertinent metal salt. The reaction usually takes a few minutes to complete and occurs at room temperature. The bio-reduction process is somewhat difficult given the variety of chemicals involved [20]. Zingiberaceae family member *C. aromatica* is found growing wild across India and is mostly cultivated in Kerala and West Bengal. It has a long history of usage as an aromatic medicinal cosmetic and is a potential medication for medical use.

The change in colour of the solution containing AgNO₃ and the extract is a key indicator for the formation of nanoparticles. The color intensity is directly proportional to the surface plasmon resonance. In the present study, the UV-Vis absorption spectra showed a peak of absorption at 417 nm that matched with the peak of the surface plasmon resonance of silver (Fig. 2). The silver is characterized using spectrophotometric absorption measurements in the wavelength regions of 400–450 nm [21]. FTIR spectrum would identify the possible interactions between silver metal and phytoconstituents of the aqueous extract of *C. aromatica*. The band at 3300 cm⁻¹ indicates O-H stretching vibrations of the hydroxyl group, H-bonded alcohols, phenols, or N-H stretching of 1° and 2° amines and amides [22]. The weak band at 2942 cm⁻¹ corresponds to the C-H stretch of alkene or O-H stretch of carboxylic acids. Peaks at 2350 and 1628 cm⁻¹ bends correspond to the C = O stretch of unsaturated aldehydes, and ketones; the peak at 1409 cm⁻¹ is assigned to the O-H bend of different phytochemicals [23, 24]. Changes in the transmittance related to the bond with N atoms indicated that these atoms are the binding sites for the AgNPs on the protein. It is thus revealed from the FTIR spectrum that phytoconstituents such as carboxylic, amine, and phenolic groups might be involved in the reduction of silver ions (Fig. 3). The FESEM and TEM high magnification images showed a dispersed dense of AgNPs at different sizes ranging from 30 to 70 nm, with an average size of 50 nm (Figs. 4 & 5). The EDX image showed a strong peak at 3.5 keV for silver ions, confirming the reduction of silver ions by the phytoconstituents of *C. aromatica* aqueous extract. Other peaks observed in the EDX between the range 0–3 keV indicate the presence of carbon, hydrogen, and other phytoconstituents present (Fig. 4).

3.2. Larvicidal activity of AgNPs against *Anopheles gambiae* larvae

The result of the larvicidal efficacy of AgNPs against *Anopheles gambiae* larvae is shown in Table 1. It is evident from the results that silver nanoparticles may be able to successfully reduce *Anopheles gambiae* larvae. Silver nanoparticle treatment results in higher larval mortality as compared to aqueous extract. Aqueous extract and distilled water were shown to have little to no influence on larval mortality. After 12

hours of exposure, higher doses of the aqueous plant extract began to kill the larvae. After 12 hours of exposure, 2.0 mg/l of AgNPs lowered larvae survival to more than 75%, while 5.0 mg/l of AgNP killed the entire larval population. After 16 hours of exposure, over 98% of the larvae were dead due to the nanoparticle at 2.0 mg/l, which killed them slowly. The most effective concentration of silver nanoparticles was 5.0 mg/l (Table 1). The LC50 value of the anti-larvae activity of the aqueous extract of *C. aromatica* and AgNPs biosynthesized using *C. aromatica* were > 30 mg//L and 3.76 mg/L after 24 h of treatment. The larvae's mortality may be brought on by the nanoparticles' capacity to puncture through the membrane protecting it. Silver ions function larvicidal by slowly releasing silver ions through oxidation inside or outside of the cell. It is widely known that the permeability of membranes in microbial and other cells is altered by silver nanoparticles [25]. Silver nanoparticles may cause denaturation in some organelles and enzymes by adhering to phosphorus- or sulfur-containing materials like DNA or proteins. Cellular function is lost because of the decreased membrane permeability and the disruption of the proton motive force, which leads to cell death [26]. The efficacy of different extracts of *C. aromatica* against mosquito larvae has been reported [27]. According to the study, the petroleum ether extract exhibited LC50 and LC90 values of 11.42 and 18.00 ppm. Further, it proves that the AgNPs biosynthesized from *C. aromatica* have an appreciable mosquito larvae activity compared to its extract.

Table 1
Larvicidal activity of silver nanoparticles (AgNPs) biosynthesized from *Curcuma aromatica*

Extract/AgNP	Concentration (mg/L)	Mortality rate (%) at different time intervals				LC50 at 24 h
		3 h	6 h	12 h	24 h	
Aqueous extract	10	-		7	20	> 30 mg/L
	20	-	7	18	27	
	30	10	12	20	32	
AgNPs	2	10	30	74	100	3.76 mg/L
	4	18	54	81	100	
	5	27	76	100	100	

4 Conclusion

The most popular method for creating environmentally friendly, green nanoparticles is the manufacturing of such particles using plant extracts. The fact that plants are plentiful, easy to get, more-safer to handle, and serve as a source of numerous metabolites makes this strategy even more advantageous. Hence, plant extracts can be effectively used in the production of silver nanoparticles as an ecologically friendly way to combat the infectious vector agent. Also, the research aims to increase the possibilities for examining the physiological and molecular processes behind the larvicidal effect.

Abbreviations

AgNPs

Silver nanoparticles

UV

Ultraviolet visible

FT

IR-Fourier transform infrared spectroscopy

EDAX

Energy dispersive X-ray analysis

TEM

Transmission electron microscopy

FESEM

Field Emission Scanning Electron Microscopy

LC50

Lethal concentration 50

°C

Degree Celsius

Ag⁺

Silver ion

ml

Millilitre

mg/L

Milligram per liter

DNA

Deoxyribonucleic acid

Declarations

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Consent to participate Not applicable

Consent for publication Not applicable.

Competing Interests The authors declare that they have no competing interests.

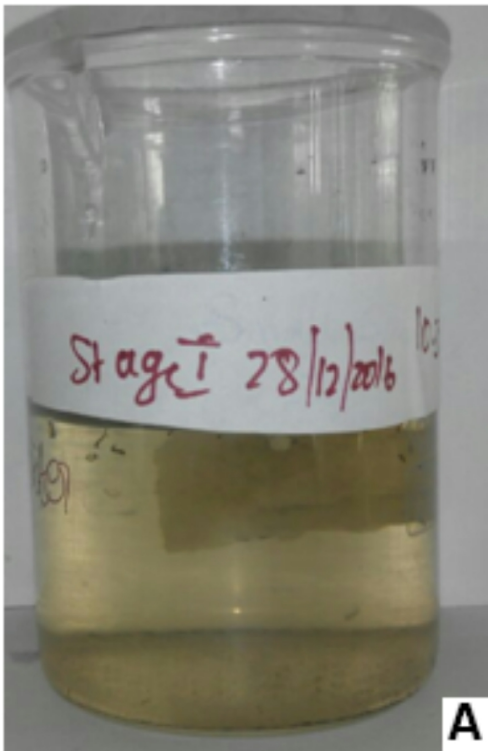
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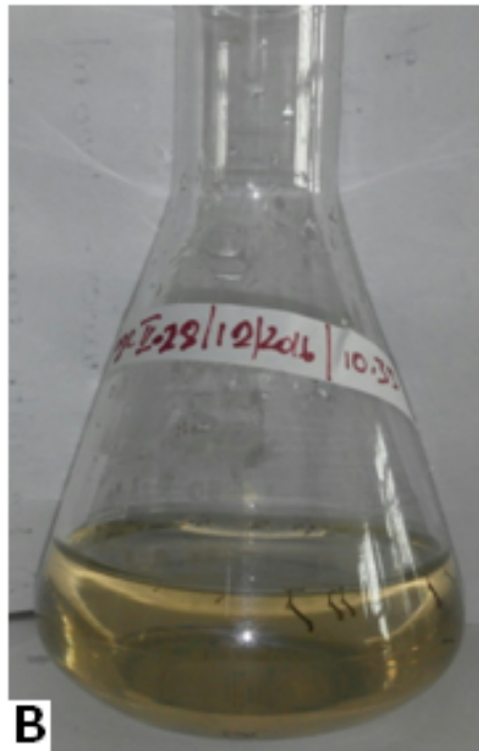
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Figures



A



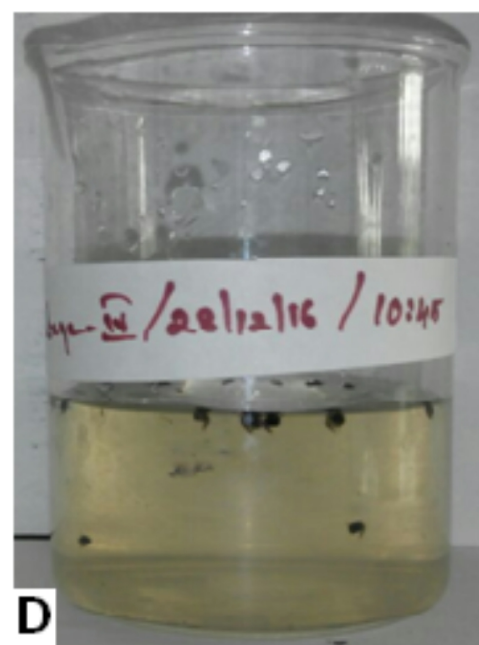
B

First instar larvae

Second instar larvae



C



D

Third instar larvae

Fourth instar larvae

Figure 1

Different stages of larval growth. A. first instar larvae, b. second instar larvae, c. third instar larvae, D. fourth instar larvae

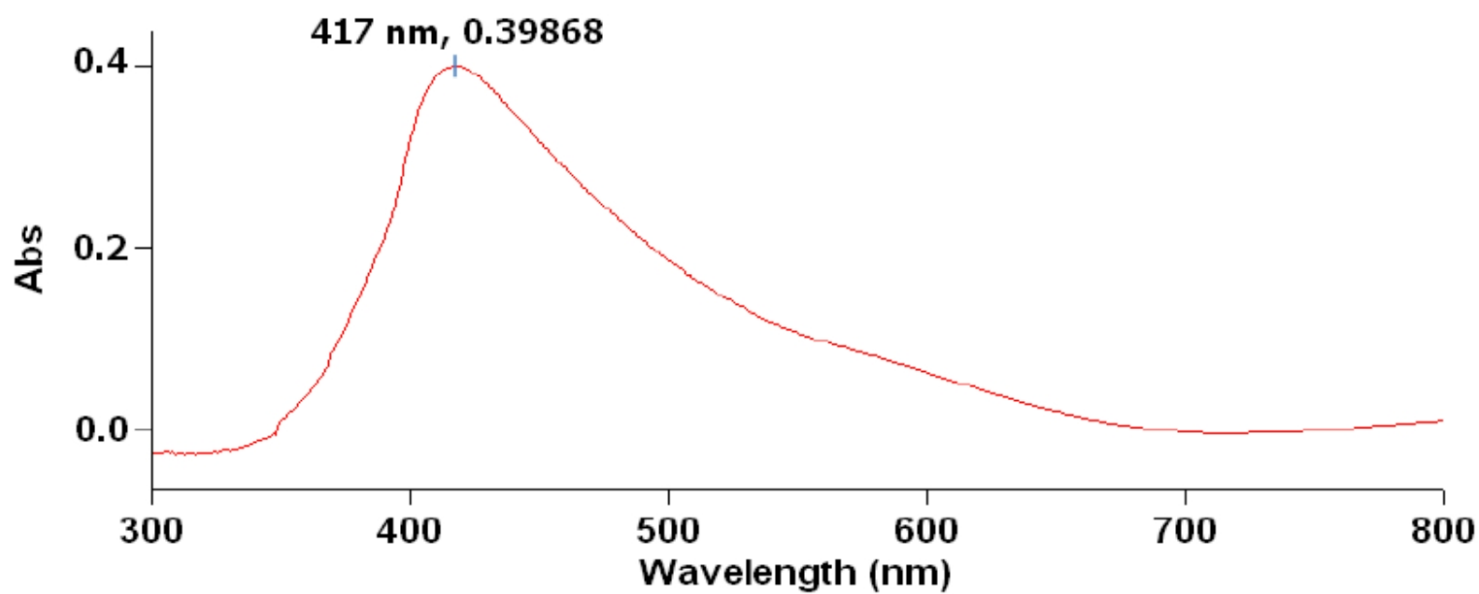


Figure 2

UV-VIS spectra of Silver Nanoparticles (AgNPs) biosynthesized from the aqueous extract of *Curcuma aromatica*

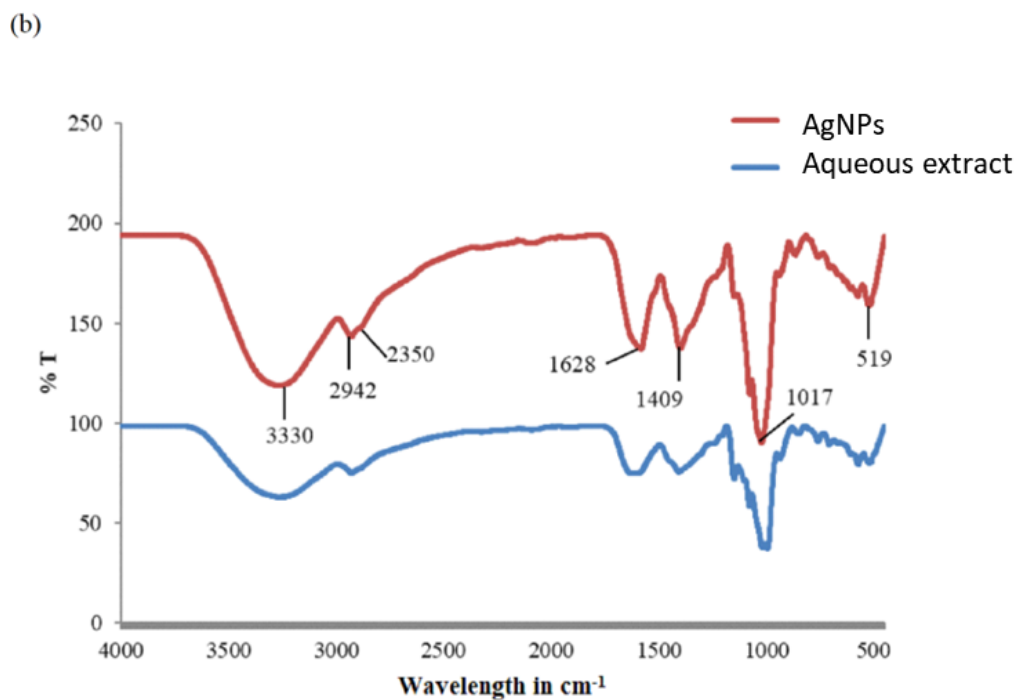
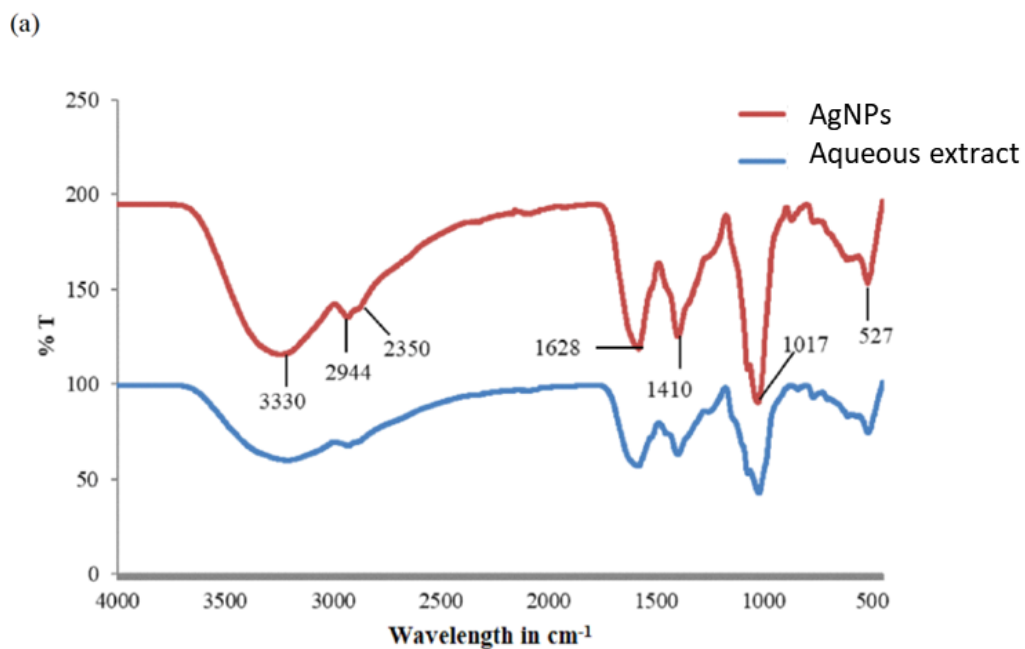


Figure 3

FTIR spectrum of aqueous extract of *Curcuma aromatica* rhizome, AgNPs solution formed the reaction of silver nitrate with aqueous extract of *C. aromatica* for 24 h (a) *C. aromatica* aqueous extract; (b) AgNPs biosynthesized from aqueous extract of *C. aromatica*

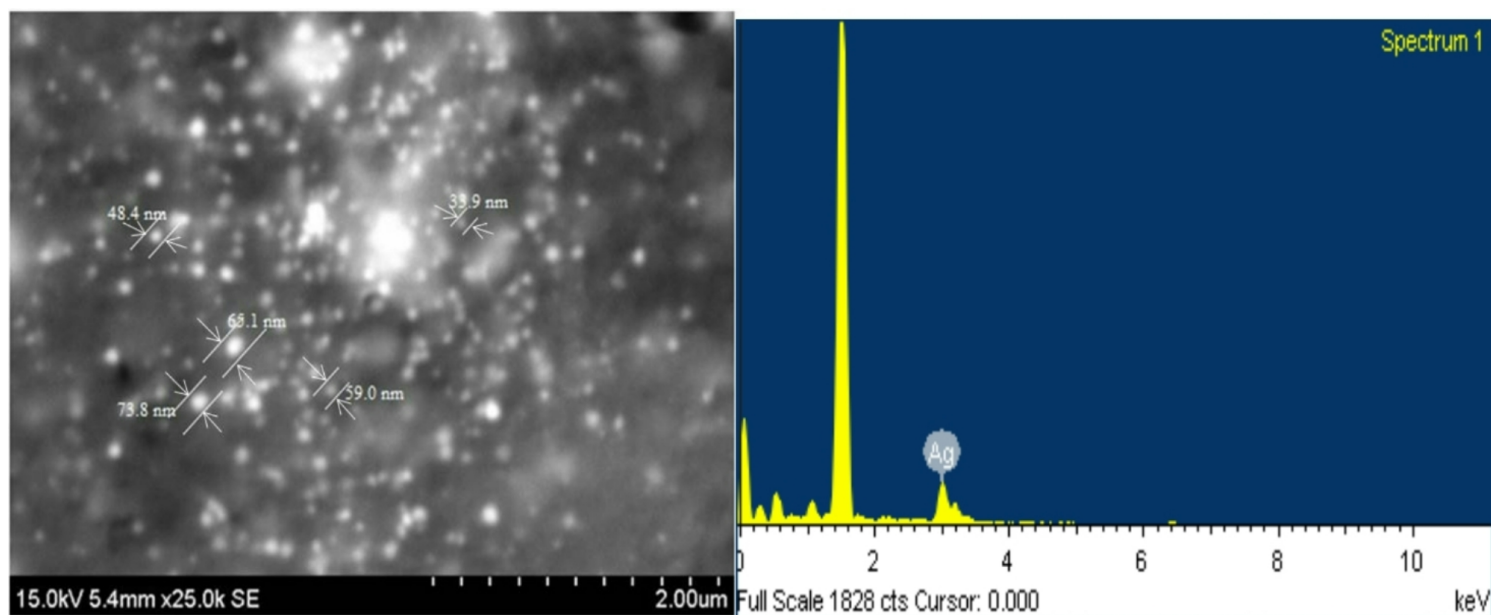


Figure 4

Scanning electron microscopic image of Silver Nanoparticles (AgNPs) biosynthesized from the aqueous extract of *Curcuma aromatica*

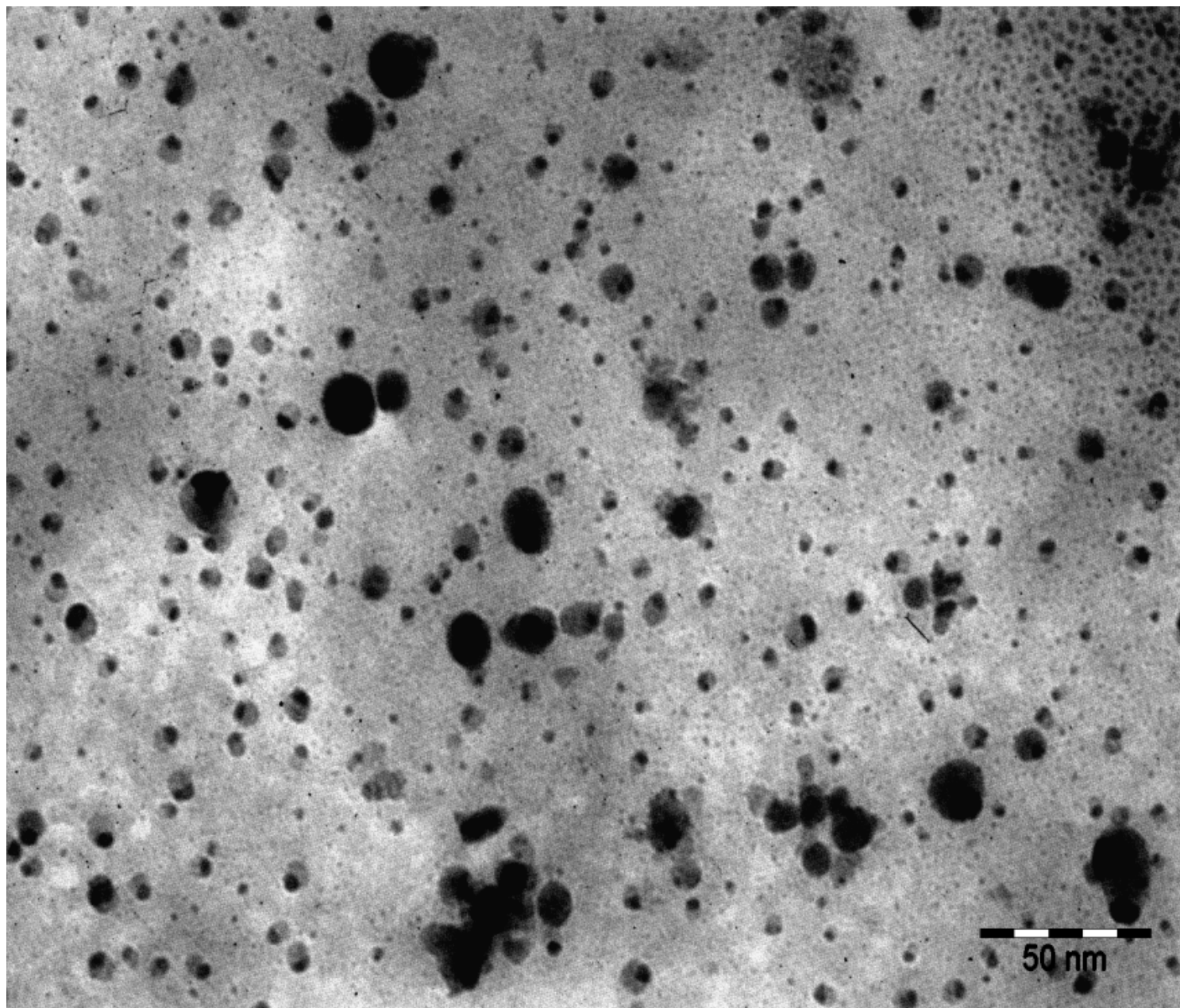


Figure 5

Transmission electron microscopic image of Silver Nanoparticles (AgNPs) biosynthesized from the aqueous extract of *Curcuma aromatica*