BIOINSPIRED SYNTHESIS OF SILVER NANOPARTICLES FOR THE CONTROL OF DENGUE AND MALARIA VECTORS

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The current study reported the biosynthesis of silver nanoparticles (AgNPs) from aqueous leaves extract of *Hippophae rhamnoides*, its characterization and mosquito larvicidal potential. The biofabricated AgNPs confirmation were characterized by UV-Visible Spectrophotometer, Fourier-transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM), Energy Dispersive X-ray (EDX), X-ray diffraction (XRD) and Thermogravimetric/Differential Thermal Analysis (TGDTA).The maximum larval mortality of AgNPs against *Aedes aegypti* with 600 ppm at 72 hrs incubation time was 93±0.96%. The *Anopheles stephensi* maximum larval mortality (%) was 95±0.56 for 72 hrs at 600 ppm. The probit analysis of biofabricated AgNPs was calculated that LC_{50} (24 hrs), LC_{90} (24 hrs), LC_{50} (48 hrs), LC_{90} (48 hrs), LC_{50} (72 hrs) and LC_{90} (72 hrs) against *Aedes aegypti* were 394.85, 1200.00, 312.86, 853.33, 267.50 and 605.89 ppm respectively and against *Anopheles stephensi* were 322.69, 988.10, 281.71, 743.15, 221.88 and 656.65 ppm respectively.

(Received November 12, 2018; Accepted January 18, 2019)

*Keywords:*Aqueous extract, Silver nanoparticles, Characterization, Aedes aegypti, Anopheles stephensi, Human health, Lethal concentration

1. Introduction

Millions of deaths every year are due to the transmission of serious human diseases. The application of man-made insecticides against vector mosquitoes results in hazards to the environment, developed insect's resistance as well as high production price. The formations of environment friendly compounds as insecticide against vector are of great importance in this part [1].The spreading risk of diseases and humans contact with vectors can only be reduced by effective mosquitocidal agents. The mosquitocidal compounds should be non toxic, non-irritating and should have effects for longer time [2].

Nanotechnology makes a well-off involvement of biotechnology and biomedical technology by producing numerous products and devices [3]. The most important benefit of using plant extracts for nanoparticles (NPs) synthesis was that they were safe, effortlessly available, harmless and nontoxic in most cases [4].

Pathogenic vectors responsible for different ailment including dengue, Japanese encephalitis, chickungunya, filariasis and malaria are commonly caused by a number of mosquito species which belong to the Culex, Aedes and Anopheles genera [5]. Mosquito control programmes were established in various countries but slight development in the malaria management and infections can be seen, causing financial and human loses [6]. Due to increase pesticides resistance, unavailability of vaccines and medicines, it was very difficult to control mosquito born diseases [5]. The current study was designed to biofabricate the silver nanoparticles (AgNPs) from aqueous leaves extracts of *Hippophae rhamnoides* Linn, its characterization and control of mosquito vectors *Aedes aegypti* and *Anopheles stephensi* of dengue and malaria.

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2. Experimental

2.1. Plant collection

The fully matured healthy leaves of *Hippophae rhamnoides* were collected from Pakistan Council of Scientific and Industrial Research (PCSIR) Skardu Gilgit Baltistan, Pakistan. The leaves were shade dried and powdered with a laboratory mill.

2.2. Leaves extraction

Twenty five gram of the leaves powdered were weighed and kept into 1L conical flask containing 500 ml double distilled water, well mixed and then boiled for 25 min. The extract obtained was filtered through muslin cloth and then filtered through filter paper (Whatman No.1).

2.3. Bioinspired synthesis of AgNPs

Plant leaves extract (5 ml) was mixed with 95 ml aqueous solution of 1 mM $AgNO_3$ and heated on horizontal shaking water bath at 75 °C for 60 min in a dark room. Reduction of $AgNO_3$ to silver ions was confirmed by change in color from colorless to brown. The fully reduced solution was concentrated on rotary evaporator (R-200, Buchi Rotavapor, Switzerland) on 50°C. The concentrated AgNPs were dried in an oven overnight at 50°C and grinded in mortar and pestle.

2.4. Characterization

The silver ions reduction confirmation was carried out by UV-Visible Spectrophotometer UV-1700 (Shimadzu, Japan) spectrum of the reaction solution after cooling at room temperature. It was measured by mixing 20 ml distilled water with 1ml sample reduced solution (AgNPs).The IR spectrum was obtained using FTIR Prestige -21 Shimadzu Japan using IR solution software. Scanning Electron Microscopic (SEM) images was carried out by JSM-5910 (JEOL, Japan) machine. The AgNPs elemental composition study was carried out with EDX model INCA 200, Oxford Instruments UK. X-Ray Diffractometer measurement of the prepared AgNPs was carried out by model JDX-3532, JEOL Japan. Diamond TG-DTA Perkin Elmer Instrument USA was used to evaluate the thermal crystallization and decomposition temperature of the AgNPs.

2.5. Larvicidal activity

Mosquitoes *Aedes aegypti* (*A. aegypti*) and *Anopheles stephensi* (*A. stephensi*) were reared in the Food Technology Center, PCSIR Peshawar, Khyber Pakhtunkhwa-Pakistan. The mosquito's vectors cyclic generations were under specified conditions of relative humidity i.e. 80-90% and temperature i.e. 25-29 °C in insectarium. The food used for the growth of larvae consisted of yeast and powdered dog biscuits (1:3). The larvicidal activity test was carried out by described method [7]. Twenty-five instar (IV) larvae of *A. stephensi* and *A. aegypti* were transferred to plastic cups (500 ml) having 249 ml (distilled H_2O) and 1 ml of sample from the desired concentration. Four replicates for each concentration were set up. Distilled H_2O having 25 larvae were used as a control. Abbott's formula (1987) [8] was applied to correct the control mortality and (Finney 1971) [9] probit analysis were used to calculate regression equation, LC_{90}, LC_{50} and Chi-square value (X2).

$$\textbf{\% Mortality} = \frac{\%\text{Mortality(sample)} - \%\text{Mortality(control)}}{100 - \%\text{ Mortality (control)}} \times 100$$

2.6. Statistical study

Statistical analysis of the study records were carried out with the help of SPSS to find the mean, standard deviation (±SD), LC_{50}, LC_{90}, regression equations and Chi-square value (X^2) values.

3. Results and discussion

3.1.UV-Visible spectroscopy

The AgNPs synthesis confirmation was carried out by a spectrum in visible range peak broadening indicated that the nature of particles (polydispersed) and the maximum absorbance occurs at 435 nm (Fig.1). However there was no broadening of peak absorption in to the leaves extract because NPs and $AgNO_3$ were absent (Fig. 2). The maximum absorption peak occurs at 435 nm representing AgNPs as previous research finding [10, 11, 12].

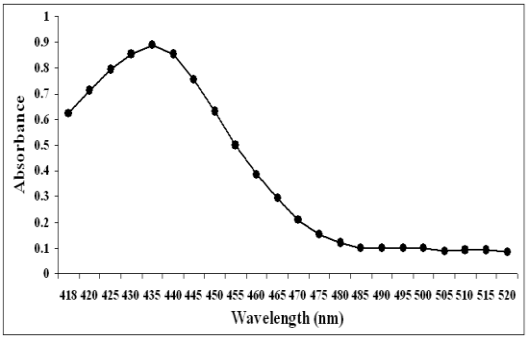

Fig. 1. UV-VIS Absorption spectra of AgNPs.

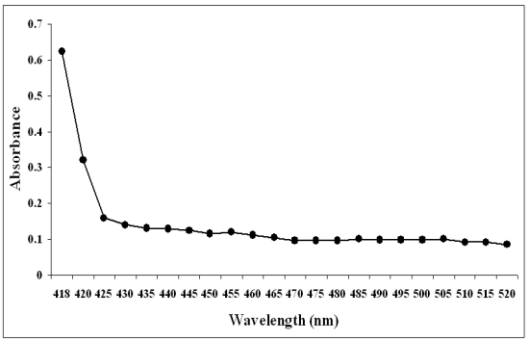

Fig. 2. UV -VIS Absorption spectra of plant extract.

3.2. FTIR analysis

Hippophae rhamnoides leaves powder FTIR spectra showed the presence of alkenes, nitrates, amino acids, organic halogen compounds, carbohydrates and ethers (Fig. 3). The prepared NPs were analyzed by FTIR to determine capping ligand of AgNPs which play a role as stabilize agent. FTIR spectrum of prepared AgNPs is visually shown (Fig. 4). The peaks values of 3309.85 cm^{-1} corresponds to hydroxyl compound, band at 2918.30 and 1708.93 cm^{-1} represent the carbonyl groups, similarly the other lower values corresponds to aldehyde and alkynes groups. It was clear that synthesized AgNPs were bounded by metabolites and proteins. FTIR peaks measurements were intended for the identification of responsible suitable biocomponents to reduce Ag^{+2} ions and capping of the bioreduced synthesize AgNPs. Carbonyl group [13] form amino acid and proteins residues were powerful affinity to fasten through metal demonstrating that the proteins may be responsible to form a film to cover the metal NPs (i.e., capping of AgNPs) to stabilize the medium and prevention of agglomeration. In our finding (Fig. 3) it was confirmed that plant leaves have carbonyl group and performed the same function. Phenolic compounds [14] in the leaves extract might improve the reduction mechanism and the occurrence of these ingredients in biological synthesis of NPs solution may act as stabilizing or capping components.

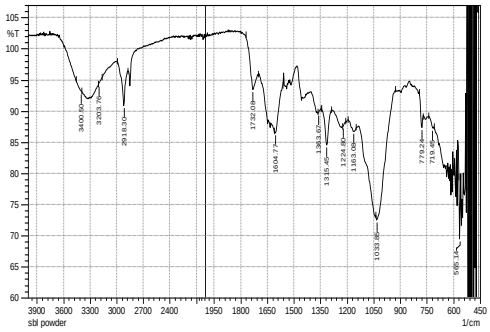

Fig. 3. FTIR of H. rhamnoides leaves powder.

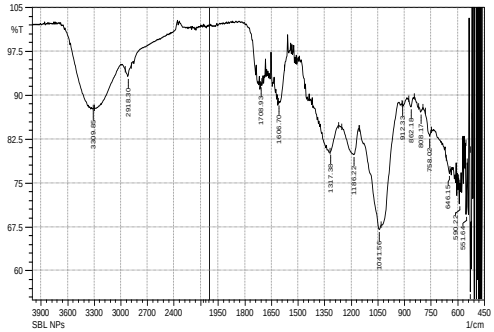

Fig. 4. FTIR spectrum of AgNPs.

3.3. SEM and EDX analysis

The images showed (Figs. 5 and 6) that AgNPs found in a number of aggregates and also in individual identity. The prepared AgNPs spherical shaped (round) and aggregate into bigger round shape distinct shape. The SEM analysis showed that the prepared AgNPs were spherical in shapes and size in the range (135- 300 nm). The EDX pattern of spectrum recorded high signals for silver. Elemental composition of AgNPs showed that besides silver other elements (Fig. 7) were also detected. The bright and sharp signals of silver correspond to the peaks in the graph confirm the formation of AgNPs. In the current study the SEM images of AgNPs were not in straight make contact with even the aggregations, indicating that proteins secreted by plant leaf extracts are the capping agent to stabilize the NPs. The EDX technique is utilize to determine the amount of silver as well as to identify additional elementary compositions in the prepared NPs. The occurrence of bioactive compounds as capping agent to AgNPs may be come from leaves extracts [15]. The AgNPs indicate an optically absorb band peaking at 3 keV which was the identification absorption peaks of AgNPs [16, 17, 18, 19, 20]. The elemental analysis other than silver showed that these elements come from the leaves aqueous extract.

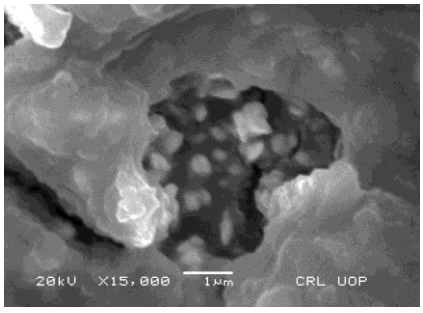

Fig. 5. SEM micrograph of AgNPs.

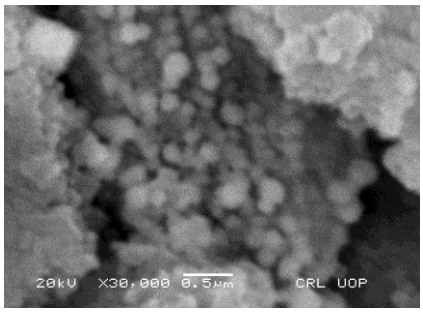

Fig.6. SEM micrograph of AgNPs.

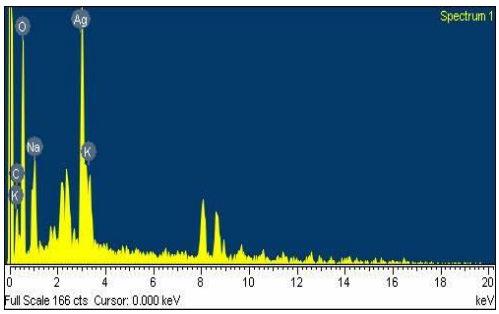

Fig. 7. EDX Spectrum of AgNPs.

3.4. XRD analysis

XRD pattern of the synthesized AgNPs revealed the presence of sharp diffraction lines at 10° to 70°, showing four strong peaks in the entire spectrum. The AgNPs have peaks of Ag at 2θ= 34.24°, 38.12°, 44.32° and 64.52°. XRD peaks clearly illustrate that the AgNPs in the current study are crystalline in nature (Fig. 8). The XRD technique is used to study the nature and crystal size of NPs. There are no peaks observed for the impurities in the XRD pattern is a sign of the purity of the AgNPs. The patterns of XRD at 2θ were 38.08, 32.96, 32.22 and 15.3 and 32.94. No peaks were recorded for the contamination in the XRD spectrum proving that the synthesized AgNPs were of high purity [21]. These findings are a close agreement to our prepared AgNPs in which the same sharp peak (38.12°) was occurred.

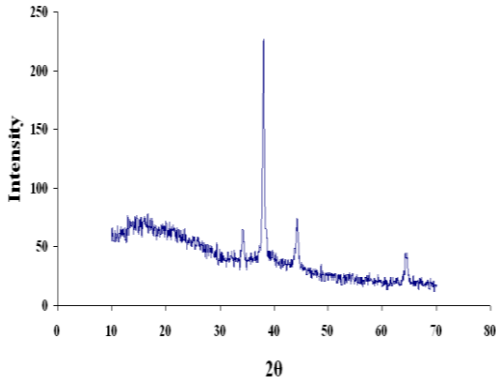

Fig. 8. XRD Analysis of AgNPs.

3.5. TG-DTA analysis

The recorded TGA and DTA spectra were carried out in the range of temperature as of 44.3241°C to 910.5593°C. The TGA curve (Fig. 9) shows that at 50.724°C, 70.034°C and 131.198°C the weight reduction of AgNPs were 8.532 mg, 8.484 mg and 8.191 mg respectively, which demonstrate no significant weight reduction. While prominent weight reduction footstep starting from 194.027°C till to 588.531°C, which showed that in this temperature range, weight was reduced from 7.601 mg to 1.002 mg, but no extra loss of weight was recorded at beyond 600°C. The loss of weight was correlated to the organic matrix ignition, which was present in the prepared AgNPs, acting as a cap and stabilizing agent. The DTA curve (Fig.9) is showing that an exothermic peak was detected between 400 to 607 °C with a maximum at about 500 °C. It was concluded that the thermal processes be able to be linked through the burnout of organic matrices occurred in the *H. rhamnoides* leaves aqueous extract and of the left over carbon residue. The main loss of weight of the sample happened in temperature between 200 and 300°C. Almost there was zero weight reduction beneath 200°C and over 300°C. [22]. It was assumed that the thermal activity can be related with the burnout of organic matter involved in the precursor powders [23]. The DTA curve [24] exhibits a wide peak in the temperature range 220-350 °C, probably linked with the removal of bound organics and water. The TGA plot of the AgNPs [25] observed a stable weight failure in the 0-100 °C temperature due to loss of moisture. These studies are a close agreement to our findings.

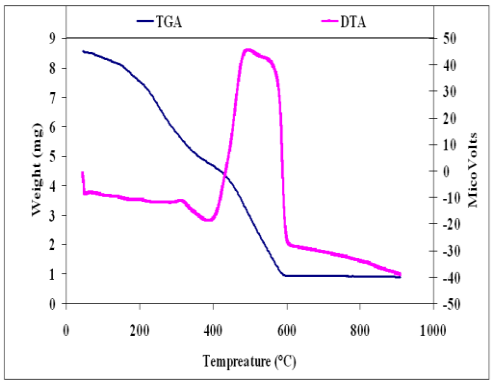

Fig. 9. TG/DTA curves of AgNPs.

3.6. Mosquito vectors of Dengue and malaria

Percent larvicidal activity of AgNPs against *A. aegypti* is shown in Fig.10. The minimum mortality was 27±0.50% at 24 hrs with 200 ppm and maximum mortality was 93±0.96% at 72 hrs with 600 ppm.

Larvicidal activity (*A. stephensi*) of AgNPs is presented in Fig. 11. The minimum mortality was 31±0.50% for 24 hrs at 200 ppm and maximum mortality was 95±0.56 for 72 hrs at 600 ppm. Silver nitrate and control (distilled water) were found no mortality. The results concluded that mortality increases when the concentration of AgNPs were increased. The probit analysis of bioinspired synthesized AgNPs is presented in Table 1.

The AgNPs may penetrate through the larval membrane and rupture the midgut epithelium due to interaction with cell molecules of some organelles resulting in death of larvae [26]. Evidenced showed that AgNPs caused loss of cellular functions as well as the proton motive force essential for ATP construction [27]. The correct mechanism of NPs has been documented that from blood circulation the NPs come out, instantly intermingle with the intestinal fluid and extarcellular matrix and finally make interactions with cells, lymphatic system and peripheral tissues leading injure them by blocking the systems [28]. The biologically reduced silver ion primary interact with cytoplasm in the interior of the cell wall and denature the ribosome, finally suppressed the expression of enzymes and proteins essential to ATP production leading to disruption of the cell [29]. The reasons by which the mortality of larvae could occur are the NPs

ability to penetrate in the course of membrane larvae. In the intracellular space AgNPs be able to attach to phosphorus containing molecules (DNA) or S-containing proteins, leading to the deterioration of some enzymes and organelles. After, disorder in proton drive force and reduction in membrane permeability leads the failure of organelles function and lastly cell death [30].

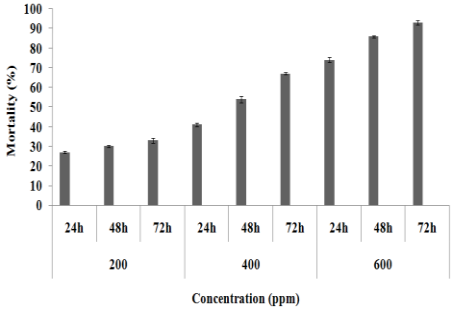

Fig.10. Mortality of NPs against A. aegypti.

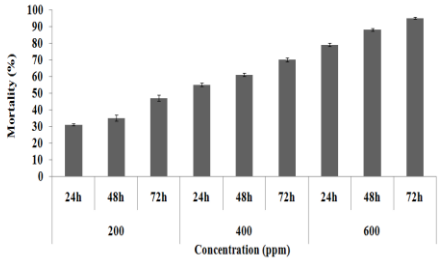

Fig. 11. Mortality of NPs against A. stephensi.

Table.1. Probit analysis of AgNPs against vector mosquitoes larvae.

***LC_{50} and **LC_{90} at different time**	***A. aegypti***	***A. stephensi***
LC_{50} (24 hrs)	394.85 ppm	322.69 ppm
LC_{90} (24 hrs)	1200.00 ppm	988.10 ppm
Regression equation	Y= -1.92+ 0.40X	Y= -1.60+ 0.39X
Chi-square value (X^2)	1.70	0.588
LC_{50} (48 hrs)	312.86 ppm	281.71 ppm
LC_{90} (48 hrs)	853.33 ppm	743.15 ppm
Regression equation	Y= -2.33+ 0.40X	Y= -2.46+ 0.41X
Chi-square value (X^2)	1.33	1.06
LC_{50} (72 hrs)	267.50 ppm	221.88 ppm
LC_{90} (72 hrs)	605.89 ppm	656.65 ppm
Regression equation	Y= -4.034 + 0.43X	Y= -1.31+ 0.41X
Chi-square value (X^2)	0.779	0.584

LC_{50}**: Lethal concentration required to kill 50 per cent of the population exposed.*LC_{90}**: Lethal concentration required to kill 90 per cent of the population exposed.

4. Conclusions

It was concluded that using aqueous extract of *Hippophae rhamnoides* leaves has been documented a new beneficiary method using non-expensive raw materials for the biosynthesis of

AgNPs. This low cost, non-complicated, less time consuming and environment friendly biosynthetic preparation gives potent applications in various human benefits related fields. This approach will enhance AgNPs sustainable management and economic viability. The efficiency here killing of mosquito larvae (*A. aegypti* and *A. stephensi*) were promising.

Additionally, to consider protection point of view and mosquito resistance appearance to traditional insecticides create natural origin larvicide which give superiority over non-natural larvicide. Non-synthetic larvicides, particularly plant origin with the purpose of extra choose, degradable and more talented within this characteristic. Overall, our current findings grow the efficient utilization of the bioinspired fabricated NPs from aqueous leaves extracts of *H. rhamnoides* in numerous human benefits related fields especially health care system and to develop new drugs in favor of human being benefits in near future.

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