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Assessment of Larvicidal Activity of Synthesized Silver Nanoparticles Leaf Extract of *Annona senegalensis* and *Cassia obstufolia* Against 4th Instar Mosquito Larvae

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Abstract: *Silver nanoparticles synthesis has been achieved using plant extract which is ecofriendly. The presents study was carried out to assess the larvicidal activity of aqueous and synthesized silver nanoparticle leaf extract of Annona senegalensis and Cassia obstufolia against fourth instar larvae. It was established that aqueous silver ions can be reduced by the extract of the plant to generate a stable silver nanoparticles. Nanoparticles were characterized using UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy, Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD) Spectroscopy analysis. The formation of the silver nanoparticle was monitored through a UV-Vis-spectrophotometer in a wavelength range of 300-900nm. Peaks were revealed at 400nm and 420nm indicating the production of silver nanoparticles. The FTIR analysis strongly supported the capping behaviour of bio-reduced synthesized silver nanoparticles which in turn imparted the high stability of the synthesized silver nanoparticles. SEM micrograph SEM analyses of the synthesized AgNPs were clearly distinguishable. The XRD study revealed the crystalline nature of the nanoparticle with a Face-Centered Cubic (FCC) structure. The fourth instar of mosquito larvae were exposed to different concentration (10-50ppm and 100-500ppm) of the synthesized and aqueous leaf extract. Maximum mortality rate of 90% at concentration of 50ppm and LC_{50} (20.00ppm) LC_{90}(46.0ppm) was achieved for AgNps of C. obstufoliaand the aqueous extract has 86.6% maximum mortality for 500ppm with LC_{50}(223.0ppm), LC_{90}(506.0ppm).while A. senegalensis revealed 75% for AgNPs and 68.3% for aqueous extract. The result of the findings suggests that, synthesized silver nanoparticles of A. senegalensis and C. obstufolia can be used as a rapid, environmentally safer and greener approach for mosquito control.*

Keywords*: Annona senegalensis, C.obstufolia ,Silver nanoparticles ,Characterization, Larvicidal activity.*

1.0 Introduction

Mosquito belongs to the phylum arthropod and is an important vector for many vector-borne diseases, including malaria, filariasis and numerous viral diseases, such as dengue fever, yellow fever, Japanese encephalitis, west Nile, Rift valley fever, zika and chikungunya (Benelli, 2017). In the template climate countries they are important as nuisance pests than as vectors (Abou-Elnaga, 2014). There are about 3000 species of mosquitoes, of which about 100 are vectors of human diseases (Pohlit*et al.,*

2011). Control measures are directed mainly against only one or few of the most important species and can be targeted at the adult or the larval stages (Michigan Mosquito Control Organization, 2013).

For spans of years, several scientists have been engaged in searching for the effective and efficient mosquito control program. The World Health Organization (WHO) expert committee felt that the resistance in vector was probably a major challenge in the struggle against vector borne diseases (WHO, 2017). The conventional insecticides are environmentally non-sustainable and harmful to both human and non-target organism moreover, most mosquitoes species are increasingly becoming physiologically resistance (Karunamoorthi, and Sabesan, 2013).

As the problem of insecticide-resistant mosquitoes to chemical agents is on the rise, natural sources, such as plant are good alternatives to control mosquito vectors.They are harmless to human, target specific, bio-degradable,ecofriendly, and cost-effective (Govindarajan, 2016b).Plants are rich sources of bioactive compounds, which can be used to develop environmental safe vector managing agents. A number of plants have been reported as excellent toxics against mosquitoes acting as adulticidal, ovicidal, larvicidal, oviposition deterrent, and reproduction inhibitors and adult repellents (Govindaranjan, and Sivakumar, 2011).

In recent years, the green synthesis of eco-friendly metal nanoparticles from various plant derived metabolites has increased interests on nanotechnology acting as a good material for vector control. The nanoparticles possess valuable properties such as catalytic, optical, antimicrobial antiviral, antiplasmodial, insecticidal and larvicidal properties (Santhosh*et al.,* 2015). The plant mediated biosynthesis (i.e. "green synthesis")of nanoparticles is advantageous over chemical and physical method, a growing number of plants and fungi have put forward are efficient and rapid extra-cellular synthesis of silver and gold nanoparticles with excellent mosquitocidal properties in both field and laboratory conditions (Dinesh *et al.,* 2015; Amerasan*et al.,* 2015).

Annona senegalensis, commonly known as wild custard apple and wild sour-sop is a shrub or small tree 2-6m tall but may reach 11m under favourable conditions (African Union Scientific, Technical & Research Commission, 2014). The bark is clean to roughish, silver gray or grey-brown, leaves are alternate, simple, oblong, ovate or elliptic, green to bluish green; plants are up to 3cm in diameter on stalks 2cm long, solitary or in corporations of 2-4, bobbing up above the leaf axils(Mustapha*,* Owuna &Uthman, *2013*). The plant is found growing throughout Nigeria and very common in Northern Nigeria, particularly in Nasarawa, Kaduna, Kano, Plateau, and Niger States and in the Federal Capital Territory, Abuja and usually known as (Hausa,Gwándàndààjìì) or (Fulani,dukuu-hi) (Mustapha, 2013).

Cassia obtusifolia family Leguminosae (Fabaceae) is generally distributed in Africa and the Americas. In Sudan it is found mostly on the clay plains of the central rain lands and in the southern regions. *C. obtusifolia* is native to tropical South America but has become widespread throughout the tropics and subtropics. However, the extent of its original distribution in the neotropics is un-known. It grows wild in North, Central and South America, Asia and Africa and is considered a particularly serious weed in many places. The species name comes from the Latin obtus (dull or blunt), and folium ((Devi, Shankar, Femina&Paramasivam, 2012).

From previous literatures, the search for a sustainable natural biodegradable, eco-friendly, and difficult to develop resistance mosquitocide is important and urgent. The current study aimed to assess the larvicidal activity of green synthesized AgNPs using aqueous leaf extract of *A. senegalensis* and *C. obstufolia* against 4^{th} instar mosquito larvae.

2.0 Material and Methods

2.1 Collection of plant materials

The fresh matured leaves of *A. senegalensis* and *C. obstufolia* were collected from Maiduguri Metropolitan Council (latitude $11^0$49' and longitude $13^0$90') Area of Borno State, Nigeria. The plant was identified and authenticated by a plant taxonomist from Lake Chad research institute and a voucher specimen with number 03-458 was deposited at the herbarium of the institute.

2.2 Preparation of plant extracts

The leaf of *A. senegalensis* and *C. obstufolia* were dried in shade and ground to fine powder in a mortar. Aqueous extract was prepared by mixing 50g each of the dried leaf powder with 500ml of water (boiled and cooled distilled water) with steady stirring on a magnetic stirrer (Veerakumar*et al.,* 2013). The suspensions of the dried leaf powder in water were left for 3hrs, filtered through whatman no 1 filter paper, and the filtrate was stored till use.

2.3 Synthesis of silver Nanoparticles

The broth solution of fresh plant leaf were prepared by taking 10g of thoroughly washed and finely cut leaves in a 300ml flask along with 100ml of sterilized double distilled water and then boil the mixture for 5min before finally decanting it. The extracts were filtered with Whatman filter paper no 1. The filtrates were treated with aqueous 1mM silver nitrate ($AgN0_3$) (21.2mg of $AgN0_3$ powder in 125ml of distilled water) solution in a flask and incubated at room temperature for 6hrs. Eighty-eight-milliliter aqueous solution of 1mM of silver nitrate was reduced using 12ml of leaves extract at room temperature for 10 minutes. A resulting dark brown solution indicates the formation of silver nanoparticles (AgNps). The obtained AgNps were centrifuged at 3,000 rpm for 45min and three-repeated wash with distilled water were performed to discard a clear supernatant solution. The obtained pellets were dried and stored for further characterization and bioassays (Veerakumar*et al.,* 2014b).

2.4 Characterization of silver nanoparticles

Synthesis of AgNps solution was observed by UV-Vis spectroscopy. The bio reduction of the Ag^+ions in solution were monitored by sampling of aliquots (1ml) of the aqueous component after 20 fold dilution and absorbance was measured using Jenway 7315 spectrophotometer in 300-900nm range, operated at a resolution of 1nm. The functional groups from synthesized nanoparticles were examined using Fourier Transform Infrared (Shimadzu-8400s FTIR) spectrophotometer at a scan range of 750-4000cm^{-1}. The surface morphology and size of the AgNps were examined using a Scanning Electron Microscope (SEM) and Energy Dispersive X-ray(EDX) spectroscopy for elemental analysis using PhenomProX. The crystalline nature of AgNps were determine using Shimadzu XRD-6100 diffractometer, operating at 45kv and 40mA. CuKr radiation with wavelength of 1.54A^0 and a step size of 0.02^0 in the 2ɵrange 5-80 degrees (Govindarajan, 2016).

2.5 Larvicidal bioassay

The larvicidal activity of the aqueous extract and silver nanoparticles from *A. senegalensis and C. obstufolia* were evaluated according to World Health Organization (WHO), guidelines for testing larvicidal. Aqueous crude extract was tested at the range of 100, 200,300,400, and 500ppm concentrations and silver nanoparticles was tested at the range of 10, 20,30,40 and 50ppm concentrations. Twenty 4^{th} instar larvae were introduced into 500ml glass beaker containing 249ml of distilled water, 1ml of desired concentration of the aqueous leaf extract and silver nanoparticles were added to each. Average of three replicates were recorded. Larval mortality were recorded 24h after exposure. Each test included a set of control groups (1Mm silver nitrate and distilled water) (WHO, 2005).

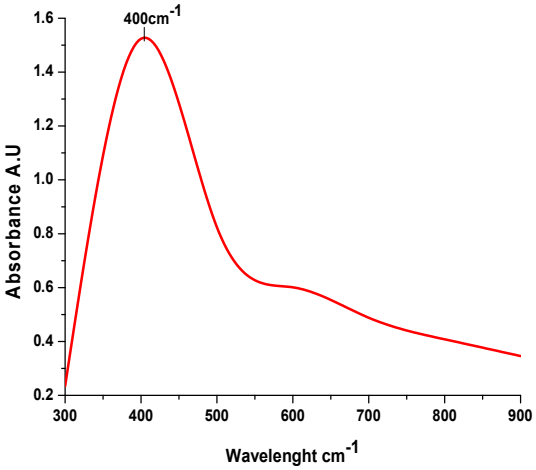
2.6 Statistical Analysis

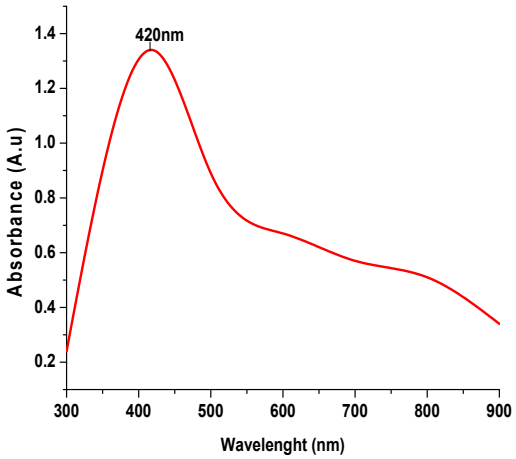
The percentage larval mortality were subjected to log-probit analysis and regression analysis for calculating LC_{50}, LC_{90} statistics at 95% confidence limits of upper confidence limit (UCL), and lower confidence limit (LCL), and chi-square values were calculated using the statistical package for social sciences (SPSS) version 26.0 software. Results with P< 0.05 were considered statistically significant.

3.0 Results

3.1 Uv-vis Spectroscopy

Evidence of reactivity in *A. senegalensis* and *C. obstufolia* leaf extract with $AgN0_3$ solution were visually indicated by a change in colour after 3hrs of incubation. The formation of the synthesized AgNps





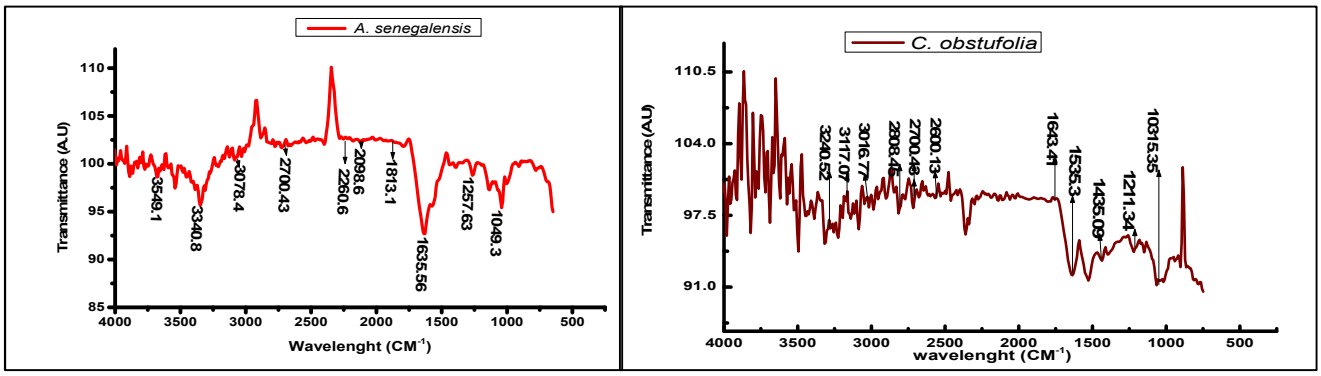
was monitored by scanning the absorption spectra in the range of 300-800nm. The most intense peak was observed at 400nm in *A. senegalensis* and 420nm in *C. obstufolia* (Fig. 1 a & b).

Fig.1 a &b: Uv-Vis absorption spectra of synthesized silver nanoparticles of *A. senegalensis* and *C. obstufolia* leaf extract.

3.2 FTIR Analysis

The FTIR spectrum of AgNPs prepared from *Annona senegalensis* extract showed the presence of different functional groups as reducing agents in the biosynthesis of AgNPs (Fig.4.7). The absorption band at 902.72(N-H) wag, 1049 cm^{-1}(CO-O-CO) streching ,1257cm^{-1}(C-O) stretching, indicates vibration due to the presence of primary amine, secondary amine, anhydride and alkyl aryl ether. Band at 1404.22cm^{-1}(C-F) stretch was due to fluro compound. The band at 1635cm^{-1}(C=C) stretching vibration correspondsto cyclic alkane. The peak at 1813 cm^{-1}(C=C) is an indicative of stretching which can be assigned to acid halide. The peaks observed at 2098cm^{-1}(N=C=S) and 2260cm^{-1}(C=N) indicates the ultraviolet region stretching vibrations assigned to isothiocynate and alkyne respectively. The peak at 2414.96cm^{-1}(O=C=O) indicatesstretchingof carbondioxide. The peak at 2700 cm^{-1}(C-H) corresponds to stretching assigned to aldehyde. Peak at 2793.02 (C-H) was due to stretching corresponding to aldehyde. The peak at 3078 cm^{-1}(C-H) was due to the stretching vibration corresponding to alkene. 3340 cm^{-1}(N-H) was assigned to the amine functional group with stretching vibration and the spectrum from C. obstufolia shows peaks at 833.28cm^{-1}(C-CL) stretch, corresponds to alkyl halides and 941.29(O-H)

bend of Carboxylic acid, 1033cm^{-1}(S=O) stretch, due to sulfoxide and 1211 cm^{-1}(C-O) stretching vibration of vinyl ether. The peak at 1435cm^{-1}(O-H) bending, is due to carboxylic acids. The strong peak at 1535cm^{-1}(N-O)stretching, vibration was due to nitro compounds. The band at 1643cm^{-1}(C=C) stretching, may correspond to alkene. The peak at 1797cm^{-1}(C-H)bending, and 2121cm^{-1}(C=C)stretching, correspond to aromatic compound and alkyne respectively. Bands at 2345.52cm^{-1}(O=C=O) stretching, and 2430.39cm^{-1}(C=C) stretching, are due to carbondioxide and carboxylic acid. The band at 2600cm^{-1}(O-H) indicates a stretching vibration of carboxylic acid. The peaks at 2700cm^{-1}(C-H) and 2808cm^{-1}(C-H) stretching, may both be assigned to the presence of aldehyde. The peaks at 3016 cm^{-1}(O-H), 3117cm^{-1}(O-H) and 3501cm^{-1}(O-H) stretching indicate the presence of alcohol intra-molecular bonding. The medium peak at 3294cm^{-1}(N-H) and 3240cm^{-1}(N-H) stretching vibration corresponds to aliphatic primary amine. (Fig.2a & b).



Fig(2a &b). FTIR spectra of synthesized silver nanoparticles using *G. senegalensis and C.obstufolia* aqueous leaf extract

3.3 Scanning electron microscopy (SEM)

SEM micrograph of the synthesized AgNps of *A. senegalensis* and *C. obstufolia* magnified at X1500 were shown in Fig. 3a&b. The visual observation of the surface morphology of the synthesized *A. senegalensis* nanoparticles showed a spherical, cubic with few cuboidal structures while the SEM representative of C. obstufolia were predominantly spherical withsome uneven structures .

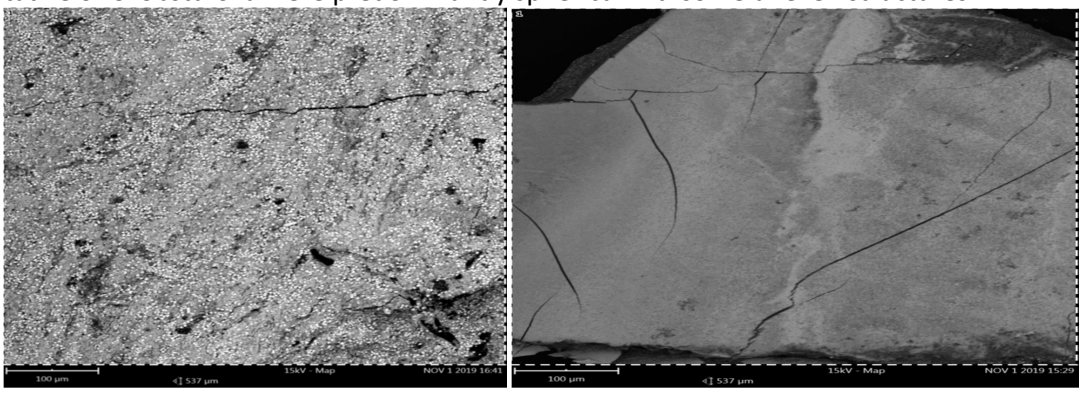


Fig3 a & b. SEM micrograph of synthesized silver nanoparticles of *A. senegalensis* and *C. obstufolia* at X1500

3.4 X-ray diffraction spectroscopy (XRD)

The crystalline nature of *A. senegalensis and C. obstufolia* synthesized AgNps were shown by XRD analysis. Diffraction peaks were observed at 2θ value corresponding to (111), (200), (220) and (311) set of lattice plane and were indexed as face-centered cubic (FCC) structure of silver nanoparticles (Fig.4a & b).

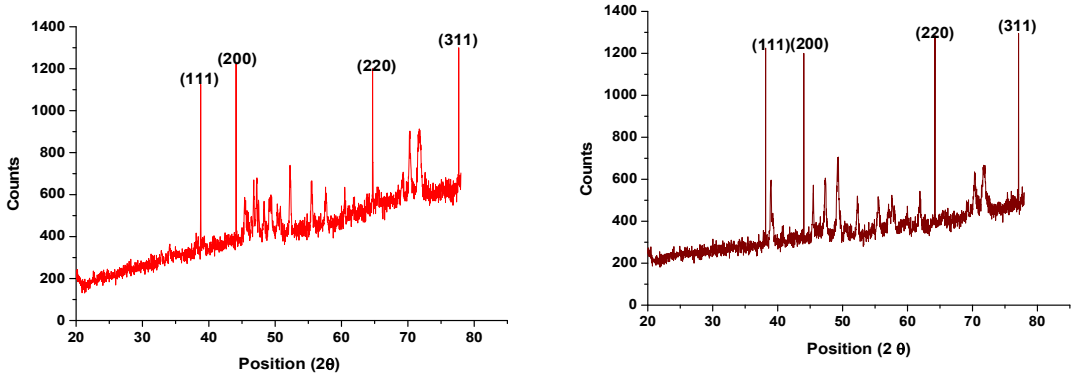


Fig.4a & b. XRD pattern of synthesized from leaf extract of *A. senegalensis and C. obstufolia*

4.0 Larvicidal bioassay

The larvicidal activity of synthesized silver nanoparticles and aqueous leaf extract of *A .senegalensis* and *C.obstufolia*. Data revealed from synthesized AgNps showed mortality rate of 75%±1.53 at a concentration of 50pm, and while the lowest mortality was 28.52±0.58 at 100ppm. The lethal concentration LC_{50} value of 30ppm and LC_{90} 60ppm, were required to kill 50 and 90% larvae respectively. The result of the aqueous extract revealed rate of 68.38% at 500ppm with LC_{50} of 310ppm and LC_{90} of 630ppm respectively. C. obstufolia was highest at 90%, 50ppm AgNPs with LC_{50} 29ppm, aqueous extract was 80.6% at 500ppm and LC_{50} of 223ppm.

Table 1 a: Larvicidal activity of *Anonna senegalensis* Aqueous and Silver Nanoparticles leaf extracts against Fourth Instar Mosquito species (20 larvae) exposed for 24hours

Concentrations (ppm)	% mortality ± SD	LC_{50}(ppm)LCL-UCL	LC_{90}(ppm)LCL-UCL	x^2(df)
	Anonna senegalensis aqueous leaf extract			
500	68.38 ± 0.58			
400	57.40 ± 1.50			
300	48.00 ± 1.53			
200	33.33 ± 1.15	310 (248 - 386)	630(517 - 873)	4.340 (4) ns
100	28.52 ± 0.58			
control	0.00 ± 0.00			
	Anonna senegalensis silver nanoparticles leaf extract			
50	75.00 ± 1.73			
40	60.25 ± 1.00			
30	50.00 ± 0.00	30 (24 - 37)	60(50 - 84)	4.361(4) ns
20	38.33 ± 0.58			

10	30.00 ± 1.53
control	0.00 ± 0.00

SD standard deviation, Values are mean ± SD of three replicates, LCL lower confidence limits, UCL upper confidence limits, X^2 chi-square test, and $p < 0.05$, level of significance.

Table1b. Larvicidal activity of *Cassia obstufolia* Aqueous and Silver Nanoparticles leaf extract against Fourth Instar Mosquito species (20 larvae) exposed for 24hours.

Concentrations(ppm)	% mortality± SD	LC_{50}(ppm)LCL-UCL	LC_{90}(ppm)LCL-UCL	x^2(df)
	Cassia obstufolia aqueous leaf extract			
500	86.67 ± 0.58			
400	76.58 ± 1.15			
300	70.33 ± 0.52	223(161- 279)	506(422 - 663)	7.354(4)ns
200	53.53 ± 0.00			
100	45.33 ± 1.00			
control	0.00 ± 0.00			
	Cassia obstufolia silver nanoparticles leaf extract			
50	90.00 ± 1.00			
40	78.33 ± 1.15	20 (14 - 25)	46 (38 - 58)	8.148(4)ns
30	75.00 ± 1.73			
20	56.67 ± 0.58			
10	48.33 ± 1.53			
control	0.00 ± 0.00			

SD standard deviation, Values are mean of ± SD of three replicates, LCL lower confidence limits, UCL upper confidence limits, X^2 chi-square test, and $p < 0.05$, level of significance.

5.0 **Discussion**

Several investigations were carried out to characterize the biosynthesized silver nanoparticles. The biosynthesis of metal nanoparticles involves the reduction and stabilization potential of plant extract and metabolites (Rajan*et al.,* 2015).

The synthesized silver nanoparticles were incubated at room temperature and within 3hrs of reaction a clear change in colour from brown to dark brown was observed. Such method was well explained by other researchers who worked with different plants (Muthukrishnan*et al.,* 2015; Kalaiselvi*et al.,* 2015). The colour change was due to the excitation of surface plasmonresonance(SPR) in metal nanoparticles (Logeswari *et al.,* 2015). The period of colour change in this study was in conformity with Muthukrishnan*et al.* (2015) and differs with Nithya and Raghavan (2014) who reported colour charge after 24 hours. The variation in bio reduction may be due to difference in Enzymes activities present in the extract of *A. senegalensis and C.obstufolia.* A sharp peak at 400nm and at 420nm were observed (Fig 1a& b). The UV-Vis band is an evidence of the presence of surface plasma resonance (SPR) of AgNps which is ranged from 420-450mm (Ramalingan*et al.,* 2014).

The IR spectroscopy study has confirmed that the carbonyl group of amino residue and peptides of proteins has a stronger ability to bind metal to prevent the agglomeration of particles, and thus stabilization of nanoparticles in the medium (Indhurmthi and Apunprasath, 2019).The identified functional groups and secondary metabolites are responsible for the reducing, capping and stabilization activity of the plant extracts in addition to prevention of aggregation (Suriyakalaa*et al.,* 2013).

The XRD patterns clearly demonstrate that the AgNPs formed in the present study were crystalline in nature. The sharpening of the peaks specifies that the particles be in a nano range. The stronger planes indicate AgNPs as a key element in the biosynthesis. Minor shift in the peak indicates presence of some strains in the crystal structure.. Selvi and Sivanmar (2012) obtained similar reports.

The larvicidal activity of aqueous and silver nanoparticles leaf extract of *A. senegalensis* at various concentrations against 4th instar mosquito larvae was presented in Table 1a & b. *A. senegalensis* and *C. obstufolia* AgNPs exhibited moderate activity than the aqueous extract. The result also revealed that C. obstufolia AgNPs is more potent as a mosquito larvicide which showed maximum mortality even at low concentaration of 10ppm. The findings of this research is consistent with the work of Roni *et al.,* (2012), also disagree with several authors (Parthiban *et al.,* 2018; Bianca *et al.,* 2018). Researchers has reported that synthesized AgNPs may has significant impact on mosquito larvae, findings of the study also in line with (AgalyaPriydarShini*et al.,* 2012, Roni *et al.,* 2012).

Conclusion

The study shows that the aqueous leaf extracts and silver nanoparticles obtained from *Annona senegalensis and C .obstufolia* present a clear larvicidal effect against 4th instar larvae. Furthermore, the effect with the synthesized silver nanoparticles is higher.. The biosynthesis of aqueous leaf extract of *A. senegalensis* and C. obstufolia has the potential to be used as a suitable alternative in mosquito larvae control.

Conflict of interest statement

We declare that we have no conflict of interest

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