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Green synthesis, characterization and larvicidal activity of CuNPs using *Hygrophila Auriculata* leaf extract against *Aedes aegypti* larva

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Abstract
Copper is a chemical element possess high thermal and good electrical conductivity. The copper nanoparticle synthesis is achieved due to the reduction of copper sulphate while the aqueous leaf extract of *Hygrophila auriculata.* Preliminary phytochemical analysis showed the presence of a maximum of phytochemicals in aqueous extracts of *Hygrophila auriculata.* The synthesized Cu nanoparticles were characterized by UV visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), EDAX and particle size analyzer. Antimicrobial activity by agar well diffusion method revealed that aqueous extract of nanoparticles showed maximum inhibitory activity against pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris* and *Klebsiella pneumonia* based on zone of inhibition. The larvicidal activities were assessed for four days against the larvae of *Aedes aegypti* with varying concentrations of aqueous extract of *Hygrophila auriculata* and LC_{50} and LC_{90} values were calculated.

Keywords: *Hygrophila auriculata,* FTIR, SEM, EDAX, *Aedes aegypti*

Introduction

Nanotechnology is a developing science of modern research. Nanotechnology involves the process of production, manipulation of nano materials size ranging from less than a micron to that of individual atom [1]. It plays an important role in the modern world of science that has been growing fast. It is most commonly used in biology, chemistry, physics, electronics, life science and engineering. More specifically, nanomaterials are used in many fields such as biomedicine for disease diagnosis and treatment, catalysis, energy production, environmental remediation and others. Eco-friendly alternatives to chemical and physical methods are biological ways of nanoparticle synthesis using microorganisms and vascular plants. The development of these environment friendly methods for the synthesis of nanoparticles is evolving as an important branch of nanotechnology [2]. These nanoparticles synthesized nanoparticles with plant extracts were found to exert biopotential like antibacterial, antioxidant and anticancer activities [3,4,5].In recent years, research in the field of agriculture has focused on the impact of certain minor elements on the economy of plants [6]. CuNPs synthesis has attracted selective interest, compared with other NPs, as their useful properties are achievable at costs lower than silver and gold [7].

Hygrophila auriculata is an important medicinal plant. It has a record of many names in the Ayurvedic literature as Ikshura, Ikshagandha and the Indian cuckoo. *Hygrophila auriculata*, a perennial angiosperm of Acanthaceae, is a wild herb. It is commonly distributed in moist places on the banks of rivers, ditches and paddy fields in India, Srilanka, Burma, Malaysia, and Nepal. It is used as medicine in like Odisha, Chhattisgarh and West Bengal. The pre-flowering or flowering succulent aerial parts are boiled and consumed by the rural people of these states. The extract of the plant also increases the haemoglobin level, at the same time does not have any side effects. The plant contains phytoconstituents like phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, alkaloids, enzymes, amino acids, carbohydrates, hydrocarbons, flavonoids, terphenoids, vitamins, glycosides, etc. It is useful in the treatment of anasaraca, diseases of urinogenital tract, dropsy of chronic Bright's disease, hyperdipsia, vesical calculi, flatulence, diarrhea, dysentery, leucorrhoea, gonorrhea, asthma, blood diseases, gastric diseases, painful micturition, menorrhagea etc,[8].

Materials and methods

Collection of plant material

The leaves of the healthy plant of *Hygrophila auriculata* were collected from the surrounding of Kalayarkurichi village, Sivakasi (Taluk), Virudhunagar district, Tamilnadu (Fig.1). The collected leaf samples were tightly packed with polythene bag and then transfer to the laboratory. The leaves were surface cleaned with running tap water for two to three times to remove soil and dust particles prior to drying. The leaves were dried at room temperature for 20 days to completely remove residual moisture and powdered using mechanical kitchen blender and then stored in airtight container.

Fig 1: *Hygrophila auriculata*

Preparation of leaf extract
10g of leaf powder was taken in 500 ml round bottom flask along with 200 ml of distilled water. The mixture was then boiled for 25 minutes in heating mantle at 80 ^{o} C until the color of the leaf extract changes to dark brown color. Then the extract was cooled to room temperature and then it was filtered with Whatman No.1 filter paper to get a pure leaf extract. The resulting product is stored at refrigerator for further analysis [9].

Synthesis of copper nanoparticles
1 mM aqueous solution of $CuSO_4$ was prepared using 100 ml double distilled water. 15 ml of *Hygrophila auriculata* leaf extract was treated with 85 ml aqueous solution of 1 mM $CuSO_4$ and incubated in dark room at room temperature for the reduction of copper ions. After 12 hours, the reduction of Cu ions was confirmed by the color change of the solution. It indicates the formation of copper nanoparticles. Then the CuNPs solution was centrifuged for 20 min at 10,000 rpm and the collected pellet was washed in double distilled water for three times to remove any unwanted biological materials. Then the pellet was taken in watch glass and it was transferred to hot air oven to dry the pellet in 70°C [10].

Phytochemical screening
The components analysed were protein, phenolic, tannins, flavonoids, carbohydrates, saponins, terpenoids, steroids, alkaloids, glycosides and quinone [11,12,13,14,15].

Characterization of copper nanoparticles
UV-Visible Spectral Analysis
Copper nanoparticles was carried out by using UV-Visible spectrophotometer with leaf extracts of *Hygrophila auriculata*. The bio reduction of precursor copper ions were monitored by sampling of aliquots at different intervals. Absorption measurements were carried out on UV-Visible spectrophotometer using UV-1700 Pharma spec, at a resolution of 1nm between 200-800 nm. Distilled water was used as blank [16].

Fourier transform infrared [FTIR] spectroscopy
The copper nanoparticles solution was centrifuged at 10,000 rpm for 30 minutes. The pellet was washed three times with 20 ml of de-ionized water to get rid of free proteins or enzymes that are not capping the copper nanoparticles. The samples were dried and ground with KBr pellets and analyzed on a SHIMADZU model in the diffuse reflectance mode operating at a resolution of 4cm^{-1}. Background correction was made using a reference blank KBr pellet [17].

Scanning electron microscopy
The morphological features of synthesized copper nanoparticles from *Hygrophila auriculata* leaf extract was identified using Scanning Electron Microscopy (SEM), Hitachi S-4500 [18].

Energy dispersive absorption X-ray spectroscopy (EDAX)
Hygrophila auriculata extract reduced copper solutions were detected by Energy dispersive X-ray spectroscopy (EDAX) analysis for the confirmation of elemental copper was carried out for the detection of elemental copper [19].

Particle size distribution
In particle size distribution, the surface area size distribution in measurements with an overall sizing range of 0.4μm to 1200μm. In the present study, the size of the experimental sample was given for PSD analysis (Shimadzu SALD-2300, Wing SALD II: Version 3.1.1) [20].

Antibacterial activity assay
The antimicrobial activity of selected medical plant against clinical pathogen was determined by using agar well diffusion method [21]. Muller Hinton agar was prepared for cultivation of the bacteria. Approximately 20ml of molten and cooled media was poured in sterilized petri dishes. Then under the aseptic condition, placed a sterile swab into the broth culture of a fresh overnight grown cultures of the bacteria, then gently removed the excess liquid by gently pressing or rotating the swab against the inside of the tube and spread it on Muller Hinton containing petri plates respectively. The five wells were cultured in the agar plates using gel puncher. The nanoparticles were placed on the agar surface. All the plates were incubated at 37°C for 24hrs.

Antioxidant activity
DPPH radical scavenging assay
The antioxidant activity of synthesized copper nanoparticles was determined based on their scavenging activity of the stable 1, 1- diphenyl-2-picryl hydrazyl (DPPH) free radical. The sample and ascorbic acid were mixed with 95% ethanol to prepare the stock solution (5mg/ml). Ascorbic acid was taken as standard. At first, five tubes were taken to make aliquots of 5 concentrations (20-100µl) with the samples. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution and to homogeneously dissolve magnetic stirrer was used. After 30minutes, the absorbance of each test tube was measured by a UV spectrometer at 517nm [22].

Hydrogen peroxide assay
The hydrogen peroxide were mixed with phosphate buffer (pH-7.4) to prepare stock solution. Ascorbic acid was taken as standard. At first, five test tubes were taken with aliquots of five concentrations (20-100µl) of the synthesized copper nanoparticles. 0.6 ml of H_2O_2 in phosphate buffer was added and the reaction mixture was incubated at room temperature for 10 minutes. Absorbance was read at 230 nm against blank. The percentage of inhibition was calculated by the following equation [23].

Insect rearing
Different larval instars of *Aedes aegypti* were collected from the Indian Council for Medical Research, Madurai and were brought to the laboratory. These eggs were kept in enamel trays containing deionized water. The eggs were maintained and reared in the laboratory and allowed to feed on brewer's yeast, dog biscuits and sucrose in 3:1:1 ratio in the laboratory at room temperature for 24 hours, before start of the experiment. The larvicidal activity of synthesized CuNPs against *Aedes aegypti* larva was evaluated by the standard procedure of with slight modifications [24].

Statistical analysis
The data obtained was subjected to probit analysis using SPSS Statistical Software Package (Version: 19.0). The LC values were estimated at 30, 50 and 90 levels. Other

statistical parameters including 95% confidential limits, chi-square, standard deviation and regression coefficient were calculated in order to compute the significance and evaluate the difference between test samples.

Result and discussion

The copper nanoparticles were biosynthesized successfully using leaf extract of *Hygrophila auriculata.* The leaf extract was mixed in the aqueous solution of copper ions, the color changed from light brown to dark. The color change was observed after 24 hours of incubation. The reductions of Cu++ ions exhibited the dark color due to excitation of Plasmon Resonance property of copper nanoparticles (Fig.2 A, B). Green synthesis of copper nanoparticles using *Cassia auriculata* leaf extract was first identified by color changes of the extract from light yellow to dark brown color. Reduction of Cu+ ion into copper nanoparticles during exposure to the leaves extract was observed as change in colour [25].

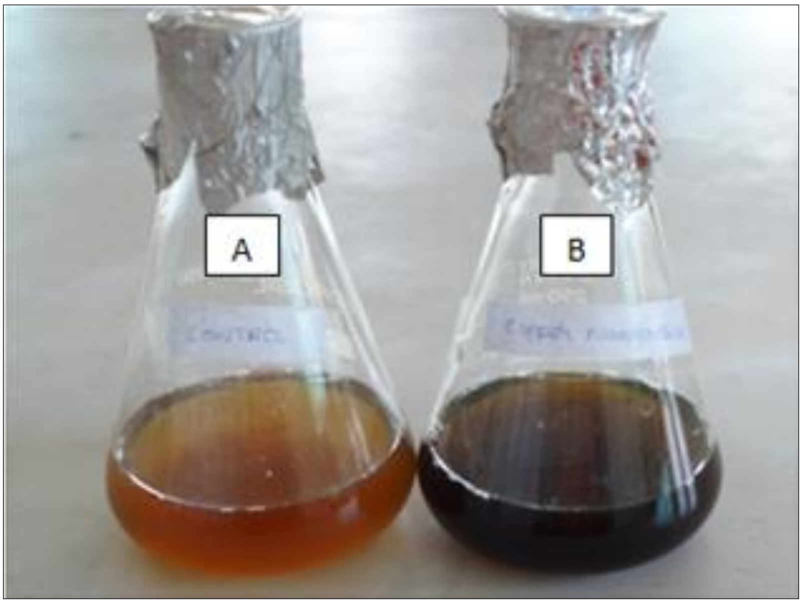

Fig 2: (A) - Leaf extract of *Hygrophila auriculata* and (B) - Copper nanoparticles

The qualitative analysis of phytochemical compounds present in the ethanol extract of *Hygrophila auriculata* plant was identified using many preliminary tests. The results exposed the presence of protein, phenolic compound, tannins, flavonoids, carbohydrates, terpenoids, saponins, alkaloids, glycosides, quinines and absence of steroid in the table 1. These phytochemical compounds were major compounds which gives important medicinal values. The preliminary phytochemical screening of different medicinal plants revealed the presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, anthraquinones and anthocyanosides [26]. The phytochemical screening of *V. lasiopus, V. auriculifera and V. galamensis* extracts contains most of the secondary metabolites like steroids, saponins, flavonoids, terphenoids and cardiac glycosides was reported by [27].

Table 1: Phytochemical analysis of active constituents in the leaf extract of *Hygrophila*

S. No	Phytochemicals	*Hygrophila auriculata*
1	Proteins	+
2	Phenolic compounds	+
3	Tannins	+
4	Flavonoids	+
5	Carbohydrates	+
6	Saponins	+
7	Terpenoids	+
8	Alkaloids	+
9	Steroids	-
10	Glycosides	+
11	Quinones	+

The green copper nanoparticles biosynthesized from leaf extract of *Hygrophila auriculata* were further confirmed by UV-Vis spectroscopy. The result obtained from UV-Visible spectroscopy analysis of the biosynthesized copper nanoparticle was showed in Fig. 3 and depicted with the peaks at 278 nm after 24 hours. This wavelength was characteristic feature of copper nanoparticles formation. Broadening of peak indicated that the copper nanoparticles present in the sample solution were polydispersed. The absorption peaks were obtained at 375 nm for copper nanoparticles using *Persea americana* seeds extract synthesis method [28]. Similarly [29] reported that the UV spectroscopic analysis of synthesized copper nanoparticles mediated by methanol extract of *Passiflora foetida* showed the maximum absorbance at 407 nm.

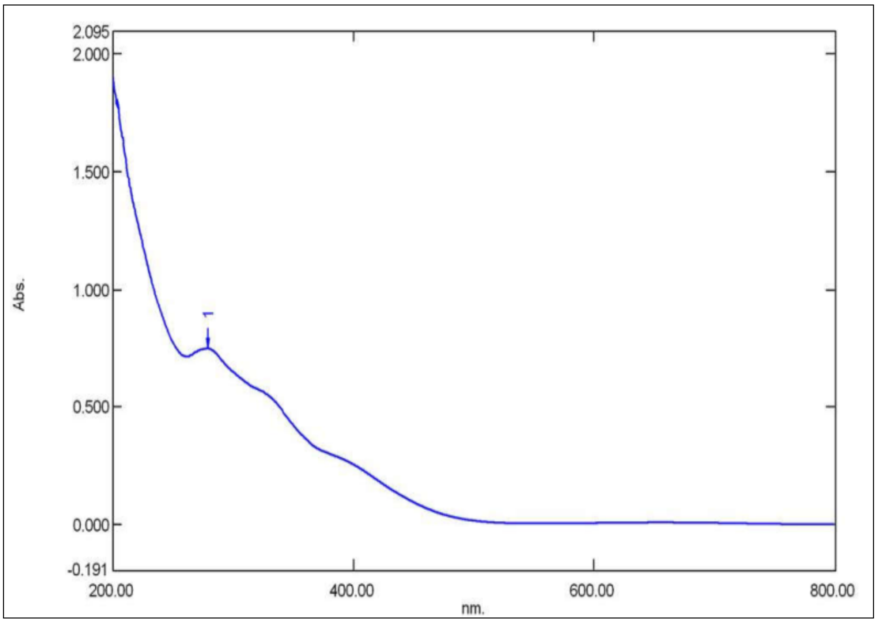

Fig 3: UV-Vis spectroscopy Analysis for *Hygrophila auriculata* copper nanoparticles

FTIR spectrum of copper nanoparticles green biosynthesized by *Hygrophila auriculata* leaf extract showed many absorption bands at 602.71cm^{-1}, 750.26 cm^{-1}, 1136.96 cm^{-1}, 1192.89 cm $^{-1}$, 1399.26 cm^{-1}, 1635.52cm $^{-1}$, 2065.62 cm-1, 3445.59 cm^{-1} were assigned to the Iodo compound C-I Stretch, Aromatic compound C-H bending, Alcohol C-O Stretch, Amines C-N Stretch, Corboxylic acid O-H bending, Alkene C-C stretch, Isothiocyanate N=C=S stretch, Alcohol O-H stretch. FTIR spectrum of Cu nanoparticles suggested that copper nanoparticles were surrounded by different organic compounds like alcohol, alkenes, amines and carboxylic acid. This result confirms that the copper nanoparticles synthesized using *Hygrophila auriculata* contain many functional groups (Fig. 4). The FTIR spectrum of aqueous extract of the *Tilia* showed five main peaks at 3419, 2952, 1760, 1688 and 1141 cm^{-1} which represented O-H stretching vibrations (alcoholic or phenolic), C–H asymmetric stretching, C=O stretching, C=C stretching and C–OH bending, respectively [30]. FTIR spectra revealed the presence of different functional groups like Alcohol (O- H stretch H-bonded, free), Alkane (C-H stretch, -C-H bending) Alkene (=C-H bending, C=C stretch), Amine (C-N, stretch) Nitro compounds (N-O stretch), Acid (O- H, stretch), Ester (C-O, stretch) obtained from *Nerium oleander* leaf extract. This functional group plays a very important role in copper nanoparticle synthesis [31].

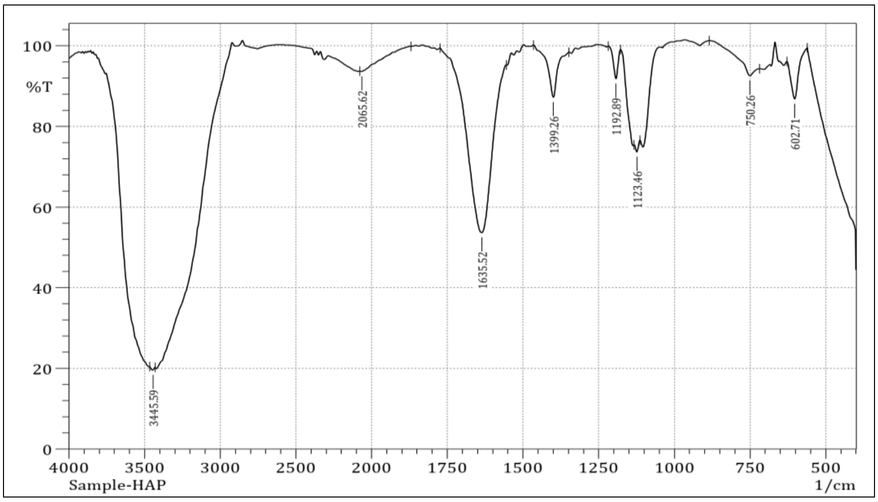

Fig 4: FTIR spectrum of *Hygrophila auriculata* copper nanoparticles

The typical SEM images of the copper nanoparticles synthesized by *Hygrophila auriculata* leaf extract was shown in (Fig. 5). The shape of the copper nanoparticles was found be spherical and the images were identified as 200 nm, 300 nm and 1µm range. The magnification of SEM was done in 50.00 K X, 40.00K X, 20.00 KX and 10.00 K X. The same result was reported by[32] in the biosynthesis and characterization of copper nanoparticles by *Artocarpus heterophyllus*. The SEM image of the green synthesized copper nanoparticles by *Persea americana* extract has spherical shape with the size range of particles from 42 to 90 nm. When the *P. americana* extract added with copper sulfate does not change nanoparticles shape but it increases the size of the nanoparticles mostly in higher concentration (50%) and aggregation for (0%) *P. americana* extract by [28].

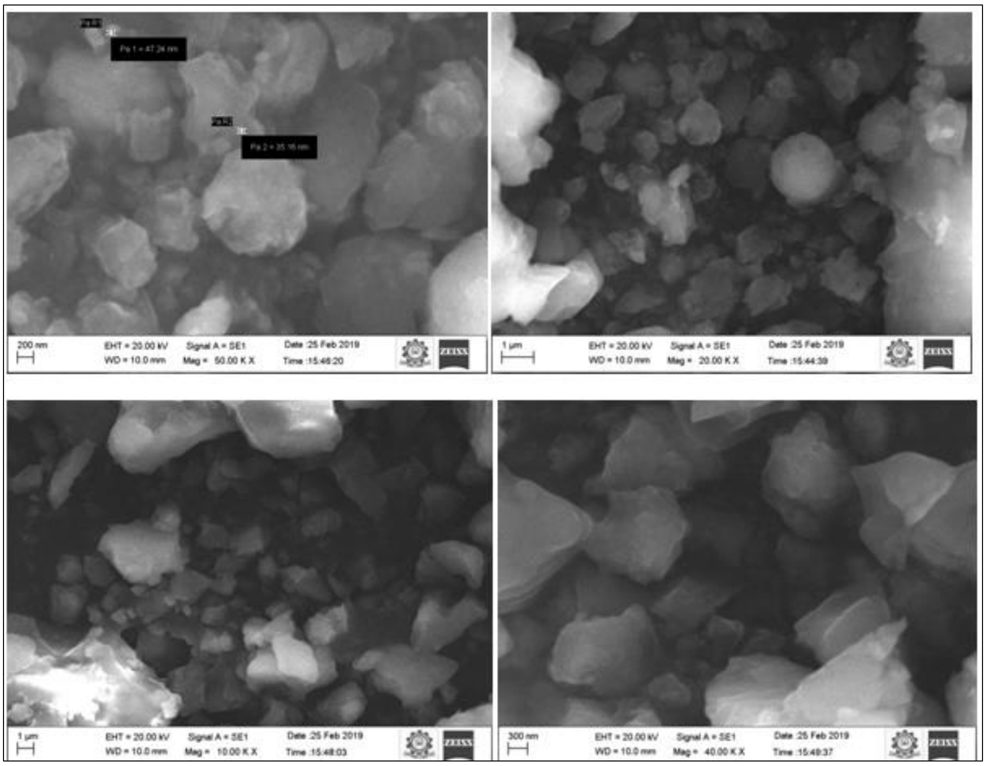

Fig 5: SEM results of copper nanoparticles from *Hygrophila auriculata*

EDAX analysis was used to confirm the presence of expected elements in the copper nanoparticle sample. EDAX result confirmed the successful synthesis of copper nanoparticle from plant extract of *Hygrophila auriculata* (Fig. 6) showed EDAX results of copper nanoparticles from *Hygrophila auriculata.* This result was confirmed by the presence of elemental copper in the sample. The EDAX of green synthesized copper oxide nanoparticles from *Acalypha indica*, which denotes that the strong signal in the copper and confirmed the formation of copper oxide nanoparticles [33].

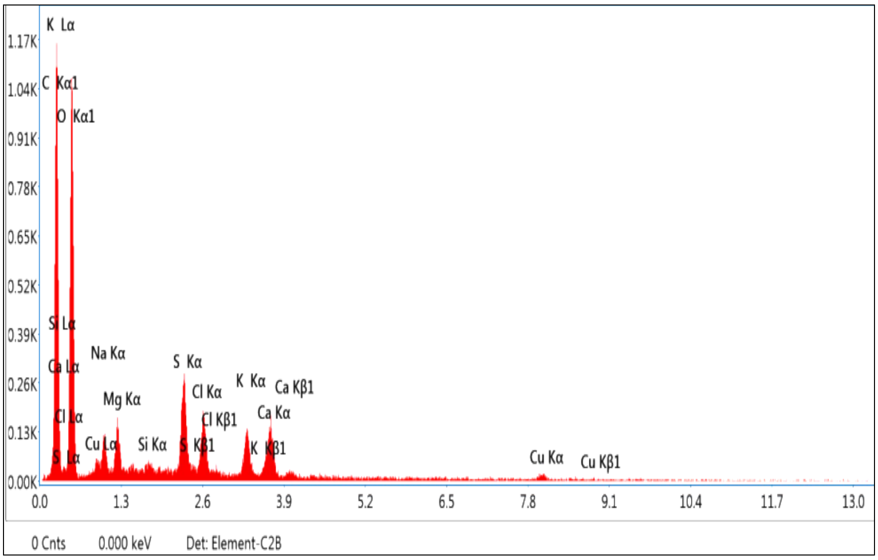

Fig 6: EDAX results of copper nanoparticles from *Hygrophila auriculata*

Particle size distribution analysis was used to characterize of nanoparticles. This result provides information about the size of the copper nanoparticles by *Hygrophila auriculata* plant leaf extract, and was found that PSD of 10%, 50% and 91% for particles 0.513, 0.918, 553.634. The mean value is 1.824 µm (Fig. 7). Similarly [34] reported that the copper nanoparticles were synthesized from *Dioscorea bulbifera* leaf extracts and particle size distribution employing dynamic light scattering exhibited the presence of particles in a range from 8 nm to 220 nm, majority being 12 to 16 nm. Bigger size might be due to capping of CuNPs by biomass which remained strongly associated in the close proximity of the nanoparticles. The copper nanoparticles using *Nerium oleander* leaf extract has an average particle size of the copper nanoparticle was found to be 139.2 nm. This particle size range of 50 to 1000 nm is generally considered as nanoparticles. This results indicates the particle size is in the nano range [35].

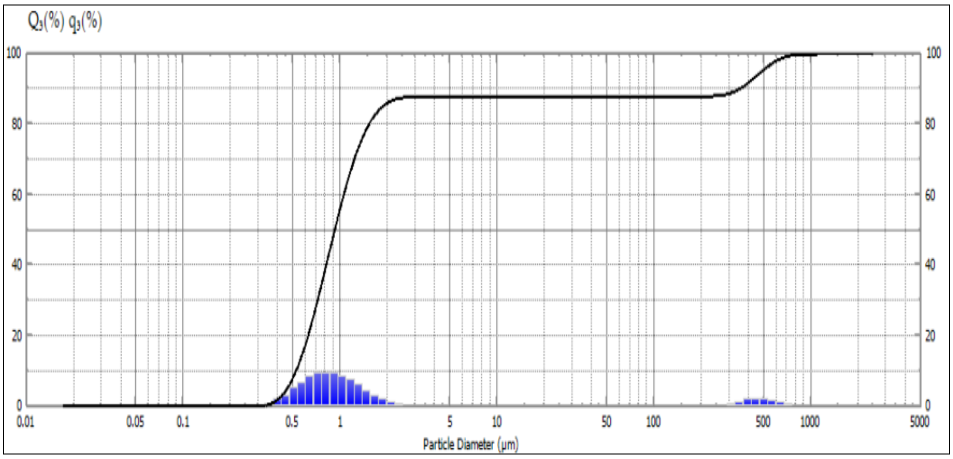

Fig 7: Particle size distribution analysis result of copper nanoparticles from *Hygrophila auriculata*

The antibacterial study was performed by agar-well diffusion method. The effect of *Hygrophila auriculata* leaf extract on standard strain of four human pathogenic *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris* and *Klebsiella pneumonia* were investigated for the antibacterial assays. Water was used as control and Tetracycline was used as standard reference antibacterial compounds respectively. In the disc diffusion method, the diameter of the inhibition zone was measured table 2. The copper nanoparticles at different concentration such as 50µl, 100µl and 150µl showed antimicrobial activity against human pathogens. *Hygrophila auriculata* was most sensitive against *Proteus vulgaris* with a maximum zone of inhibition in diameter (8mm, 8mm and11mm) compared with other strains. The antibacterial activity of synthesized copper nanoparticles using *Vitis vinifera* leaf extract was evaluated against *E. coli*, *S. aureus and K. pneumonia* with zone of inhibition of 14 mm, 16mm and 12 mm respectively [36]. The Cu-NPLs showed an effective broad-spectrum antibacterial activity. The Cu-NPLs at 100 mg produced a higher antimicrobial effect (14 mm) than the Fluconazole (75 mg/mL) in the inhibition of the growth of *B. subtilis* (9mm) [30].

Table 2: Antibacterial activity *Hygrophila auriculata* (Zone of Inhibition)

S. No	Particulars	Concentration (µg)	Zone of inhibition (mm)			
			P. aeruginosa	*S. aureus*	*P. vulgaris*	*K. pneumoniae*
1	Control	-----	---	---	---	---
2	Antibiotic	50	7	8	8	9
3	Plant extract	50	5	5	6	6
4	$CuSO_4$	50	7	7	8	8
5	Copper nanoparticle (T_1)	100	6	6	8	7
6	Copper nanoparticle (T_2)	150	8	8	11	9

The free radical scavenging activity of copper nanoparticles of *Hygrophila auriculata* leaf extract was calculated by DPPH (1, 1-diphenyl-2-picrylhydrazyl) method. The leaf extract showed higher percentage of inhibition and had good antioxidant activity with various concentrations of 20 to 100 µl when compared with standard ascorbic acid. About 19 to 54% of free radical was observed in copper nanoparticle (Fig. 8). The antioxidant activity of CuNPs and aqueous leaf extract of *E. prostrata* was assessed by DPPH free radical scavenging assay. The mean percentage inhibition values were 32, 34, 41, 46 and 53%; and 29, 32, 37, 43 and 48%, respectively. The control values of ascorbic acid were 85, 87, 89, 92 and 95% with the increasing concentrations of NPs [37]. The significant antioxidant activity and the DPPH radical scavenging assay from *Cassia fistula* and the antioxidant activities were strongly correlated with total phenols [38].

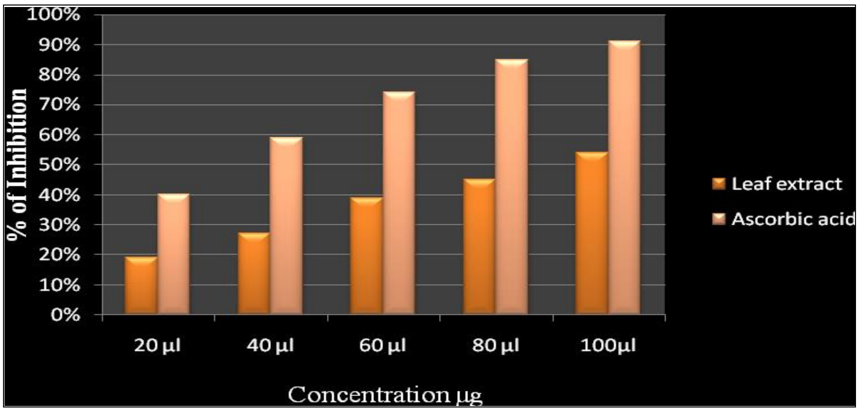

Fig 8: Antioxidant activity of copper nanoparticles *Hygrophila auriculata* and ascorbic acid by DPPH assays

The antioxidant activity of copper nanoparticles of *Hygrophila auriculata* leaf extract was determined by hydrogen peroxide scavenging activity method. H_2O_2 scavenging activity of synthesized copper nanoparticles compared with standard ascorbic acid. The percentage of inhibition of free radicals increased with increase in concentration of samples. The concentration from 20 µl to 100 µl of green synthesized copper nanoparticles showed 28 to 59% of copper nanoparticles (and Fig. 9). The leaf extract of *Hibicus rosa sinensis* with CuNPs was shows good anti-oxidant activity from FRAP and Hydrogen peroxide scavenging assay [39].

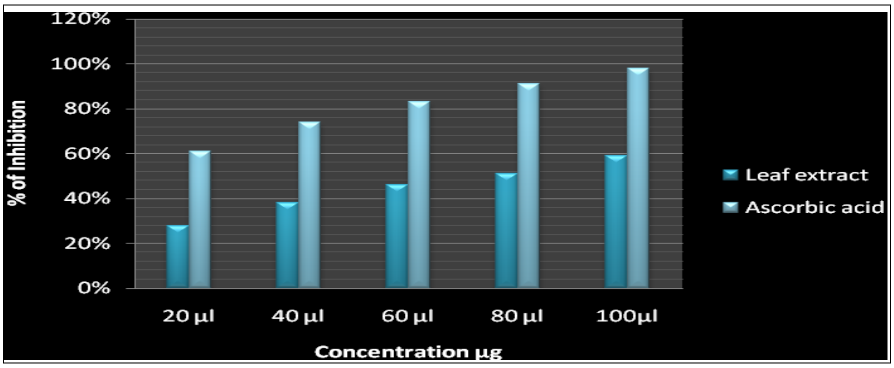

Fig 9: Antioxidant activity of copper nanoparticles *Hygrophila auriculata* and ascorbic acid by Hydrogen peroxide assays

The larvicidal activity of leaf extracts and copper nanoparticles synthesized from *Hygrophila auriculata* plant extracts against the second instar larvae of *Aedes aegypti* was recorded. The *Aedes aegypti* mosquito larval mortality increased from 2-8 larvae with the concentration from 10-100 ppm of leaf extract of *Hygrophila auriculata* (Table 3). The *Aedes aegypti* mosquito larval mortality increased from 2-9 larvae with the concentration from 1-10 ppm of copper nanoparticles synthesized from plant extract of *Hygrophila auriculata* (Table 4). The mosquito larvicidal activity of aqueous leaf extracts and synthesized copper nanoparticles of *A. heterophyllus* showed 100% mortality first to fourth instars larvae of *A. aegypti* at the concentration of 10 mg/l [32].

Table 3: Effect of plant extract (*Hygrophila auriculata)* on the larval mortality of second instar of *Aedes aegypti*

Day	Control	Concentration of plant extract (*Hygrophila auriculata)* in ppm									
		10	20	30	40	50	60	70	80	90	100
1	0	0	0	0	1	1	1	2	2	2	2
2	0	0	1	0	+1	+1	+1	+1	+1	+1	+2
3	0	1	0	+2	0	+1	+2	+2	+2	+2	+1
4	0	+1	+1	+1	+2	+2	+2	+1	+2	+3	+3
Total	0	2	2	3	4	5	6	6	7	8	8
Mortality%	0	20%	20%	30%	40%	50%	60%	60%	70%	80%	80%

Table 4: Effect of copper nanoparticles synthesized from plant extract *Hygrophila auriculata* on the larval mortality of second instar of *Aedes aegypti*

Day	Control	Concentration of the copper nanoparticles (ppm)									
		1	2	3	4	5	6	7	8	9	10
1	0	0	0	0	1	1	0	2	2	1	2
2	0	0	1	0	+1	+1	+2	+1	+2	+2	+2
3	0	1	+1	1	+1	+1	+1	+2	+1	+2	+2
4	0	+1	+1	+2	+1	+2	+2	+1	+2	+3	+3
Total	0	2	3	3	4	5	5	6	7	8	9
Mortality%	0	20%	30%	30%	40%	50%	50%	60%	70%	80%	90%

The LC_{50} value of the plant extract of *Hygrophila auriculata* against *Aedes aegypti* in first, second, third, and fourth day was in the order 191.276, 169.253, 91.268 and 44.742. LC_{90} value of the plant extract of *Hygrophila auriculata* against *Aedes aegypti* in first, second, third, and fourth day was in the order 664.420, 1230.602, 541.932, 209.507. The LC_{50} value of the synthesized copper nanoparticles using plant extract of *Hygrophila auriculata* against *Aedes aegypti* in first, second, third, and fourth day was in the order 23.527, 13.295, 8.873and 4.299. LC_{90} value of the synthesized copper nanoparticles using plant extract of *Hygrophila auriculata* against *Aedes aegypti* in first, second, third, and fourth day was in the order 89.532, 84.645, 55.779 and 21.065 (Table 5).

The adult mortality of *Aedes aegypti* mosquito was not found in plant extract *Hygrophila auriculata* and copper nanoparticles synthesized by *Hygrophila auriculata* plant extract at various concentrations. The total mortality of *Aedes aegypti* mosquito increased from 20 - 80% with the concentration of plant extract of *Hygrophila auriculata* from10 – 100 ppm. The total mortality increased from 20 - 90% with increasing concentration of copper nanoparticles biosynthesized by plant extract of *Hygrophila auriculata* from 10-100 ppm. Adult emergence was found to be 80% in 10 ppm, 80%in 20 ppm, 70% in 30 ppm, 60% in 40 ppm, 50% in 50 ppm, 40% in 60 ppm, 40% in 70 ppm, 30% in 80 ppm, 20% 90 ppm, 20% in 100 ppm in plant extract of *Hygrophila auriculata* (Table 7). Adult emergence was found to be 80% in 1 ppm, 70%in 2 and 3 ppm, 60% in 4 ppm, 50% in 5 and 6 ppm, 40% in 7 ppm, 30% in 8 ppm, 20% in 90 ppm, 10% in 10 ppm in copper nanoparticles synthesized plant extract of *Hygrophila auriculata* (Table 6). The larvicidal activity of CuNPs synthesized from marigold and sunflower mediated CuNPs showed increasing mosquito larval mortality with increasing concentration of CuNPs from 1 mg/L to 10 mg/L. However, maximum significant ($p<0.05$) mortality was recorded at higher concentration (10 mg/l) for both petal extract of marigold and sunflower mediated CuNPs at 24 h of incubation. After 72 hrs of incubation, marigold mediated CuNPs showed almost 80% enhancement of mortality. However, when concentration increased to 10 mg/l, only 34.48% mortality increased with respect to 1 mg/L CuNPs. On the other hand, mortality rate with sunflower mediated CuNPs showed only

15% and 23% during 24 hrs and 72 hrs of incubation, respectively [40].

Table 5: LC_{50} & LC_{90} value of *Hygrophila auriculata* leaf extract and Copper nanoparticles

ample	Time period	Lethal concentration (ppm)				
		LC_{50}	**95% CL**	**LC_{90}**	**95% CL**	**Chi Square**
Hygrophila auriculata leaf extract	1 Day	191.276	110.216-125493.125	664.420	222.890-826491200.000	1.262
	2 Day	169.253	92.876-10956.032	1230.602	302.345-99142896.000	1.131
	3 Day	91.268	62.217-276.210	541.932	210.258-30377.201	1.171
	4 Day	44.742	30.337-62.896	209.507	120.255-974.527	2.045
Copper nanoparticles	1 Day	23.527	11.927- 209152102.944E+07	89.532	24.325-100000002.004E+12	3.444
	2 Day	13.295	8.734-77.805	84.645	28.167-21831.793	2.589
	3 Day	8.873	6.014-27.054	55.779	21.164-3756.159	2.152
	4 Day	4.299	2.840-6.066	21.065	11.945-104.41	3.361

Table 6: Effect of various concentrations of plant extract of *Hygrophila auriculata* on the total mortality and adult emergence on the second instar larvae of *Aedes aegypti*

Parameters	Control	Concentration of plant extract of *Hygrophila auriculata* in ppm									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Larval period in days	4	4	4	4	4	4	4	4	4	4	4
Larval mortality	0	2	2	3	4	5	6	6	7	8	8
Adult mortality	0	0	0	0	0	0	0	0	0	0	0
Total mortality%	0%	20%	20%	30%	40%	50%	60%	60%	70%	80%	80%
Adult emergence%	100%	80%	80%	70%	60%	50%	40%	40%	30%	20%	20%

Table 7: Effect of various concentrations of copper nanoparticles on the percentage of total mortality and emergence on the second instar larvae of *Aedes aegypti.*

Parameters	Control	Concentration of the copper nanoparticles in ppm									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Larval period in days	4	4	4	4	4	4	4	4	4	4	4
Larval mortality	0	2	3	3	4	5	5	6	7	8	9
Adult mortality	0	0	0	0	0	0	0	0	0	0	0
Total mortality%	0%	20%	30%	30%	40%	50%	50%	60%	70%	80%	90%
Adult emergence%	100%	80%	70%	70%	60%	50%	50%	40%	30%	20%	10%

Conclusion

In conclusion, our study shows a simple, cost-effective method for the synthesis of CuNPs from the leaves of *Hygrophila auriculata*. The biosynthesized copper nanoparticles had shown the best potential effect of characterization of UV-Visible spectroscopy, FTIR, SEM, EDAX and particle size analyser. The properties of plants belonging to Acanthaceae possess potential antibacterial activity and controlling vector mosquito *Aedes aegypti* can be developed as ecofriendly larvicides.

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