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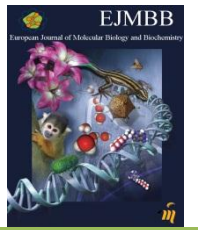

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM LEAVES EXTRACT OF *MILLIETTA PINNATA L.* AGAINST DENGUE CAUSING MOSQUITOES

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ABSTRACT

Mosquitoes transmit serious human diseases, causing millions of deaths every year. The use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides synthesized from natural products for vector control have been a priority in this area. In the present study, silver nanoparticles (Ag NPs) were green-synthesized using a leaves extract of *Millietta pinnata* screened for larvicidal activity against third instar larvae of mosquitoes. The synthesized Ag NPs were characterized by using UV–vis absorption, SEM and FTIR techniques. The textures of the yielded Ag NPs were found to be spherical and polydispersed with a mean size in the range of 2–10 nm. Larvae were exposed to various concentrations of methanolic extract of *M.pinnata* and synthesized Ag NPs for 24 h, and the maximum mortality was observed from the 0.04mg and 0.05mg synthesized Ag NPs against the third instar larvae of *Aedes aegypticus* mosquitoes within six hours. These results suggest that the synthesized Ag NPs have the potential to be used as an ideal eco-friendly approach for the control of mosquitoes.

INTRODUCTION

Mosquito-borne diseases are endemic over 100 countries, transmitting the illness to more than 700 million individuals annually inflicting mortality of nearly two million individuals and a minimum of one million children die every year, leaving 2100 million individuals in danger around the world [1]. Vector control is an essential requirement in control of epidemic diseases such as malaria, filariasis and dengue that are transmitted by different species of mosquitoes. Emergence of insecticide resistance, harmful effect on non-target organisms and environment necessity an urgent search for development of new and improved mosquito control methods that are economical and effective as well as safe for non-target organisms and the environment. Application of chemical insecticides provokes undesirable effects of chemical resistance, toxicity to non-target organism and environmental and human health concerns [2,3]. In this regard, nanoparticles exhibits important role in the several aspects such as drug delivery, diagnostics, antimicrobial activities and tissue engineering [4,5]. Synthesis of nanoparticles by using chemical and physical methods requires high pressure, energy, temperature and toxic chemicals. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as natural capping agents. Synthesis of nanoparticles using plant extract is the most adopted method of eco-friendly production and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of

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several metabolites [6]. The plant-mediated synthesis is a rapid, flexible and suitable process for large-scale production of nanoparticles. Nowadays, plant parts like seed, leaf, bark, stem and fruit extracts have been effectively used for synthesis of nanoparticles [7]. Among nanoparticles, silver nanoparticles have been used enormously due to their potent larvicidal activity.

Millettia pinnata Linn. Panigrahi is a medium sized glabrous tree belonging to the family Fabaceae .It is popularly known as Pongam in tamil .The medicinal tree is native to Western Ghats and chiefly found in tidal forests of India [8]. Historically, Pongamia has been used as folk medicinal plant, particularly in Ayurveda and Siddha systems of Indian medicine [9]. All parts of the plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, itches, abscess, painful rheumatic joints wounds, ulcers, diarrhea etc., [10] Extract of the plant possess significant anti-diarrhoeal, anti-fungal, anti-plasmodial, anti-ulcerogenic, anti-inflammatory and analgesic activities [11]. Phytomedicine represents one of the most imperative fields of traditional medicine all over the world. To uphold the proper use of phytomedicines and to find out their potential use as a source of new drugs, it is essential to study medicinal plants, which have traditional reputation. As a part of this, for the first time the present experiment was conducted to investigate the larvicidal activity of *M. pinnata* leaves extracts.

MATERIALS AND METHODS

Aedes aegypticus mosquitoes were collected from Kovilambakkam sewage area, Chennai. It was reared in lab with yeast and biscuit as a feed.

Plant materials

The plant species of *Milliettapinnata*were collected from Chennai region of Tamil Nadu, India. It was identified and authenticated by the Taxonomist, St. Joseph's College, Tiruchirappalli, Tamilnadu, India. The specimen voucher number is KD001.

Plant sample extraction

The leaves were cut into pieces and shadow dried at room temperature. The dried leaves were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. 100 g of crushed leaves were continuously extracted with 95% methanol using soxhlet up to 48 h. The extract was filtered and concentrated in rotary evaporator at 35-40 ºC under reduced pressure to obtain a semisolid material, which was then lyophilized to get a powder (28.5%, w/v).

Phytochemical analysis

The methanolic leaves extract of *Millietta pinnata* subjected to following test for the identification of its various active constituents by standard method. Alkaloids were identified by Dragendroff's test, flavonoids by lead acetate test, carbohydrates by Fehling's test, proteins by Million's test, phenols by Libermann's test and tannins by Ferric chloride test. Saponins, phytosterol, terpenoids and tannins were identified by Harborne method.

Gas Chromatography - Mass Spectrometry (GC- MS):

The GC-MS analyses were carried out in Perkin Elmer, auto system XL GC+ with the following parameters: Carrier gas: Helium with a flow rate of 0.7 ml/min, Column temperature: 180°C for 5minutes, 180-260°C at 3°C/min., 260°C for 5minutes 260-280 at 0.2°C/min, and280°C for 5 minutes. Injector temperature was 280°C and detector temperature was 290^{o}C. Volume injected: 1µL of sample, Ionization potential: 70 eV, Ion source temperature: 290^{o}C.Interpretation of mass spectrum GC-MS was conducted by using the database of National Institute of Standard and Technology (NIST) having more than 62000patterns.

Synthesis of Silver Nanoparticles (AgNPs):

In a typical synthesis of silver (Ag) nanoparticles, the leaf extract (50 ml) was added to 450 ml of 1 M AgNO3 aqueous solution and kept at room temperature [12]. After 1 hour the colour of the solution changed from colourless to honey brown indicating the formation of silver nanoparticles. This is confirmed and characterized by UV-Visible spectroscopy, SEM and FTIR.

Larvicidal Bioassay:

Larvicidal bioassay of individual plant extracts was tested against third instar larvae. The tests were conducted in glass test tube. Standard WHO protocol with slight modifications was adopted for the study. Ten replicates and a control were run simultaneously during each trial [13,14]. For control, 1.0 ml of leaf extract was dissolved in 10mL of de-ionized water. Mosquito immature particularly early third instar larvae were obtained from laboratory colonized mosquitoes of F1 generation. Twenty healthy larvae were released in each glass beaker and mortality was observed 24 and 48 h after treatment at 1000ppm. A total of three trials were carried out. However, when the control mortality ranged from five to twenty per cent, the observed percentage mortality was corrected by Abbott's formula [15].

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae x 100}}{\text{Number of larvae introduced}}$$

RESULTS and DISCUSSION

Plants and their products have been used for many years for human health. Many plants possesses various novel medicinal values, but still not explored and utilized. Variousvaluable phytochemicals are derived from plants which acts as a drug that are currently used in more countries in the world [16]. The present study reveals that methanol extract of *M. pinnata*leaves exhibited the presence of Alkaloids, Flavanoid, Phenol, Steroid, Saponins and Carbohytrates. GC-MS analysis reveals the

presence of 10 bioactive compounds and these compounds are very significant importance in controlling the growth of mosquito larvae (figure:1)(table:1). Biological methods of nanoparticles synthesis using plant or plant extract have been suggested as possible eco-friendly alternatives to chemical and physical methods. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications in the field of Medicine and Agriculture [17].The change of colour indicates the formation of silver nanoparticles (SNPs) (13). Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability (figure:2).The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. In the present study reveals that silver nanoparticle was synthesized from *M.pinnata*. The reduction of silver nitrate using the plant leaf extract was viewed by the colour change in the reaction solution. Silver nanoparticles (AgNPs) appear honey brown in colour in aqueous medium as a result of Surface Plasmon vibrations.The synthesized silver nanoparticles of *M.pinnata* leaf extract was analyzed using UV- Vis absorption spectrophotometry from 400nm-700nm. The result was observed at 440 nm (Figure 3). Almost all similar results were observed in various medicinal plants namely *Cleodendrum inerme*, *Euphorbia hirta* and *Argimone maxicana* [18].

The SEM images showed different shapes of AgNPs in *M.pinnata* leaves extract which has been used as reducing and capping agents .This analysis shows uniformly distributed silver nanoparticles on the surfaces of the cells (Figure 4) and individual spherical polydispersed AgNPs as well as number of irregular in shaped aggregates. The size of the silver nanoparticles was found to be 5-50 nm, with an average size of 10 nm. The larger silver particles may be due to the aggregation of the smaller ones. However, on most occasions, agglomeration of the particles was observed probably due to the presence of a weak capping agent which moderately stabilize the nanoparticles [19]. FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules [20] FTIR gives the information about functional group present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic $AgNO_3$ to elemental silver. Our study suggested that the FTIR analysis confirmed that the bioreduction of silver ions to AgNPs is due to the reduction by capping material of the plant extract. A typical FTIR spectrum obtained from green silver nanoparticles is shown in the absorption bands at 3407.66, 3178.50, 2896.42, 2059.54, 1648.44 cm−1. The intense band at 3407.66 corresponds to N-H stretching, 3178.50 for =C-H Medium stretching, 2896.42 Carboxylic acid H-C=O:C-H stretching, 2059.54 corresponding to C-H stretching, 1738 due to stretch vibration of -C=O, the band at 1648.44 corresponds to amide, arising due to carbonyl stretch in protein. The bands at 2896.42 and 2059.54 correspond to H-C=0: C-H stretch (Aldehydes). The 3407.66l N-H groups are responsible for reducing the Ag+ ions to atoms and suppressed bands are responsible for stabilising the nanoparticles (Figure :5)

Bio larvicidal activity of mosquito 3rd instar larva was observed in the following concentration of *M.pinnata* leaf extract and *M.pinnata* silver nanoparticles extract treated mosquito larvae [21,22] as follows:

0.01mg concentration of leaf extract treated mosquito larva shows13 larvae death out of 20 and 13 out of 20 in 0.01 mg concentration of green silver nanoparticle treated mosquito larvae.0.02mg concentration of leaf extract treated mosquito larva shows 14 larvae death out of 20 and 15out of 20 in 0.02 mg concentration of green silver nanoparticle treated mosquito larvae.0.03mg concentration of leaf extract treated mosquito larva shows17 larvae death out of 20 and 18 out of 20 in 0.03 mg concentration of green silver nanoparticle treated mosquito larvae.0.04mg concentration of leaf extract treated mosquito larva shows 18larvae death out of 20 and 20out of 20 in 0.4 mg concentration of green silver nanoparticle treated mosquito larvae.

0.05mg concentration of leaf extract treated mosquito larva shows20 larvae death out of 20 and 20 out of 20 in 0.05mg concentration of green silver nanoparticle treated mosquito larvae(graph:1&2).

The findings of present study is very unique with previous reports. studied the larvicidal activities of different solvent leaf extracts of *Elaeagnus kologa*in which methanol, ethyl acetate and acetone extracts showed 100% in 15 and 20 mg/ml concentrations against *Aede saegypti.* .The experiment reveals that the crude chloroform extract of *Millettiadura* (M. dura) showed high activity (LC^{50} = 3.5 $_{\mu}$g/ mL at 24 h) against second-instar larvae of *Ae. aegypti* [21] and the larvicidal efficacy of leaf extract of *Acacia ferruginea* (A. ferruginea) showed LC^{50} values of 5 362.6 ppm against late third instar larvae of *Cx. Quinque fasciatus*[23].

S.NO	COMPOUNDS	RT
1.	Phenol 2-(1,1 dimethyl 2-propenyl 3,6-dimethyl	10.55
2.	Thiabendazole	11.15
3.	Cuvebene	11.98
4.	Phenylchromone	12.52
5.	6,8-Tridecadien 2-ol,acetate	14.15

6.	Oleic acid	15.93
7.	1-Docosanol formate	17.53
8.	3,7,11Trimetydodeca tetraenal	19.07
9.	Dodecanoic acid dodecyl ester	20.35
10.	Genisteine	21.63

Figure 1. GC-MS ANALYSIS

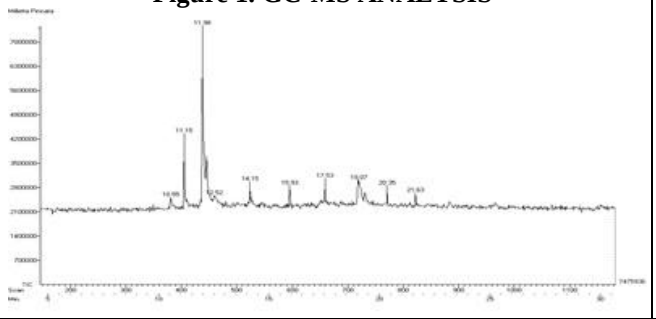

Figure 2. Green Silver Nanoparticle Synthesis

Figure 3. UV- VIS Absorption Spectrophotometry

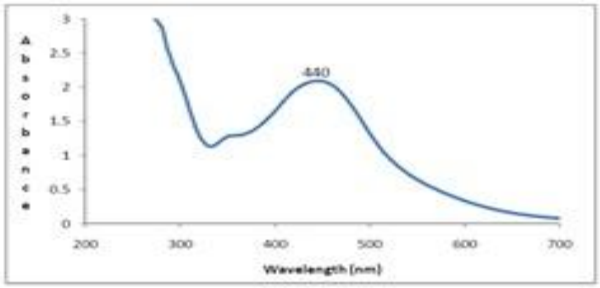

Figure 4. SEM Analysis

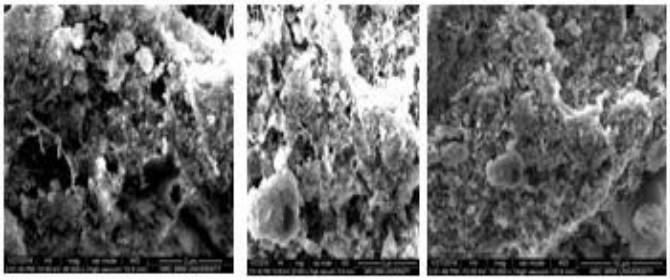

Figure 5. FTIR

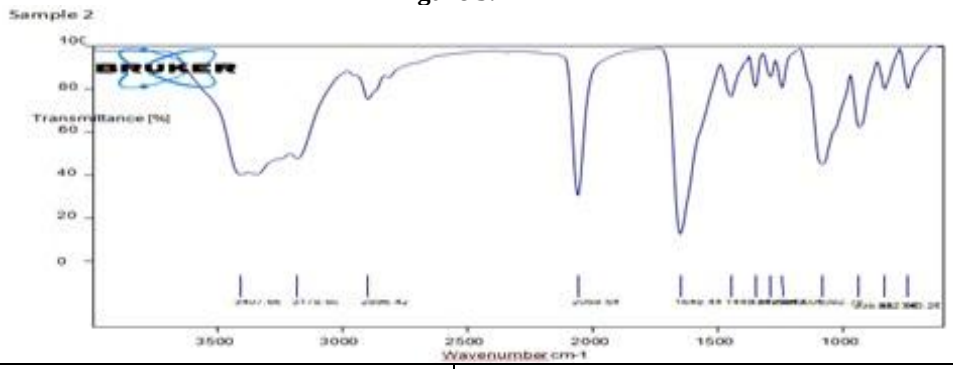

Figure 6. Larvicidal activity of crude extracts

Figure 7. Larvicidal activity of AgNPs

CONCLUSION

The present investigation revealed that the methanolic leaves extract of *M.pinnata* possesses ten potent phytochemical constituents which exhibited high potent biolarvicidal activity against *Ae. aegypti*. The presence of these bioactive constituents justifies the use of the plant leaves for mosquitocidal activities and synthesized green silver nanoparticle shows high efficacy than plant extract for larvicidal, skin repellent and

Oviposition activities which have no side effects as well as very useful for society .

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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