

MOSQUITO LARVICIDAL ACTIVITY OF COST-EFFECTIVE BIO-SYNTHESIZED SILVER NANOPARTICLES USING *Mentha piperita* EXTRACT AGAINST *Aedes aegypti*

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ABSTRACT

The major trajectories for the transfer of assorted diseases together with protozoal infection, break-bone fever are Mosquitoes. Dengue fever has been one of the pathogenic infection carried by *Aedes aegypti* and is reported to increase forcefully around the world. Target species and a biodegradable quest for control agents from a natural product are highly crucial as there are no vaccines or drugs are available for Mosquito-borne diseases. The biosynthesis of metal-based nanoparticles has attained attention due to its non-toxic nature. The present study assessed the phytochemical screening of *Mentha piperita* leaves which shows the presence of bioactive phytochemicals. Green incorporated silver nanoparticles were described by UV-Visible, FT-IR and SEM analysis. The fluid concentrates of *Mentha piperita* leaves show generally excellent enemy of larvicidal action against the dengue vector *Aedes aegypti* hatchlings.

Keywords: *Mentha piperita*, Phytochemical Screening, Silver Nanoparticles, UV- Visible Analysis FTIR, SEM, EDAX, and Larvicidal Activity.

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INTRODUCTION

The mosquito transmits genuine human illnesses causing a large number of passing's consistently. Dengue fever has become a significant general medical issue, particularly with increasingly extreme types of the ailments¹. Since successful antibodies or medications are not accessible for the anticipation and treatment of these ailments, vector control has been embraced as the primary way to deal with diminished transmission². Mosquito protection techniques are being used in the most prevalently chemical method. But prolonged usage of liquid mosquito repellants and anti-mosquito coils paves the way for illness in the respiratory system. The vast majority of the engineered synthetics are costly and ruinous to the earth and poisonous to people and other non-target life forms³. In this way vector control systems, particularly viable, minimal effort, non-toxic, and highly essential. The present study attempted to screen and evaluates the efficacy of the plant extract with the nanoparticles against larvae to control the dengue vector⁴. Nanomaterials can be used for a variety of technological applications and that has led to a tremendous increase in commercial value⁵. The diseases Malaria, Dengue (Mosquito-borne diseases) are responsible for the major mortality, morbidity, and economic loss.⁶ Ancestors used several culinary herbs for flavoring foods and above all used them for medicine⁷. *Mentha piperita* is one of the most important culinary herbs due to both of its flavoring and fragrance properties its medicinal property had been attained our attention to use it. Silver Nanoparticles were derived from the silver nitrate solution added to the leaf extract of *Mentha piperita*.⁸⁻¹³ This shows an effective result against the larvae of *Aedes aegypti*.

EXPERIMENTAL

Sample Preparation

The medicinal plants used in this study are the leaves of *Mentha piperita* collected from Periyakaruppur village, Tiruchirappalli, Tamil Nadu, India. The solvent broth has been done by using the dried leaves

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of *Mentha piperita* using double distilled water. The leaf broth was preserved in bottles at a lower temperature for further analysis.

Qualitative Analysis

Phytochemical screenings were performed using a standard procedure (Harborne method).

Quantitative Analysis

Total Tannins, Saponins, Flavonoids and Terpenoids were determined using standard methodologies.

Characterization of Silver Nanoparticles

UV-Visible Spectra

The development of nanoparticles was confirmed by the UV- Visible spectroscopy. UV-Visible Spectra has been recorded for the synthesized nanoparticle using UV-Visible 1700 (Shimadzu).

FT-IR Assessments

FT-IR estimations were done to recognize the major useful primary functional groups inside the plant extricate and their conceivable inclusion in the combination and adjustment of silver nanoparticles. The presence of biomolecules liable for the reduction of Ag^+ ions to AgNPs inside the pattern was analyzed in FTIR.

SEM Analysis

Surface Morphology along with the topography of the sample can be obtained by this characterization method. The SEM image obtained for the synthesized nanoparticle is given.

Larvicidal Activity

The technique for trying out the larvicidal movement the crude extracts was barely modified from those of the WHO (WHO, 1996). The differently concentrated solutions were produced by diluting a regarded quantity of crude extract in water and saved in a refrigerator at 15°C . Ten healthful overdue 4th instars larvae have been added into each trying out cup which containing 100 ml of dechlorinated water with a solution. A measured quantity of inventory solution was brought to achieve the preferred concentrations. The investigations have been done with a collection of different dilutions. To determine the massive variations in the mortality of mosquito larvae between the standard and the various dilutions. Regression Analysis was used to calculate LC_{50} values and to find out the fatal concentrations of the sample on *Aedes aegypti* mosquito larvae.

RESULTS AND DISCUSSION

Qualitative Analysis

The qualitative screening revealed that the existence of different phytochemicals in the aqueous extract of *Mentha piperita* leaves (Table-1). Visual observation of phytoconstituents in *Mentha piperita* leaves is shown in Fig.-1.

Table-1: List of Phyto-constituents Present in the *Mentha piperita* Leaves Aqueous Extract

S.No.	Phytochemical Constituents	Samples
1	Tannin	+++
2	Phlobatannin	++
3	Saponin	++
4	Flavonoids	+++
5	Steroids	-
6	Terpenoids	++
7	Cardiac glycosides	+++
8	Leuco anthocyanin	+

9	Anthocyanine	+
10	Anthroquinone	++
11	Protein	+++
12	Coumarin	+++
13	Glycoside	-
14	Phenol	+++
15	Alkaloids	+++
16	Xanthoprotein	+
17	Emodine	++
18	Carbohydrate	+

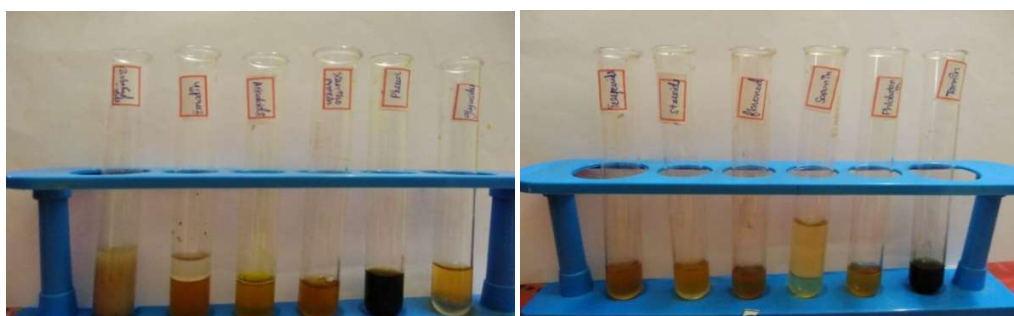


Fig.-1: Visual Observation of Qualitative Analysis on Aqueous Extract of *Mentha piperita* Leaves, Strongly present = +++, moderately present = ++, slightly present = +, absence = -

Quantitative Analysis of Aqueous Extract of Samples

Quantitative evaluation shows the quantity of following phyto-constituents present in the extract of *Mentha piperita* leaves shown in Table-2. Visual observation of phyto-constituents in *Mentha piperita* leaves is shown in Fig.-2.

Table: 2 Quantity of the Phytochemical Constituents in the Aqueous Extract

S. No.	Phytochemical Constituents	Samples (mg/g)
1.	Flavonoid	0.013
2.	Tannin	0.002
3.	Saponin	0.008
4.	Alkaloid	0.005
5.	Phenol	0.003
6.	Terpenoid	0.001



Fig.-2: Quantitative Analysis of Phyto-constituents in *Mentha piperita* Leaves

Synthesis of Silver Nanoparticle

The AgNO_3 solution and then leaf extract is prepared by using double distilled water. The required amount of the leaf extract was added to the AgNO_3 solution gradually with constant agitation. After 30 minutes, the color of the solution changed from colorless to dark brown color, indicates the development of AgNPs. The colloidal solution was analyzed by a UV-Visible spectrophotometer.

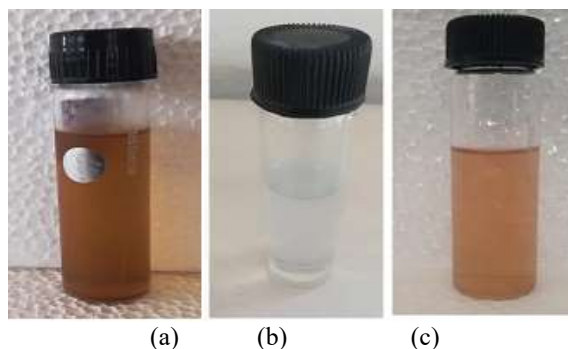


Fig.-3:(a) *Mentha piperita* Leaves Extract, (b) Silver Nitrate Solution, (c) Silver Nanoparticles

UV- Visible Spectra Spectroscopy

The broad absorption peak is observed at 430 nm from the UV- Visible Spectrum which proves the development and sustainability of the biosynthesized silver nanoparticles using *Mentha piperita* leaves¹⁴. Earlier confirmation is only the visual observation. This confirms the reduction of silver into silver nanoparticles.

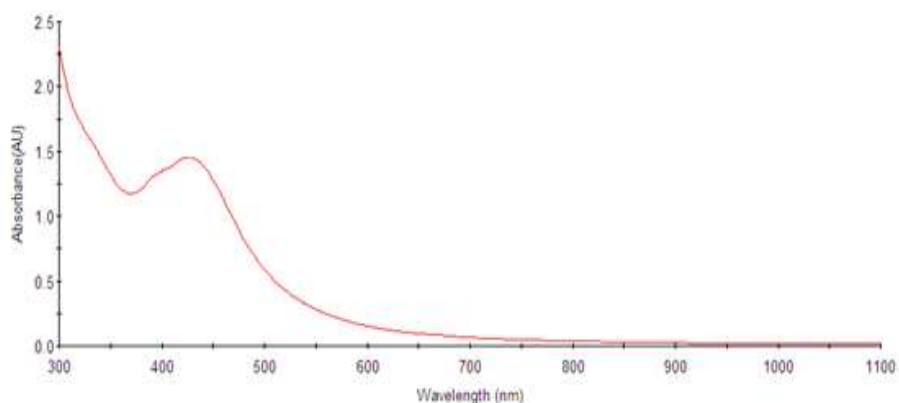


Fig.-4: UV- Visible Spectra of the Spectrum of Silver Nanoparticles

FT-IR Analysis

This shows the existence of secondary metabolites contains functional groups that are accountable for the formation of silver nanoparticles. Table-3 shows various frequencies corresponding to the observed bands.

Table-3: Various Frequencies Obtained From FT-IR Spectrum of Synthesized Silver Nanoparticles

Frequency	Band
3441.16cm ⁻¹	Strong, broad O-H stretching alcohol
2077.79cm ⁻¹	Strong N=C=S stretching isothiocyanate
1634.81cm ⁻¹	Medium C=C stretching conjugated alkene
1405.54cm ⁻¹	Medium O-H bending alcohol
1114.46cm ⁻¹	Strong C=O secondary alcohol
1030.73cm ⁻¹	Strong CO-O-CO anhydride
662.74cm ⁻¹	Strong C-Br stretching halo compound

SEM ANALYSIS

SEM image represents the eco-friendly green synthesized silver nanoparticles using *Mentha piperita* leaves broth. Most of the particles were observed to be spherical. Since nanoparticles are very distinct, it indicates the stability of nanoparticles formed by the secondary metabolites which reduce silver ions to AgNPs. Applied voltage and details of magnification used for imaging were intimated in the images.

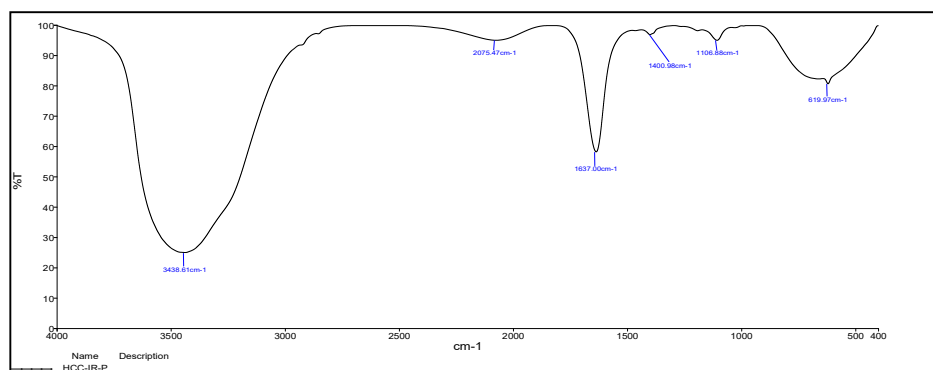


Fig.-5: FT-IR Band of Synthesized Silver Nanoparticles

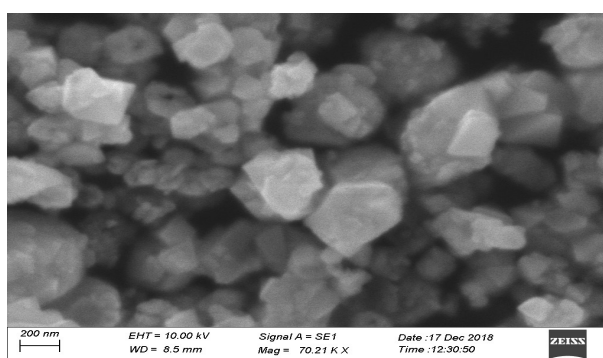


Fig.-6: SEM Image of Synthesized Silver Nanoparticles

Larvicidal Activity

The mortality rate of *Aedes aegypti* larvae by synthesized silver nanoparticles using *Mentha piperita* leaves is shown in Table-4. The death rate of all hatchlings of *Aedes aegypti* at 4 % solution was altogether higher than the death rate at all other concentrations of silver nanoparticle synthesized using *Mentha piperita* leaves extract. The highest death rate was found at 72 hours than those at 12, 24 and 48 hours.

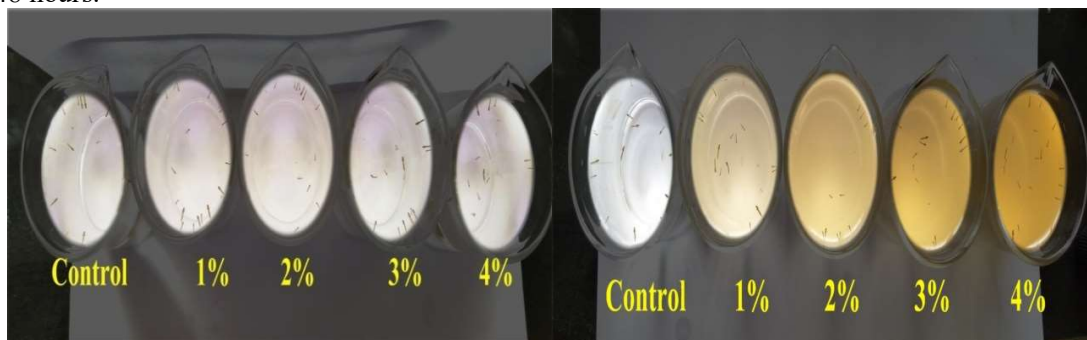


Fig.-7: Larvae before Incubation

Fig.-8: Larvae after Incubation

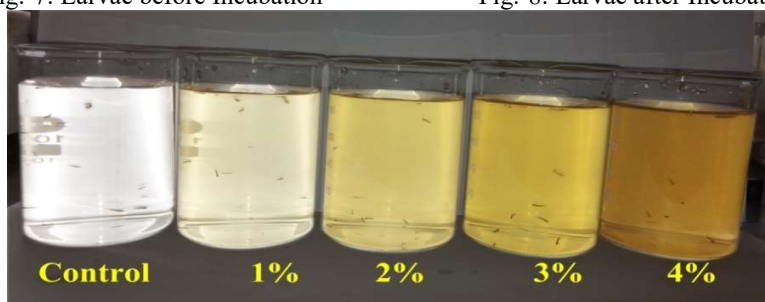
Fig.-9: % Mortality Rate of *Aedes aegypti* Larvae after the Addition of Synthesized Silver Nanoparticles

Table 4: Mortality Rate of *Aedes aegypti* Larvae by Synthesized Silver Nanoparticles Using *Mentha piperita* leaves

Concentration (%)	Number of exposed Larvae	Hrs.							
		12		24		48		72	
		Mortality	% of Mortality	Mortality	% of Mortality	Mortality	% of Mortality	Mortality	% of Mortality
0 (Control)	10	0	0	0	0	3	10	5	50
1	10	1	10	3	30	5	50	6	60
2	10	2	20	4	40	7	70	7	70
3	10	4	40	7	70	9	90	9	90
4	10	5	50	8	80	10	100	10	100

The LC₅₀ values and regression equation and regression coefficient values of synthesized silver nanoparticles using *Mentha piperita* extract against *Aedes aegypti* were presented in Table 5. Regression linear value is calculated based on % of mortality.

Table-5: LC₅₀ Value, Regression Linear and R² Value of Tested Larvae

Hours	LC ₅₀ value (%)	Regression Linear	R ² Value
12	4.00	y = 13x - 2	0.9826
24	2.30	y = 20x + 4	0.9709
48	1.00	y = 18x + 32	0.9878
72	0.15	y = 13x + 48	0.9826

From Table-5 it revealed that LC₅₀ values were slowly reduced during exposure and there is an effective relationship between death rate (Y) and concentration (X) having regression coefficient value roughly similar to one in each case. LC₅₀ value of *Mentha piperita* was 4.00, 2.30, 1.00 and 0.15 ml for 12, 24, 48 and 72 hours respectively. The lower LC₅₀ value has potential larvicidal activity. The potential larvicidal activity was observed at 72 hours of exposure which has the lowest LC₅₀ value of 0.15.

CONCLUSION

Bio-insecticides from plant beginning may contribute viable, economical and safe techniques for vector management. The Phytochemical screening of *Mentha piperita* leaves indicates that it has considerable amounts of phytochemical constituents in it. The quick biosynthesis of AgNPS provides an easy, financially affordable, secured, effective and ecologically agreeable course for the synthesis of nanoparticles. *Mentha piperita* shows an extraordinary ability to integrate the silver nanoparticles at optimum conditions. The SPR band at 430 nm indicates the amalgamation of silver nanoparticles. SEM image confirms that biosynthesized Silver nanoparticles were spherical. The larvicidal activity indicates that the *Mentha piperita* has efficient mosquito larvae management. These discoveries might facilitate with being applied in incorporated management techniques to increase the most extreme effect on vector control.

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