

# Biosynthesis of Silver Nanoparticles from Aqueous Leaf Extract of *Mentha Piperita* and Its Antimicrobial Activity against Intestinal Pathogens

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**Abstract:-** In the present study biosynthesis of silver nanoparticle from *Mentha piperita* were studied for its antibacterial activity against pathogenic microorganisms by well diffusion method and MIC. Nanoparticles were characterised by spectroscopic analysis. FTIR analysis showed functional group of this leaf extract. SEM analysis of biosynthesized AgNPs revealed that the size and shape of AgNPs were changed in plant extract concentrations. The biosynthesized Silver nanoparticles of *Mentha piperita* were showed for its antibacterial activity against three gram positive and two gram negative pathogenic microorganisms. The results of this study showed that the gram negative bacteria *Shigella* sp. was very effective with a zone of inhibition 15mm in diameter. The majority functional groups are alkenes and alkynes. In SEM analysis oval shaped nanoparticles of *Mentha piperita* were found in 5µm. The present result clearly indicates that the biosynthesis of AgNPs were strong antibacterial activity and also these Silver nanoparticles may be used in effluent treatment process to bring down the microbial load, and also in medical application.

**Keywords:-** Silver Nanoparticles, Well Diffusion, MIC, *Mentha Piperita*, SEM.

## I. INTRODUCTION

Nanotechnology is an associative field of research combining biology chemistry physics and materials science with particles sizes up to 100 nm (Cai *et al.*, 2008). In the span of Nano technology, research and nanoparticles is growing day to day with increasing demand. It finds extensive application in Nano medicine and emerging new field. It is chiefly concerned with synthesis of nanoparticles variables size, shapes, chemical composition and controlled disparity and their potential use for human benefit.

In recent years plant mediated biological synthesis of nanoparticles is acquiring sacrifices due to its simplicity and eco friendliness (Faroquet *et al.*, 2010). Nanoparticles are being evolved for divorced applications in the present world such as drug targeting and deliveries (Patel *et al.*, 2005). Among various kinds of inorganic metals such silver, gold,

lead etc. have different antimicrobial activity against microorganisms.

The biosynthesis of nanoparticle, which is an emerging highlight of the interaction of Nanotechnology and biotechnology has becomes a competent replacement for obtaining safer and more benign products not only for the environment but also for the people that potentially will use them (Mubarak Ali *et al.*, 2011). The nanoparticles are active against certain type of organisms like bacterial, fungi, yeast. Among these organisms, the use of plants and their extracts offer various advantages over other traditional methods and other biological system.

*Mentha piperita* is commonly known as peppermint. It is a prime therapeutic herb that belongs to Laminacea family.

### ➤ Scientific Classification

- Kingdom - Plantae
- Phylum - Angiosperm
- Order - Lamiales
- Family - Laminacea
- Genus - *Menthapiperita*

It is a hybrid mint with vast therapeutic benefits. Peppermint oil is composed basically of menthe (37%), methyl acetate (17%) and menthone (12%). Other elements include salvianolic acid dehydrosalvinic acid luteolin glucuronide, luteolin, isomethyl acetate, isomenthol, protein, piper tone, pyrimidine and limnone.

The remedial use of peppermint was an astringent, vermifuge, antiseptics, carminative, medicament, antiemetic, diaphoretic, analgesic (Paula Gardiner *et al.*, 2000). The leaf extract can hold, antioxidant antiallergic, antiplasmic, antiandrogneic, antitumorogenic, anticatarrhal. In reality this kind was used to treat disparity of digestive problems such as diarrhoea, colic in infants, indigestion, nausea, vomiting, anorexia, and morning sickness and also to reduce gas. It is used to treat irritable bowel syndrome.

Hence the present study focuses and evaluates the potency of the leaf extract of *Mentha piperita* and the biosynthesis was characterized by SEM analysis based on literature survey. The extract of this herb can also bring sown

the arsenic induced toxicity, glucose, cholesterol. The volatile oil of peppermint was useful in revitalize mind improving food, relaxing tension and anxiety (Ilmbergerl *et al.*, 2001).

## II. MATERIALS AND METHODS

### A. Collection and Authentication of Plant Materials

The leaves of *Mentha piperita* commonly known as pepper mint were collected from the local market at Udiyankulangara and were authenticated from the department of Botany Nesamony Memorial Christian College, Marthandam.

### B. Preparation of Leaf Extract

The collected leaves of *Mentha piperita* were washed three times with distilled water monitored by shade air drying and grinded to fine powder and stored in an aseptic bottle for further use. Weighted 20 grams of powder was suspended to the 100ml sterile distilled water and kept for boiling in 500 ml conical flask for 20 minutes. After cooling sample was filtered with Whatmann No.1 filter paper and preserved in sterile bottle as filtrate.

### C. Synthesis of Silver Nanoparticles From *Mentha Piperita* Leaves

Silver nitrate ( $\text{AgNO}_3$ ) in 1mM concentration was added to the preparation and volume up to 200 ml by the addition of sterile distilled water. Samples were then centrifuged 10,000 rpm for 30minutes and supernatant was again heated to 95 ° C till the brown colour of representative silver nanoparticles was formed. Cooled the sample immediately then freeze at 4 °C.

### D. Collection of Clinical Isolates

Bacterial isolates were collected from S.P hospital, Parassala, Trivandrum District and further periodic subculture in nutrient agar slants and preserved at 4°C to keep the strains viable.

### E. Antibacterial Activity of Synthesised Nanoparticles

Antibacterial activity of *Mentha piperita* was prepared by using agar well diffusion method (Anushia *et al.*, 2009). Five bacterial pathogenic strains two gram positive and three gram negative strains such as *Staphylococcus aureus*, *Streptococcus sp.*, *Klebsiella pneumoniae*, *Salmonella sp.* and, *Shigella sp.* were used in this analysis. The media used for antibacterial test was Nutrient Broth. The test bacterial strains were saturated within the Nutrient broth and incubated at 37 °C for 24 hrs. Following incubation the culture tube was inspect with the turbidity level.

Fresh bacterial isolates of 0.1 ml having 108 CFU were dispersed on Muller Hinton agar (MHA) plate using sterile swab. Wells of 6 mm diameter were bored off into medium

with sterile cork borer and charged with 50µl of plant extracts by using micro pipette in each well in aseptic condition. Plates were then put up in refrigerator to allow pre-diffusion of extract for 30 min. Further the plates were incubated in an incubator at 37 °C for 24 hrs. The antibacterial activity was assessed by measuring the zone of inhibition.

### F. MIC (Minimum Inhibitory Concentration)

#### ➤ Tube Dilution Method

This test is to verify the least concentration of an antimicrobial that will inhibit the clear growth of the microorganism after 24 hours of incubation. Ten test tubes were taken and arranged in series. Add 1.6 ml nutrient broth on first and tenth test tube, remaining test tubes was added with 0.9ml nutrient broth. Keep it for sterilization. After sterilization, except ninth test tube add 200 µl extract and 200 µl DMSO reagent (dimethyl sulphoxide) by using micropipette and the final volume of 1.8 ml. Ninth test tube is kept as control. Then 0.9ml from the first test tube to second and 0.9ml from second to third and serially dilute up to eighth test tube, 0.9ml were discarded from eighth and tenth test tube using micropipette. Then add 100 µl cultures to all the test tubes except nine and ten. Then incubate it at 37°C for 24hours. After 24 hours of incubation add resazurin dye to all the test tubes excluding ninth. Then incubate for 6-8 hrs at 37°C. After incubation the least concentration of the extract completely inhibited is recorded as MIC (Das *et al.*, 2013).

### G. MBC (Minimum Bactericidal Concentration)

The MBC of the extract was committed by sub-culturing 100 µl of the test dilutions from MIC tubes on to Muller Hinton agar dish and was incubated at 37°C for 24 hours. The highest dilution that provide little or negative bacterial colony on the plate was reported as MBC.

### H. Spectroscopic Analysis

#### ➤ FTIR (Fourier Transform Infrared Spectrophotometer)

ATR model FTIR Spectrophotometer (Bruker Alpha T) was used for the analysis of the dried leaf powder. The spectrum 1000-3500 nm by employing standard KBr pellet technique and was recorded using Attenuated Total Reflectance (ATR) technique beach measurement and computerized study along the IRPAL.

#### ➤ SEM (Scanning Electron Microscopy)

Scanning Electron Microscopic analysis was done using SEM machine. Fine coating of the sample was plot on a carbon coated copper grid by releasing a very small amount of the sample on the grid, additional solution was withdrawn using an absorbent paper and then the fine coating on the SEM grid was permitted to dehydrated by placed under a mercury lamp for 5 minutes and the measurements were performed on Tescan VEGA3 analytical instrument.

### III. RESULTS

#### A. Colour Change

The reduction of Ag<sup>+</sup> into silver nanoparticles during contact with aqueous extract of *Mentha piperita* was able to be followed by the colour change. The fresh extract of

*Mentha piperita* was green in colour. Rather, following the incorporation of silver nitrate within the leaf extract and stirring for 72 hours at room temperature, the extract changed green colour to dark brown colour. The periodical analysis of colour change of the leaf extract with silver as control was observed and results were displayed in plate 1



a) *Mentha piperita* plant

b) Control

c) Extract with silver nitrate

Plate 1 *Mentha piperita* leaf extract

#### B. Antibacterial Activity of Synthesised Nanoparticles

The silver nanoparticles of *Mentha piperita* were carry out for antibacterial activity against the clinical isolates such as *Staphylococcus aureus*, *Streptococcus* sp., *Salmonella* sp., *Shigella* sp. and *Klebsiella pneumoniae*. The silver Nanoparticles from *Mentha piperita* manifest that it was

extremely sensitive against *Shigella* sp. and *Salmonella* sp. with a zone of diameter 15mm and 13 mm respectively. The other three pathogens such as *Klebsiella* sp., *Streptococcus* sp. and *Staphylococcus aureus* were resistant against the synthesised nanoparticle of *Mentha piperita*. The results were shown in Plate 2, Table 1.

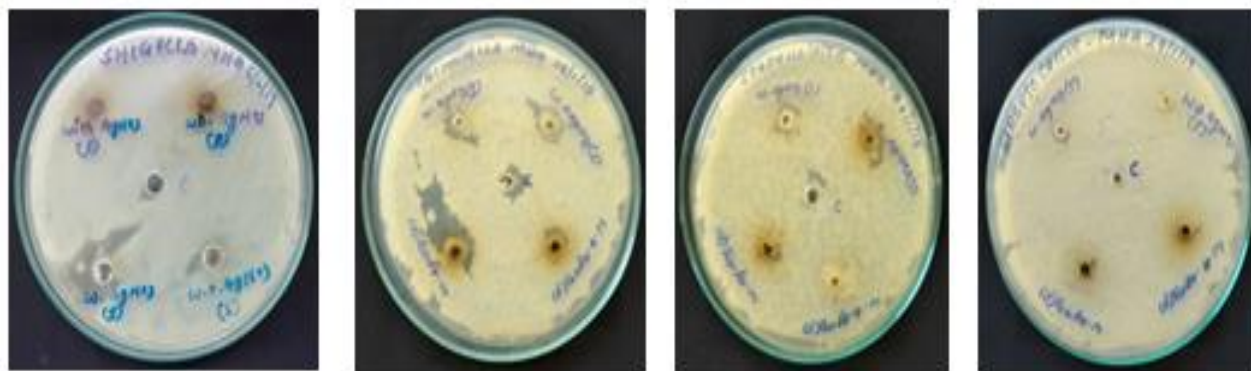


Plate 2 Antimicrobial activity of synthesised nanoparticles

SI No	Organism	Zone of inhibition (mm)	
		<i>Mentha piperita</i> of synthesised nanoparticles	<i>Mentha piperita</i> Extract
1	<i>Shigella</i> sp.	15 ±0.2	No zone
2	<i>Salmonella</i> sp.	13 ±0.3	3 ±0.2
3	<i>Klebsiella pneumonia</i>	No zone	8 ±0.4
4	<i>Staphylococcus aureus</i>	No zone	No zone
5	<i>Streptococcus</i> sp.	No zone	No zone

Table 1 Antimicrobial activity of synthesised nanoparticles



**C. MIC (Minimum Inhibitory Concentration)**

The tube dilution assay was performed to detect the minimum inhibitory concentration (MIC) of the leaf extract against the test strain *Shigella* sp. The results were concluded and argued in terms of change in colour of the tubes after addition of resazurin dye. The results were presented in plate 3, table 2.

The growth of *Shigella* sp. was inhibited at 100 mg and 50 mg, concentration of silver nanoparticles of *Mentha piperita* leaf extract in the first and second tubes showed no turbidity. Based on visual inspection it is claimed that 100 mg and 50 mg, would be the minimum inhibitory concentration (MIC) against *Shigella* sp. For further confirmation of a bacterial growth inhibition resazurin dye (20 µl) was added as a growth indicator to tubes showing reduction of bacterial growth based on turbidity. The pink colour was the indicative of presence of bacterial growth and blue colour showed inhibition of bacterial growth. Here the silver nanoparticles of *Mentha piperita* leaf extract of first and second tubes showing blue colour and rest of the concentration showing pink tube indicating bacterial growth.



Plate 3 MIC of synthesised nanoparticles

Bacterial strain	Synthesised nanoparticle extract (mg/ml)	MIC
<i>Shigella</i> sp.	100	+
	50	+
	25	-
	12.25	-
	6.12	-
	3.06	-
	1.53	-
	0.76	-
	control	+
	100	+

Table 2 MIC of synthesised nanoparticles

+ = no bacterial growth  
 = bacterial growth

**D. MBC (Minimum Bactericidal Concentration)**

Minimum bactericidal concentrations of the plant extract against the test organism *Shigella* sp. were 100mg and 50mg respectively were shown in plate 4.



Plate 4 MBC of synthesised nanoparticles

**E. FTIR Spectroscopy**

FTIR Spectrum was practiced to recognize the functional group of the active constituents in accordance with the peak value in the region of infrared radiation. The findings of FTIR peak values and functional groups were illustrated in table 3 and figure 1. This method was substantiating to be a stable and precise method for detection of bimolecular composition. The interferogram exhibit a wide band at 3274 cm<sup>-1</sup> is assigned to Amines. The sharp peak located at 1633 cm<sup>-1</sup> is related to the presence of alkenes. The another absorption bands were 2923 cm<sup>-1</sup> to alkane, 2119 cm<sup>-1</sup> to alkyne, 1549cm<sup>-1</sup> to amides/ aromatic rings, 1404cm<sup>-1</sup> to alcohol, 1443cm<sup>-1</sup> to ethyl, 1248cm<sup>-1</sup> to phenol and 1041cm<sup>-1</sup> to ether.

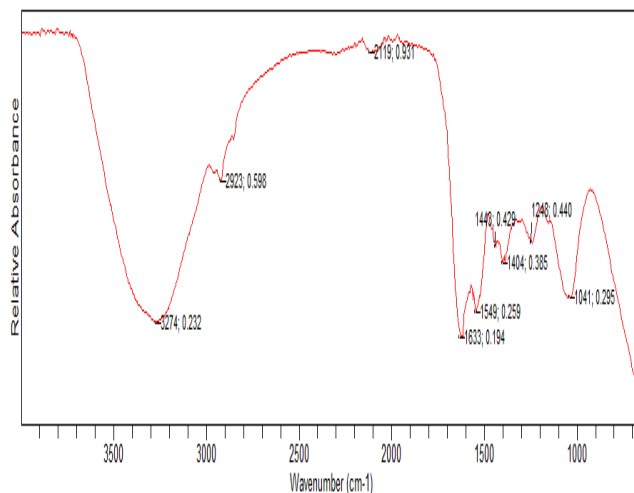


Fig 1 FTIR spectrum of synthesised nanoparticles

SI No	Peak value	Stretching	Interpretation
1	3274	NH	Amines
2	2923	C-H	Alkanes
3	2119	C≡C	Alkynes
4	1633	C=C	Alkenes
5	1549	C-H	Amide/ Aromatic compound
6	1404	C-Cl	Alcohol
7	1443	C-O	Ethyl
8	1248	C-O	Phenol
9	1041	C-O	Ether

Table 3 FTIR spectrum of synthesised nanoparticles

#### F. SEM (Scanning Electron Microscope)

SEM analysis under the optimal experimental conditions, was performed to characterize the morphology of the synthesized Silver nanoparticles. SEM image of the prepared nanoparticles showed that biologically synthesized silver nanoparticles were almost mono dispersed and approximately of oval size from 20–50 nm ranges, as displayed in Fig. 2.

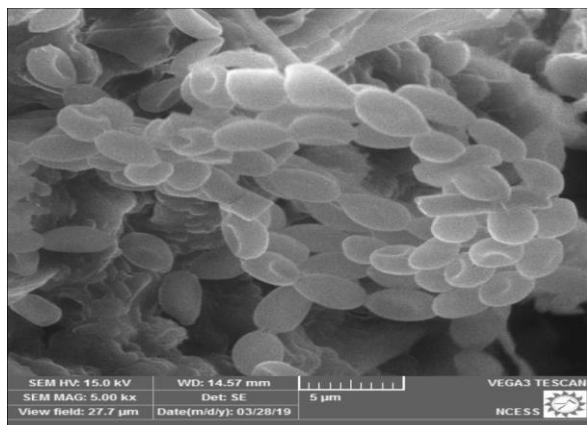


Fig 2 SEM images of synthesised nanoparticles

## IV. DISCUSSION

In the present study, aqueous extract of synthesised *Mentha piperita* nanoparticles provides an environment friendly, simple and efficient. During silver nanoparticles synthesis, it needs stabilizing agent or capping agent. When silver nitrate is dissolved in water, it leads to the formation of free silver ion and free nitrate ions (Fu *et al.*, 2006 and Jha *et al.*, 2009). When silver nitrate was diminished with aqueous extract of *Mentha piperita* leaves, silver nanoparticles synthesis was recorded. Hence, it was specified that the presence of electron donor group in aqueous extract of leaves which fundamentally act as capping agent or stabilizing agent.

FTIR studies were accomplished to inspect possible bio reducing agents present in the silver nanoparticles. FTIR results revealed that absorption bands at 3274 $\text{cm}^{-1}$ , 2923  $\text{cm}^{-1}$ , 2119  $\text{cm}^{-1}$ , 1633  $\text{cm}^{-1}$ , 1549  $\text{cm}^{-1}$ , 1404  $\text{cm}^{-1}$ , 1443  $\text{cm}^{-1}$ , 1248  $\text{cm}^{-1}$ , 1041  $\text{cm}^{-1}$  which are associated with amines, alkanes, alkynes, alkenes, amides, alcohol, ethyl, phenol, ether respectively. From this result it was inferred that few proteins and metabolites such as flavonoids and phenols are there in the leaf extract of *Mentha piperita* and may accountable for the reduction and capping of AgNPs, proved by Shankar *et al.*, (2004). The phenolic compounds can hold hydroxyl and carboxyl groups, which have the potentiality to bind metals.

Nanoparticles of *Mentha piperita* appears remarkable activity as a result of the leaf can hold innumerable potent compounds like menthol, menthone, menthyl acetate, menthofuran, and limnone. The biosynthesis of leaf extract of *Mentha piperita* exhibit powerful activity against *Shigella* sp., *Salmonella* sp., *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Streptococcus* sp. Corresponding this result, the pharmacological activity of *Mentha piperita* against infectious bacteria was reported by Deans and Baratta, (1998).

The present outcome revealed that there is no bacterial growth in 100mg and 50 mg concentration of MIC of the *Mentha* leaf extract. It was specified that the silver nanoparticles have well anti-bacterial activity. It was proved in several prior studies (Crabtree *et al.*, 2003 and Hamouda *et al.*, 2000). In SEM analysis the synthesised silver nanoparticles produced were of oval in shape with size range from 20 to 50 nm in size. SEM analysis of Rajkumar *et al.*, (2011) of the synthesized silver nanoparticles was clearly distinguishable and the structures were triangles, pentagons, and hexagons.

Hence, metal nanoparticles were practiced for disease diagnosis and pharmacological activities. Therefore it can be concluded that aqueous extract of the above mentioned leaves are the prime synthesis of nanoparticles as green touch. Subsequent study and characterization of these *Mentha* species with tests in vivo conditions are also needed to enhance the antioxidant potential which may be employed for preservation of fresh and treated foods as well as in pharmaceutical application.

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