

# Synthesis of silver nanoparticles from leaf extract of mentha piperita, and evaluation of anthelmintic activity on cestode and nematode parasites of country fowl.

Dr. Y Sunila Kumari, A.K. Sunitha , G. Sucharitha,

Department of Zoology, Oucw Koti, Hyderabad.

## ABSTRACT

Plant based nanoparticles are considered to be potential agents to control the drug resistant helminthes *Mentha piperita* leaf extract was used to prepare silver nanoparticles to control helminthic burdens. Different concentrations of aqueous extracts of *Mentha piperita* and silver nanoparticles of *mentha piperita* were tested against cestode and nematode parasites collected from country fowl. The produced silver nanoparticles were characterized by using FTIR which are useful to detect the functional groups of *Mentha piperita*. *Mentha piperita* proves its feature to prepare silver nanoparticles having anthelmintic activity when compared with standard drug, piperazine citrate. The death of the parasites was observed to be dose dependent, by increasing the dose at each level time taken for the death of parasitic cestode and nematode worms was shorter. Death was noted by observing the movement of parasites arresting by the paralytic effects.

## INTRODUCTION

Helminth diseases are caused by parasitic worms known as ‘helminthes’. There are 3 categories of worms like round worms ,tape worms and flukes. These worms causes heavy economic loses in the form of retarded growth, reduced weight gain, decreased egg production, mucoid diarrrohea, anemia, weakness, paralysis and high mortality rate amongst food animals. Poultry ranks high in their ability to convert feed into high energy food products (meat and egg) for consumption.

Helminthiasis is considered as one of the most significant constraint on poultry production in India. Climatic conditions of India are favourable for faster larval stage development in helminth parasites. Large number of parasites causes devastating effects on growth, egg production and overall health, gastrointestinal parasites which invade chickens enhance their adaptation to long living and existence in chicken. It has been reported that

parasitic infections result in immunosuppression, especially in response to vaccines against some poultry diseases.

Nanotechnology involves the aspects of nanoscience – like designing, synthesis, characterization. Nanoparticles are designed to interact with cells and tissues at molecular level of cell. Nanotechnology has applications in drug and gene delivery, biodetection of pathogens, detection of proteins and probing of DNA structure, tumour detection. Nanoparticles are synthesized on industrial scale, they are also produced as byproducts in the manufacture of other materials. Nanoparticles are produced by Top-Down processes. Top-down refers to mechanical crushing of source material using a milling process. Nanoparticles synthesis is preferred as it is cost effective and environment friendly and safe.

## MATERIALS AND METHODS

### Materials Required:

- Silver nitrate (99%)
- Distilled water
- Fresh *Mentha* leaves

### Procedure:

#### 1. Preparation of *Mentha piperita* plant leaf extract:

Fresh mint leaves were collected from the University College for Women, Koti and were identified from department of Botany. The leaves were collected are brought and washed with distilled water thrice. These leaves are kept for shady drying and after complete drying. The mint extract was prepared as per description of N.A Tambe et.al. The leaves are then grinded to fine powder and stored in plastic bottle till further use. Then weigh 20-25 grams of leaf powder and suspend it in 250ml distilled water and then it is kept it for boiling for 30minutes at 80°C in 500ml Erlen Meyer flask. Then after 20 minutes the sample is then kept for cooling then kept in a sterile bottle as filterate.



**Leaf extract of *mentha piperita* Silver nanoparticle solution of *mentha piperita*  
Biosynthesis of Silver Nanoparticles (AgNp's):**

AgNp's were synthesized according to the protocol outlined by Ahmad et al. Firstly, we have to prepare an aqueous solution of AgNO<sub>3</sub> having a molarity 1mm which is useful to synthesize the desired Nanoparticles. Take 10mg of AgNO<sub>3</sub> in 50ml of distilled water. Then add 10ml of the filtered solution for production of silver Nanoparticles of *Mentha*. The solution was incubated at room temperature for 16hours at dark condition (sayeeda sultana et al.). There conforms the colour change the formation of silver Nanoparticles of *Mentha piperita*.

## 2. Collection of parasites from infected fowl:

The parasites are obtained from the freshly slaughtered country fowl. These are examines for helminthic infections of various types of cestodes and nematodes occurs in different organs and more commonly in the intestine. The chickens were humanely slaughtered by cervical dislocation and their gastrointestinal tracts dissected out and observed for the parasites of cestode of genus *Railletina tetragona* nematode of genus *ascaridia galli*. They are removed carefully by using a sterilized forceps. They are then taken on to the sterilized petridishes. Then add silver Nanoparticles solution of *Mentha piperita* the petridishes of varying concentrations 10,20 and 50mg/ml in each petridishes.

Fig.1 Collection of parasites in chicken intestine



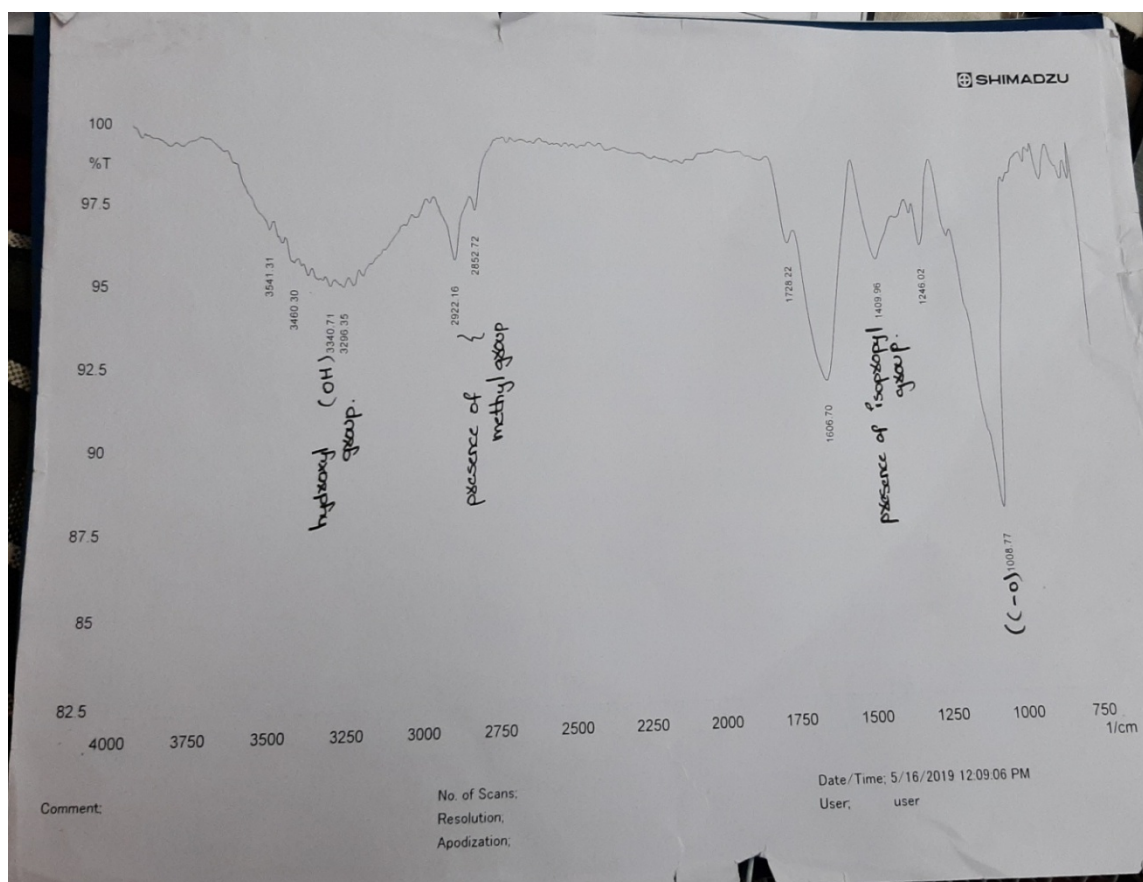
**Nematodes** (*Ascaridia galli*) **Cestode** (*Railletina sps*)

## 3. Characterization of nanoparticle of *Mentha piperita*:

Characterization by Fourier Transformed Infrared Analysis (FT-IR):

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. For the identification of the functional groups of *Mentha piperita* Fourier Transformed Infrared Analysis (FT-IR) analysis was adopted. The plant powder was characterized by using the attenuated total reflectance (ATR) technique FT-IR spectrum was taken in the mid IR region of 400–4000 cm<sup>-1</sup> for the sample prepared mixed with the potassium bromide in the ratio of 1:200. Wave length Fourier

transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. It provides the information about the structure of a molecule could frequently be obtained from its absorption spectrum. A small quantity of the *Mentha piperita* extract was mixed in dry potassium bromide . The mixture was thoroughly mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a potassium bromide thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Bruker, Germany Vertex 70 infrared spectrometer. The sample was scanned from 4000 to 400 cm-1 [5].



### FTIR analysis of leaf extract of *mentha piperita*

The result of the FTIR spectra of the *mentha piperita* exhibited prominent peaks with (3541.31,3460.30,3340.71,3296.35,2922.16,2852.72,1728.22,1606.70,1409.96,1246.02,1008.71 )different values the spectrum showed strong absorption band at 1008.77.

The spectral band shows the presence of functional groups which are responsible for reduction of metal ion. Peak was absorbed at 3340.71 represents presence of hydroxyl groups.

The peaks at 2922.16, 2852.72 represent presence of methyl groups. The peak at 1409.96 represents the presence of isopropyl groups. The peaks around the 1008.77 will represent the presence of (c-o) bond.

FTIR analysis was done at Osmania University in chemistry department, Hyderabad.

## RESULTS AND DISCUSSION

Table: Comparative Anthelmintic Potential of Green Synthesized Silver Nanoparticles in cestodes and Nematodes.

Test Substance	Concentration (mg/ml)	Cestodes(minutes) <i>Raillietina sps</i>	Nematodes(minutes) <i>Ascaridia galli</i>
Control		-	-
Standard(Piperazine citrate)	20	49( min)	48(min)
<i>Mentha piperita</i> - Aqueous extract	10	35(min)	38(min)
	20	26(min)	34(min)
	50	24(min)	28(min)
<i>Mentha piperita</i> - Silver Nanoparticles	10	28(min)	24(min)
	20	19(min)	16(min)
	50	11(min)	9(min)

From the result of the anthelmintic activity of the green synthesized silver nanoparticles of *Mentha piperita* it was found that the time taken for death of Nematodes was 24 min and 16 min and 9 min respectively for different concentrations(10 and 20 and 50 mg/ml) of nano particles. And time taken for death was 38min and 34min and 28 min respectively for different concentrations (10 and 20 and 50 and mg/ml) of aqueous extract of *mentha piperita*. From the data obtained, it was observed that the silver nanoparticles exhibited highest Anthelminthic activity at concentration (50 mg/ml) by producing paralytic effect much earlier and death was observed by movement arresting with less time.

From the result of the anthelmintic activity of the green synthesized silver nanoparticles *mentha piperita* it was found that the time taken for death of Cestodes was 28 min and 19 min and 11 min respectively for different concentrations(10 and 20 and 50 mg/ml) of nano particles. And time taken for death was 35 min and 26 min and 24 min respectively for different concentrations (10 and 20 and 50 and mg/ml) of aqueous extract of *mentha piperita*. From the data obtained, it was observed that the silver nanoparticles exhibited highest Anthelminthic activity at concentration (50 mg/ml) by producing paralytic effect much earlier and death was

confirmed by movement arresting with less time. Silver nanoparticles using *M.piperita* leaves showed better anthelmintic potential when compared with the aqueous extract of *M. piperita* leaves. Nanoparticles showed least time to cause paralysis and death of the worms followed by aqueous extract. Overall the anthelmintic assay revealed the concentration-dependent nature of the aqueous extracts and silver nanoparticles. From this study it may be concluded that, in addition to plant products silver nanoparticles using *M.piperita* leaves have more anthelmintic activity. The present study is to the best of our knowledge, the first to report the vermifugal potential of Pudina leaf assisted green synthesized silver nanoparticles in Cestodes and Nematodes.

### CONCLUSION

The present study demonstrated the bio reductive synthesis of nano sized silver particles and it may be concluded that, in addition to products of plants, silver nanoparticles using *Mentha piperita* leaves have more anthelmintic activity. Biogenic synthesis is also favoured due to its lower toxicity to environment denoting its merit and prompt for preference in various industrial and medical applications. Silver nanoparticles, obtained by a green chemistry synthetic route exhibited significant anthelmintic activity in vitro. By using the green synthesis we can effectively control the mortality rates of poultry fowls.

From this study it may be concluded that, in addition to plant products silver nanoparticles using *M.piperita* leaves have more anthelmintic activity. The present study is to the best of our knowledge , the first to report the vermifugal potential of *M.piperita* leaves assisted green synthesized silver nanoparticles in cestodes and nematodes, of poultry.