



## SCREENING OF IN-VITRO ANTHELMINTIC ACTIVITY OF SILVER NANOPARTICLES OF *EUCALYPTUS GLOBULUS* AGAINST PHERETIMA POSTHUMA

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### ABSTRACT

The biological syntheses of silver nanoparticles emerge as an eco-friendly and exciting approach in the field of nanotechnology. The human nature has never been free of health related problems neither has found single ever curing drug. Plants have been used until today as immediate remedy for various ailments. In the current study, experiments were conducted to evaluate the possible anthelmintic activity of silver nanoparticles of the leaves of plant *Eucalyptus globulus*. The prepared nanoparticles showed particle size 259.5 nm and zeta potential -27.9 mV. Various concentrations (10, 20, 30, 40, 50  $\mu\text{g/ml}$ ) of silver nanoparticles were tested and results were expressed in terms of time for paralysis and time for death of worms.

**KEYWORDS :** *Eucalyptus globulus*, silver nanoparticles, Anthelmintic activity.

### INTRODUCTION:

Helminthes infection are the important and among the most common infections in human being, affecting a large proportion of the world's population. The disease is highly prevalent particularly in third world countries due to poor management practices. In developing countries they pose a large threat and public health and contribute to prevalence of anemia, malnutrition, eosinophilia and pneumonia. Anthelmintic are drugs that expel parasitic worms (helminthes) from the body. Anthelmintic are drugs that may act locally to expel out worms from the GIT. Most of the existing anthelmintic produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea.

Helminthes infections, commonly called helminthiasis are among the most important animal diseases inflicting heavy production losses causing more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites.<sup>(1)</sup> The disease is highly prevalent particularly in third world countries due to poor management helminthiasis practices.

Nanotechnology is a broad interdisciplinary area of research, development and industrial activity which has grown very rapidly all over the world for the past decade. Silver nanoparticles (AgNPs) are very important among the most widely used metal nanoparticles. The use of plant extracts to synthesize nanoparticles is receiving attention in recent times because of its simplicity. Also, the processes are readily scalable and may be less expensive. Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. A number of plant extract mediated synthesis of AgNPs have been reported in the literature but no any method available of silver nanoparticles of *Ricinus communis* as an anthelmintic agent. There are several techniques that are known to produce extract into nanoparticles and to improve the activity of extracts. One of the current methods used to generate extract nanoparticles is by high pressure homogenization (HPH)<sup>(2-5)</sup>

The *Myrtaceae* family includes 140 genera and about 3800 species distributed in tropical and subtropical regions of the world. The essential oil 1, 8-Cineole is the pharmaceutically active component of *Eucalyptus* oil. alcohols, aldehydes, terpenes, ketones are extremely use full due to their antiviral,

antibacterial, antiseptic, anti-fungal, anti-inflammatory, disinfectant and sedative properties. In Ethiopia there are many plants with wide traditional use mainly to treat different diseases. However, almost all of them are not studied involving scientific methods. This shows scientific information regarding the medicinal plant should be incorporated as there is less know how in the people and less documented information.<sup>(6-12)</sup>

*Eucalyptus* plant is one amongst the victims. It is distributed in all most every part of the country than any plant species. However, with respect to its potential use there is insignificant information about it.

Therefore, the aim of this study is to assess the anthelmintic activity of silver nanoparticles from *Eucalyptus globulus* leaf extract. Taking into account the physicochemical properties of silver nanoparticles of *ricinus communis* with paralyzing capacity against helminthes have led to increase in the research on herbal nanoparticles and their potential application as anthelmintic.

### MATERIAL AND METHODS:

#### Collection and Identification:

The leaves of *Eucalyptus globulus* were collected in Kodoli, Kolhapur District, and State of Maharashtra. These specimens were identified by in the Herbarium of Department of Pharmacognosy, Vasantidevi Patil Institute of Pharmacy Kodoli.



Fig. No 1 Leaves of *Eucalyptus globulus*

**Preparation of Extract:**

Plant leaves were washed thoroughly with distilled water. The dried leaves of *Eucalyptus globulus* were finely grinded using electrical grinder and stored in air tight containers for further use. The pulverized plant material (250 g) was extracted with Soxhlet extraction method by using methanol as a solvent. The separated extracts were then filtered through Whatman's No. 1 filter paper. Finally extract dried at room temperature. Dried extract was collected in an air tight container and stored at 4°C till further analysis.



**Fig. No 2 Soxhlet Extraction Apparatus**

**Test drug: (Silver Nanoparticles)**

The leaves of plant *Eucalyptus globulus* was dried in sunlight and powdered coarsely. The powdered *Eucalyptus globulus* was extracted with Soxhlet extraction method by using methanol as a solvent. Then this extract was filtered and powder was dried in shade. And then a different concentration (10, 20, 30, 40, 50 µg/ml) of silver nanoparticles was prepared by diluting the stock solution with normal saline.

**Reference drug:**

Albendazole was prepared by dissolving them in normal saline at a concentration of 20 µg /ml.

**Normal control:**

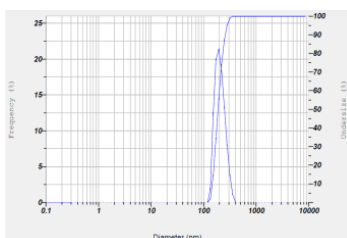
Normal saline was prepared and used to treat the normal control group.

**Animals:**

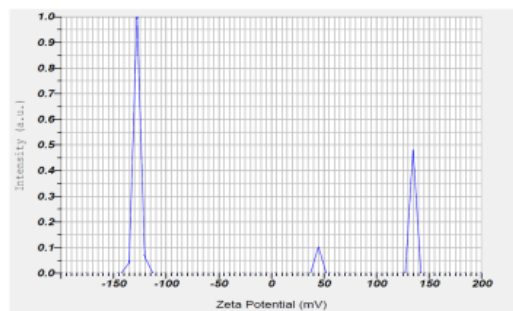
Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. The earthworms were collected from moist soil and washed with normal saline to remove all fecal matter. The earthworms of 9-10 cm in length and 0.3-0.4 cm in width were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity.

**Formulation of silver herbal nanoparticles:**

For the nanoparticle preparation, around 10 gm of the dried powder is boiled with 100 ml of distilled water. To 10ml AgNO<sub>3</sub> solution, on addition of 100 ml of plant extract herbal nanoparticles prepared by using high pressure homogenizer. The particle size and zeta potential of selected nanoparticles shown in table no. 2



**Fig. 3 Particle size distribution**



**Fig. 3 Zeta potential distribution**

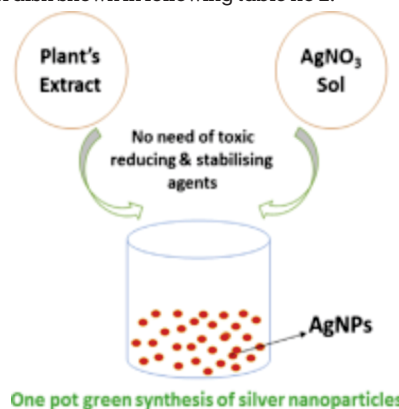
**Table No.2 Particle size and zeta potential analysis**

Particle Size (nm)	Zeta Potential (mV)
259.5	-27.9

**Anthelmintic activity:**

For the anthelmintic activity of plant extract of silver nanoparticles of *Eucalyptus globulus*, Indian adult earthworms (*Pheretima posthuma*) of 9-10 cm in length and 0.2-0.4 cm in width were used. The animals were divided into seven groups containing six earthworms in each group. A different dilution of nanoparticles (20, 40, 60, 80 and 100 µg /ml) has been made and then the volume was adjusted to 100 ml with normal saline water. All the dilutions of nanoparticles and standard from the above results, it is concluded that the plant extract silver nanoparticles of leaves of *Eucalyptus globulus* have potent anthelmintic activity when compared with the conventionally used drug.

Further studies using in vitro models are required to carry out and drug solution were freshly prepared before starting the experiments. Different concentrations of silver nanoparticles and standard drug solution were poured in different petri dishes. All the earthworms were washed in normal saline before they were released into 10 ml of respective formulation. Observation were made for the time taken to paralyze (Paralysis was said to occur when the worm did not revive even in normal saline) and death (Death was concluded when the worms lost their motility followed with their body colors fading away). All the results were expressed as a mean 1 animal in each Petri dish shown in following table no 2.

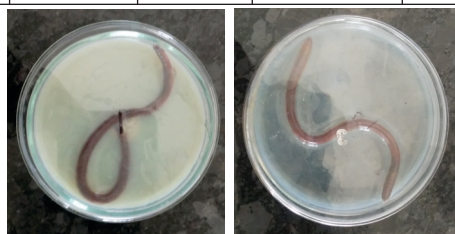


**Fig. 4 Green synthesis of herbal nanoparticles**

**Table-1 In- vitro anthelmintic activity of extracts of *Eucalyptus globulus*:**

Group	Treatment of extracts	Concentration (µg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1.	Normal control	---	---	---
2.	Albendazole (Standard)	20	20.46±0.73	57.13±0.22

3.	Alcohol	10	65.30±0.63	92.23±0.54
		20	54.36±0.44	86.21±0.27
		30	46.89±0.87	78.14±0.66
		40	38.81±0.96	68.28±0.28
		50	27.21±0.54	52.18±0.52



10 µg/ml

20 µg/ml



30 µg/ml

40 µg/ml



50 µg/ml

Standard drug (Albendazole)  
10 µg/ml

Fig. No 5 Screening of anthelmintic activity

### RESULT AND DISCUSSION:

The observed response of worms in case of paralysis there was significant variation among the result produced by extract at different concentrations like 10, 20, 30, 40, and 50 µg/ml. The prepared nanoparticles showed particle size 259.5 nm and zeta potential -27.9 mV. The silver nanoparticles showed more significant effect on paralyzing worms. (Table 2) This result may lend support for the traditional use of the plant as an anthelmintic. The presence of phytochemicals is directly related to medicinal activity of the plant, in our concern the inhibition data recorded in anthelmintic. Tannins can also react with nematode's cuticle and toughens the skin. Alkaloids act on central nervous system and caused paralysis of the worms.

Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anthelmintic activity observed.

### CONCLUSION:

Throughout screening of literature available on *Eucalyptus globulus* it's very useful in treating many diseases. The wormicidal activity of silver nanoparticle extracts of plant of *Eucalyptus globulus* suggests that it is effective against parasitic infections of humans. The data presented in table and observations made there of lead to the conclusion that the different degree of helminthiasis of the different

concentrations are due to the level of tannins present in compound.

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