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GREEN SYNTHESIS OF SILVER NANOPARTICLES OFRICINUS COMMUNIS AND INVESTIGATION OF IN-VITRO ANTHELMINTIC ACTIVITY AGAINST PHERETIMA POSTHUMA

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ABSTRACT

Development of anthelmintic drugs leads to evaluation of medicinal plants as a source of anthelmintic. In view of this, an attempt has been made to study the anthelmintic activity of leaves of plant of ***ricinus communis*** silver nanoparticles. In the current study, experiments were conducted to evaluate the possible anthelmintic activity of silver nanoparticles of the leaves of plant extract of ***ricinus communis***. Various concentrations (10, 20, 30, 40, 50 µg/ml) of nanoparticles were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendazole used as a reference standard and saline as a control group. The prepared nanoparticles showed particle size with mean diameters 328.4 nm and zeta potential -19.6 mV.

KEYWORDS: ***Ricinus communis,*** 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.[1] Anthelmintic or anthelmintic are drugs that expel parasitic worms (helminthes) from the body. Anthelmintic is 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. Hence there is an increasing demand towards natural anthelmintic.

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.[2-5] 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.[5] 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).

The leaves of plant ***ricinus communis*** are used as anthelmintic agent traditionally to eradicate or reduce the number of helminthes parasites in the intestinal tract. The ***Ricinus communis*** has been cultivated Mediterranean region, and found in many places such as India, China, Europe and United States. The glossy leaves are 15–45 cm (5.9–17.7 in) long, long-stalked, alternate and palmate with five to twelve deep lobes with coarsely toothed segments. In some varieties they start off dark reddish purple or bronze when young, gradually changing to a dark green, sometimes with a reddish tinge, as they mature. Methanolic extracts of the leaves of *ricinus communis* were used in antimicrobial, antifungal activities. The leaves contain tannins, saponins, terpenoids, reducing sugars, flavonoids, alkaloids and anthraquinones.[6-10] Also it is having an antimicrobial activity with this objective this study focuses on the validation of the traditional use of *ricinus communis* as anthelmintic agent.

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 ***ricinus communis*** 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 ricinus communis.

Preparation of Extract

Plant leaves were washed thoroughly with distilled water. The dried leaves of *R. communis* were finely grinded using electrical grinder and stored in air tight containers for further use. The pulverized plant material (250g) 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 silver nanoparticles

The leaves of plant ***ricinus communis*** was dried in sunlight and powdered coarsely. The powdered ***ricinus communis*** was extracted with Soxhlet extraction method by using methanol as a solvent. Then this extract was filtered and powder from each solvent was dried in shade. And then this dry powder used for preparation of silver nanoparticles.

Reference drug

Albendazole was prepared by dissolving them in normal saline at a concentration of 20 mg/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 10 ml $AgNO_3$ 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.

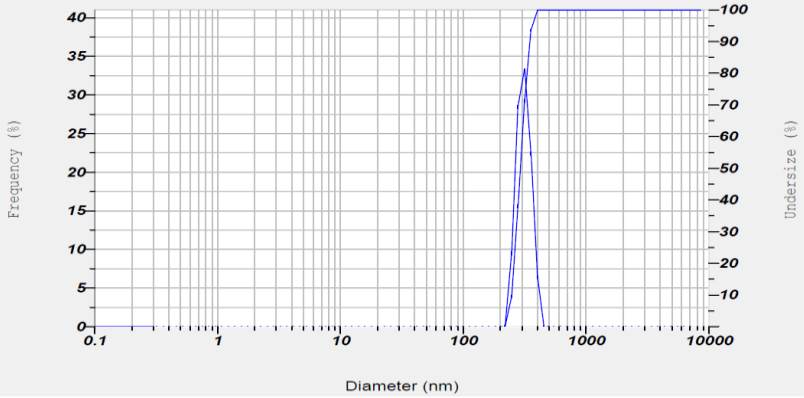


Fig. 3: Particle size distribution.

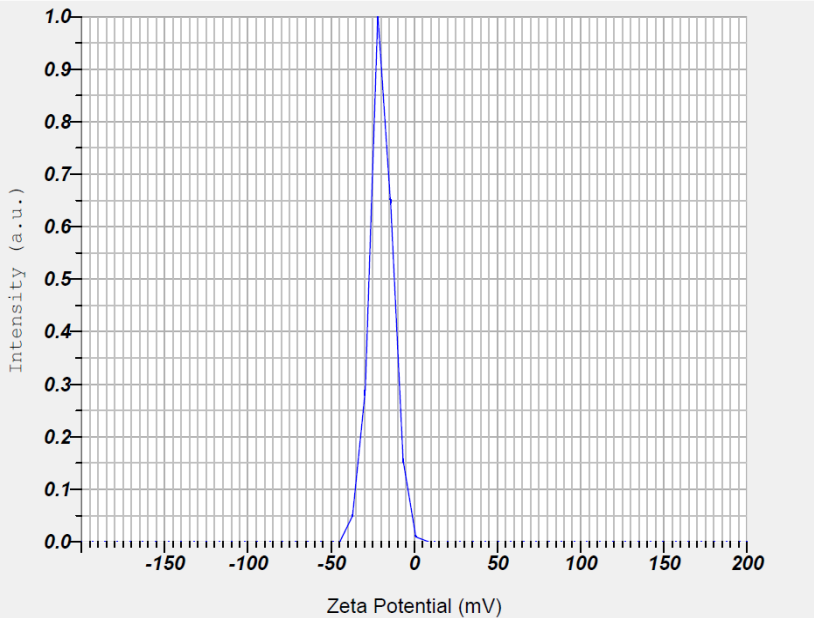


Fig. 3: Zeta potential distribution.

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

Particle Size (nm)	Zeta Potential (mV)
328.4	-19.6

Anthelmintic activity: For the anthelmintic activity of plant extract nanoparticles of ***ricinus communis***, 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 as (20, 40, 60, 80 and 100µg/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 methanolic extract of plant of ***ricinus communis*** have potent anthelmintic activity when compared with the conventionally used drug. Further studies using in vivo models are required to carry out and drug solution were freshly prepared before starting the experiments. Different nanoparticle dilutions 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.

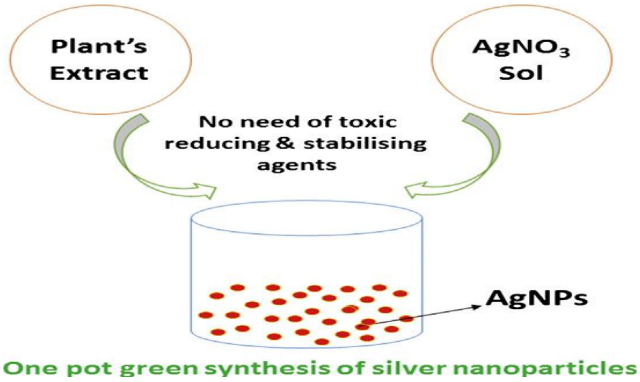
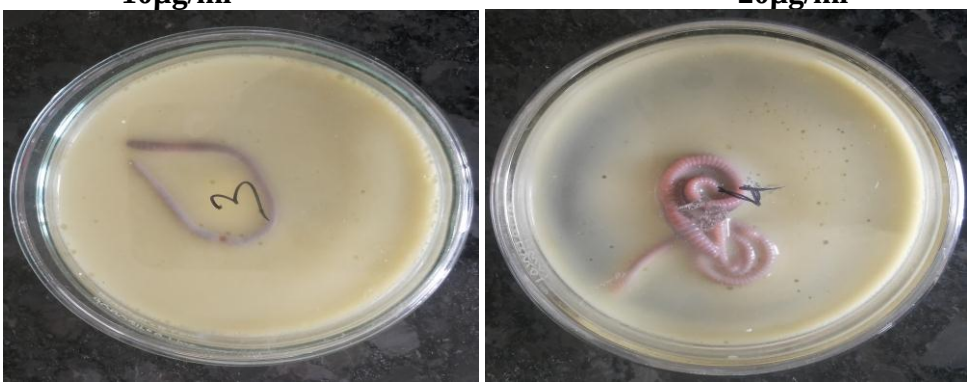


Fig. 4: Green synthesis of herbal nanoparticles.

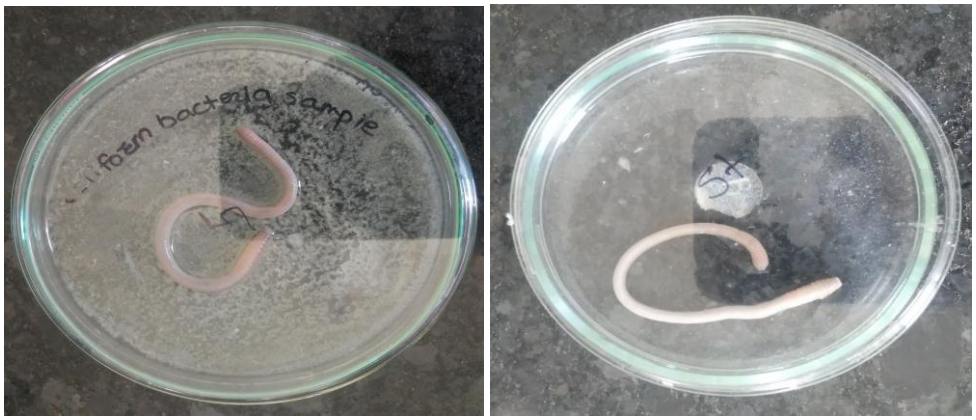
Table 2: *In-vitro* Anthelmintic Activity of Extracts of *Ricinus Communis.*

Groups	Treatment of nanoparticles	Concentration (µg /ml)	Time taken for paralysis (min)	Time taken for death (min)
1.	Normal control	---	---	----
2.	Albendazole (Standard)	10	19.41±0.63	47.23±20.23
3.	Herbal nanoparticles	10	55.30±0.65	81.23±0.35
		20	44.36±0.43	56.21±0.23
		30	41.86±0.85	71.23±0.56
		40	32.81±0.92	75.23±0.23
		50	24.31±0.14	62.13±0.65

10µg/ml **20µg/ml**



30 µg /ml **40 µg /ml**



50 µg /ml **Standard (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 nanoparticles at different concentrations like 10, 20, 30, 40, and 50µg/ml. The prepared nanoparticles showed particle size 328.4 nm and zeta potential -19.6 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.

Tannins, the secondary metabolite, occur in several plants have been reported to show anthelmintic property by several investigators. Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of parasite, and cause death. 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 ***Ricinus communis*** it's very useful in treating many diseases. The wormicidal activity of silver nanoparticle extracts of plant of ***Ricinus communis*** 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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