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GC-MS of Silver Nanoparticles Conjugated with *Leucaena leucocephala* Seed, and its effect on *Ascaridia galli* (Chicken Nematode) – An *In Vitro* Study

Sunitha A.K.* and Sunila Kumari Y.

Department of Zoology, Osmania University College for Women, Hyderabad, Telangana 500095, India

**Corresponding Author*

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Abstract: Helminth infections, frequently paralyze poultry industry causing huge economic losses. *Ascarida galli* is one of the nematode infection which causes ascaridiasis in poultry. Quite a number of synthetic anthelminthic compounds are known, which are known to control these infections. However, on the flip side, these compounds are known to cause anthelminthic resistance. To combat this menace, plant based anthelminthic are frequently being used and new sources are constantly explored. In this regard, it was found that tannin based plant extracts were found to be quite effective, and so *Leucaena leucocephala* was selected for the present study. Silver nanoparticles, infused with *Leucaena* seeds were used to study the *in vitro* anthelminthic property on *Ascaridia galli*. The treatment was found to be effective and 95 % mortality was noticed. Statistical analyses was done using Graphpad prism, and the results were found to be statistically significant at $P < 0.05$ and $P < 0.01$. As the *Leucaena* was found to be quite effective, GC –MS (Gas chromatography followed by Mass Spec) was performed to analyse the phytochemical constituents. The GC-MS chromatogram, revealed the presence of tannins and also tetraborane as the main components. The present *in vitro* anthelminthic activity observed on *Ascaridia galli* may be attributed to tannin content and also to tetraborane present in the seeds of *Leucaena leucocephala*. This study, establishes the fact, that *Leucaena leucocepahala* can be used as a potent plant based anthelminthic.

Keywords: *Leucaena leucocephala*, *Ascaridia galli*, Anthelminthic, GC-MS, Silver nanoparticles, Nematode infection

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Introduction

Ascaridia galli is the nematode parasite, which infect the chicken frequently. It is a common nematode infection reported world over (Gamit *et al.*, 2017). This infection causes greater losses to poultry industry (Gasaliyu *et al.,* 2022) and therefore must be addressed with high priority. The infection of *Ascaridia galli* can be fast and can spread easily because of the direct life cycle and also the eggs are highly resistant to the environmental conditions. As this is a problem all

over the world, different deworming strategies, have been employed in different parts of the world. Studies by Tarbiet *et al.* (2016), suggest that conventional anthelmintic drug such as fenbendazole was frequently used and was quite effective in treating the infection. Sharma *et al.* (1990) used Ivermectin, an intra-cutaneous anthelmintic drug to treat *Ascaridia* infection. In addition to these drugs, there are several anthelmintic drugs which are routinely used to treat the infections, such as Levamisole (LEV), piperazine (PIP) and flubendazole (FBZ) plus LEV-PIP (Feyera *et al.,* 2021). Though these synthetic anthelmintics are quite effective in controlling the helminth infections and also their spread, however, there are risks of developing resistance. Patel *et al*. (2018) suggested that the continuous use of synthetic anthelmintics leave residues in the poultry, which in long term cause carcino-genesis and also resistance to these anthel-mintics. In this scenario, the use of alternate treatments, plant based compounds are being screened for anthelminthic activity and is gaining more of prominence in recent times (Zafar *et al.,* 2006; Nabukenya *et al.,* 2014; Hartady *et al.,* 2021). In this regard, literature survey has shown that plants which contain tannins seem to exhibit potent anthelminthic properties. Therefore we selected *Leucaena leucocephala* (River tamarind) for the present investigation as it is known to contain tannins (Osborne and McNeill, 2001).

Materials and Methods

Leucaena leucocephala is commonly known as Subabul tree. It is a common tree found in and around Hyderabad, India. The plant was identified with the type of inflorescence. The seeds were carefully collected from the campus of Osmania University College for Women, Hyderabad in bulk and packed in Ziploc bags. They were brought to the research laboratory in the Department of Zoology, Osmania University College for Women, Hyderabad. Later they were thoroughly cleaned in water and shade dried for a period of one week. The seeds were powdered using mortar and pestle and were further processed for preparing extracts using water and ethanol and also for the synthesis of silver nanoparticles (AgNps)

Collection of Parasites (*Ascaridia galli*)

The intestine of freshly slaughtered fowls were collected from local slaughter house and washed with normal saline solution. These intestines were then dissected and the nematodes, are *Ascaridia galli* were extracted. The average size of the nematode was found to be 5 cm. These parasitic worms were used to test the anthelmintic activity with plant extracts *in vitro.* The identification of nematodes was done in the Department of Zoology, Osmania University College for Women, Hyderabad by using Phase Contrast Microscope.

Preparation of silver nanoparticles

For the green synthesis of silver nanoparticles, we used the crude aqueous extracts of plant *Leucaena* seeds. Synthesis of silver nanoparticles, was prepared as per the standard protocol suggested by Kotakadi *et al.* (2014). The formation of the AgNPs was preliminarily detected by the change in colour from yellow to dark brown. After preparation, a volume of 20 ml of each concentration of silver nanoparticles solution was added to each of the petri dish for experiment-tation. The green-synthesized nanoparticles were separated by centrifugation at 15,000 rpm for 20 min. This process was repeated thrice to get rid of free silver associated with AgNPs. The final green-synthesized silver nanoparticles (AgNPs), were freeze-dried and stored at 4°C until further use.

GC MS of Leucaena seed silver nanoparticles

Gas chromatography followed by Mass Spec (GC-MS), is a versatile analytical technique which identifies the unknown compound in a mixture of substances. The results are accurate, as it measures using mass /charge (m/z) and can detect smallest of concentrations. In the present study as the plant infused silver nanoparticles, showed promising results, it was intended to learn about their chemical composition and also to identify certain unique compounds. Thus GC-MS

Fig. 1: *Ascaridia galli.*

Table 1: Mortality of *Ascaridia galli* after exposure to different concentrations of *Leucaena leucocephala* seed extract

S. No.	% Concentration of the Seed extract with Silver nanoparticles	Volume added	Number of parasites	Mortality in minutes
1	10%	20 ml	4	25
2	20%	20 ml	4	22
3	30%	20 ml	4	20
4	40%	20 ml	4	20
5	50%	20 ml	4	15
6	Standard Albendazole	20 ml	4	40

analysis of *Leuacaena* silver nanoparticles of seed was performed.

Statistical analyses

Statistical analyses was done using Graphpad prism, online software tool and the results were found to be significant at $P < 0.05$ and $P < 0.01$.

Results and Discussion

In the present study, *Ascaridia galli* (Fig. 1) was collected from the intestines of chicken. They were treated with different dosages of silver nanoparticles of seed extract of *Leucaena* (subabul). For the *in vitro* study, approximately 3-4 parasites were taken into each of the petri dish and were tested with different dosages (10%, 20,%,30%, 40%, 50%) of silver nanoparticles of *Leucaena* seed extract. 20 ml of each concentration was added to the petridishes. When the parasites were treated with silver nanoparticles of *Leucaena* seed extract there was almost 90%-95% mortality reported after a time lapse of 22-25 min (Table 1, Figs. 2, 3) suggesting that silver nanoparticles of seed extract was more effective in causing the death of the parasites *in vitro.* For all these treatments, Albendazole, was used as standard anthelminthic drug.

In the present study, the efficacy of green synthesized silver nanoparticles, as anthelminthic, using *Leucaena* seed extract was investigated. Results, showed that the plant (seed) based nanoparticles, proved to be quite effective in controlling the *Ascaridia galli* infection *in vitro.* Though the plant based anthelminthics were effective in controlling the parasitic infections, on the flip side, these plant materials are required in bulk quantities to process them into herbal extracts, as the bioactive compound found in them majority of times is quite low (Zhang and Li.,

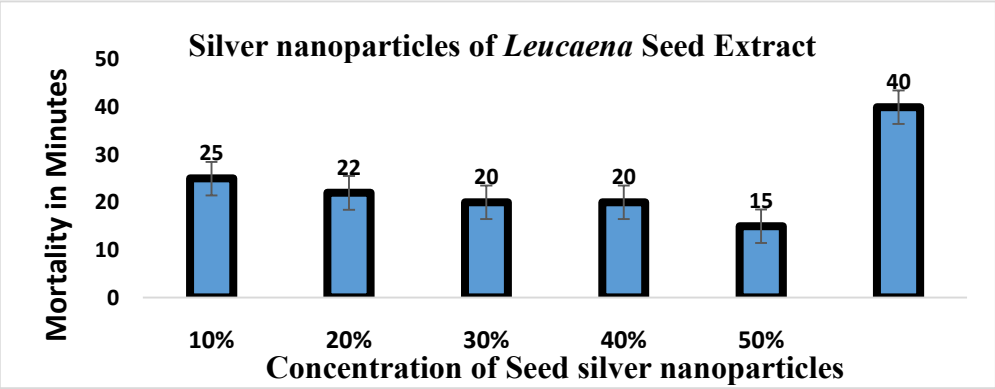
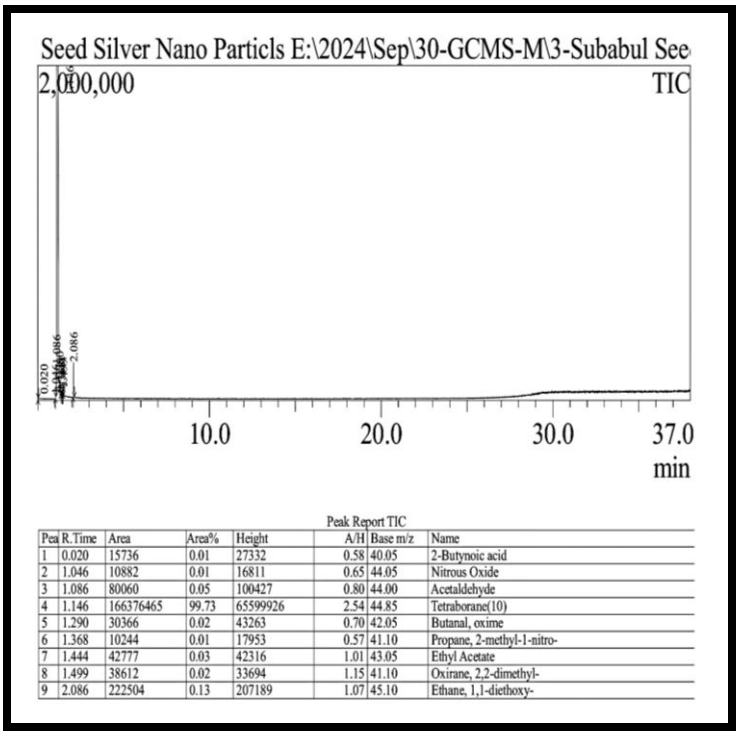


Fig. 2: Mortality of *Ascaridia galli* after exposure to different concentrations of *Leucaena leucocephala* seed extract.

Fig. 3: Mortality of *Ascaridia galli* after exposure to *Leucaena leucocephala* seed extract.

2010; Patel *et al.*, 2019). Moreover, the extraction processes sometimes are quite cumbersome, more time consuming and also expensive (Altemimi *et al.*, 2017). The conventional methods of extractions using different organic solvents which are known to be hazardous and environment toxic (Suan, 2013). Therefore in this scenario, it was felt that, if there could be a method, to enhance the productivity of the plant extracts and also to minimize the quantity of plant material, which have the same or enhanced effect would be more advantageous for this study. Therefore silver nanoparticles, infused with plant extracts were used. Apart from being less toxic and eco-friendly, the green synthesized silver nanoparticles have a two way advantage as silver being downsized to nanosize having inherent therapeutic value, and when the capping agent is a plant extract, their combined synergistic effect has much greater impact than as individual bioactive compounds hence, vastly exploited in medicine and biology (Majeed *et al.*, 2022). Based upon these suggestions, silver nanoparticles conjugated with *Leucaena* seed extract were synthesized and were tested for their anthelminthic activity, against *Ascaridia galli.* The study revealed that silver nanoparticles infused with *Leucaena* seed extracts exhibited significant mortality against the nematode. It was interesting to see that the seed extract was found to be more effective as they may seem to contain greater proportions of the phytochemicals, which may be more stable (Li *et al.*, 2023) and also contain certain additional constituents absent in leaves, which makes seeds more potent as anthelminthic agents. When silver nanoparticles infused with *Leucaena* seed extract was subjected to GCMS, it showed that almost 99 % of the seed extract has the main ingredient to be as Tetraborane (Fig. 4), which is known to be highly toxic to biological systems, however,



Peak Report TIC

Peak	R.Time	Area	Area%	Height	A/H	Base m/z	Name
1	0.020	15736	0.01	27332	0.58	40.05	2-Butynoic acid
2	1.046	10882	0.01	16811	0.65	44.05	Nitrous Oxide
3	1.086	80060	0.05	100427	0.80	44.00	Acetaldehyde
4	1.146	166376465	99.73	65599926	2.54	44.85	Tetraborane(10)
5	1.290	30366	0.02	43263	0.70	42.05	Butanal, oxime
6	1.368	10244	0.01	17953	0.57	41.10	Propane, 2-methyl-1-nitro-
7	1.444	42777	0.03	42316	1.01	43.05	Ethyl Acetate
8	1.499	38612	0.02	33694	1.15	41.10	Oxirane, 2,2-dimethyl-
9	2.086	222504	0.13	207189	1.07	45.10	Ethane, 1,1-diethoxy-

Fig. 4: GC- MS of silver nanoparticles *of Leucaena* seed extract.

literature survey has revealed that it has been used as anticancer, anti HIV and also as anti-rheumatoid agent (Hawthorne, 1993; Qian *et al.*, 2017; Axtell *et al.*, 2018). Celebi *et al.* (2024) have revealed the anti-bacterial properties of boron derivatives. These authors, have extensively studied the anti-bacterial effect of boran compounds upon pathogenic biofilms and concluded that boron and its derivatives are more effective on inanimate surfaces than living cells. Therefore based upon these evidences, it may be said that the present anthelminthic property of silver nanoparticles of *Leucaena* seed extract may be attributed to its chemical ingredient tetra-borane.

The present study establishes the anthelminthic property of *Leucaena* on *Ascaridia galli*, which may be attributed to the phytochemical components, precisely due to tannins and also due to tetraborane, which is present in large quantities in the seeds. The silver nanoparticles when conjugated or capped with seed extract, their therapeutic effect was further enhanced due to the synergetic effect, suggesting that *Leucaena leucocepahala* seeds can be a potent anthelminthic agent.

Conflict of Interest

The authors declare no conflicts of interest.

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