Indian J. Applied & Pure Bio. Vol. 39(3), 2023-2029 (2024). **ISSN: 0970-2091**
A web of Science Journal

Green synthesis of Metal nanoparticles using leaves of *Leucaena leucocephala* (River Tamarind)

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Abstract

Leucaena leucocephala, plant is commonly called as the subabul tree, which is known to have a quite a number of medicinal properties. In the present study, it was intended to study the anthelminthic properties against *ascardia galli* using the leaves of the leaf extract *in vitro*. As observed in many studies, metal nanoparticles infused with plant extracts are quite efficient than the bulk material, and therefore it was intended to synthesize silver and copper nanoparticle sussing the leaves of subabul tree. The green synthesis of nanoparticle was done following a standard protocol. SEM and EDX spectral analysis was performed to confirm the size, shape a and synthesis of silver (AgNps) and copper nanoparticles (CuNps). SEM images revealed the size of silver nanoparticle to be 1 and 2 µm. and the shape was found to be plate like. SEM studies of copper nanoparticles, revealed the size to be 100nm and 200 nm. Their shape was found to be flower like. The study was successful in synthesizing the silver and copper nanoparticles infused with *Leucaena leucocephala leaves,* which will further be tested for their anthelminthic activity *in vitro.*

Key words : *Leucaena leucocephala,* Silver nanoparticles, copper nanoparticles, *Ascaridia galli,* SEM, EDX.

Ascarida galli, is the common nematode infection of chicken reported all over the world[6]. This is most common parasitic infection, known to cause serious health conditions, such as weight loss, low egg production and in severe cases causing increased mortality. These factors directly cause huge economic loss to the poultry industry and therefore must be dealt seriously. To treat this infection, levamisole (LEV) and piperazine (PIP) are generally used and additionally drugs such as benzimidazole (BZ) anthelmintic, flubendazole (FLBZ) (Flubenol®) have also been approved for the treatment of this infection[15]. Though there are quite a number of synthetic anthelminthics, available, there seems to be a new problem brewing, which is the drug resistance against these synthetics. There is a growing evidence of majority of the helminth parasites including *Ascaridia galli* developing resistance, which has been reported worldwide[3,7,16]. To overcome this menace of drug resistance, more and more researchers, and poultry farmers all over the world, are adopting natural methods and also employing plant based pharmaceuticals, Moreover plant based anthelminthics can offer a cost effective natural resource and are also can be more effective than synthetic anthelminthics and so these seems to be a much better option than routine drugs. Moreover it is interesting is say that if materials are made up of nanoparticles and used then they are known to have a relatively larger surface area when compared to the same volume of material made up of bigger particles and additionally if the nanoparticles are synthesised using metal ions infused with plant extracts, they seem to be more effective in their efficacy as therapeutic agents as suggested by different authors such as Sharma, *et.al*,[14]; Abdelghany, *et al*.[1]; Goodsell[5]. There are quite a number of plant based nanoparticles which have been investigated for the anthelminthic properties. Authors such as Nur Hayati Azizul *et.al,*[12] have reported the beneficial uses of seeds the plant, *Leucaena leucocephala*. However not many studies have been reported where leaves of *Leucaena leucocephala* have been used to synthesize the nanoparticles and also to study their anthelminthic properties. These studies clearly indicate and highlight the importance of plant infused with metals such as silver and copper nanoparticles as effective anthelminthics. Therefore the present study was taken up to investigate the plant based metal nanoparticles using the leaves of *Leucaena leucocephala.*

Collection of plant material :

Leucaena leucocephala is commonly known as Subabul tree. It is a common tree found in and around Hyderabad. The plant was identified with the type of Inflorescence and the leaves were carefully collected from the campus of Telangana Mahila Viswavidyalayam (TMV, Hyderabad) in bulk and packed in Ziploc bags. They were brought to the research laboratory in the department of Zoology, TMV, Hyderabad. Later they were thoroughly cleaned in water and later shade dried for a period of one week. Later the leaves were powdered using mortar and pestle and were further processed for synthesis of silver nanoparticles (AgNps) and copper nanoparticles (Cu Nps) as per the standard protocol.

Synthesis of Silver nanoparticles (AgNps):

For the green synthesis of silver nanoparticles, we used the crude aqueous extracts of plant *Leucaena leucocephala* leaves. Synthesis of silver nanoparticles, was carried out as per the standard protocol suggested by Venkata Subbaiah *et. al.* (2014). The formation of the AgNPs was preliminarily detected by the change in colour from yellow to dark brown. After preparation, 20 ml was used. The green-synthesized nanoparticles were separated using 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 then stored at 4 °C until further use.

Synthesis of Copper nanoparticles (CuNps):

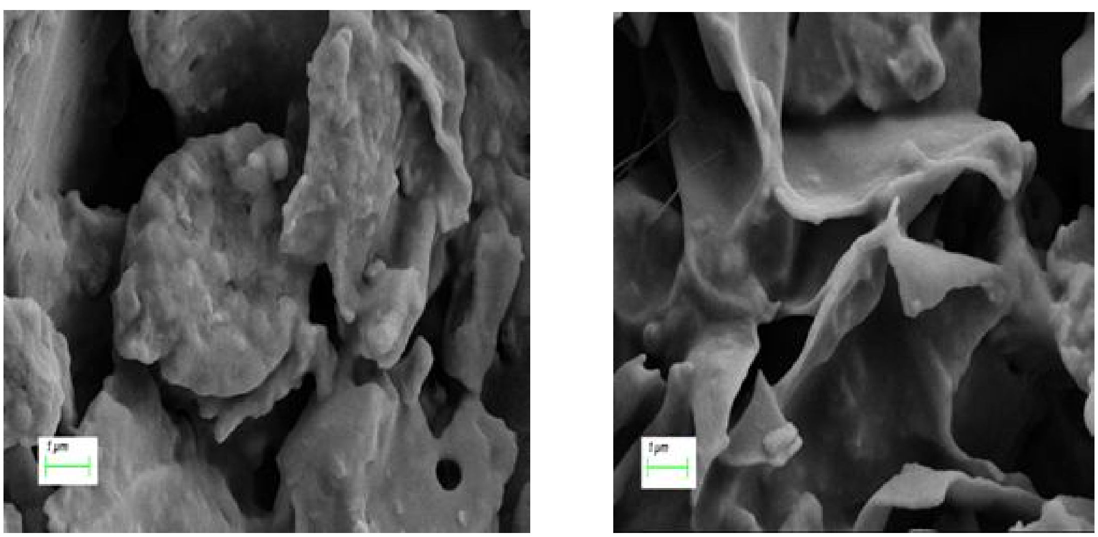
Green Synthesis of Copper Nanoparticles: For the green synthesis of Copper nanoparticles (Fig. 1), 50 ml of plant aqueous extract was mixed with 200 ml of 0.2 M $Cu(NO_3)_2 \cdot 3H_2O$ solution (1 : 4) slowly drop wise with constant stirring. The mixture was incubated at room temperature for 24 h. The colour change was noted after 30 min and also after 60 min for confirmation. The change in colour from blue to light brownish indicated the formation of CuNPs. Then, the solution was centrifuged for 15 min at 10000 rpm. The obtained Cu NPs were washed by deionized water and ethanol to remove any impurities. After the nanoparticles were prepared, they were allowed to dry and stored until further use.

SEM and Spectrometer (EDX) analysis :

As SEM produces images by scanning the surface of the material using high energy electron beams. In the present study after the synthesis of nanoparticles, lyophilisation was done using Virtis Benchtop Machine. SEM and Edx Analysis was done using Carl ZEISS EVO-18, Germany, and FESEM make. Samples were prepared as per the protocol, where in a small drop of the sample was added to the grid. The sample was allowed to dry for 5-7 mintes under a mercury lamp.

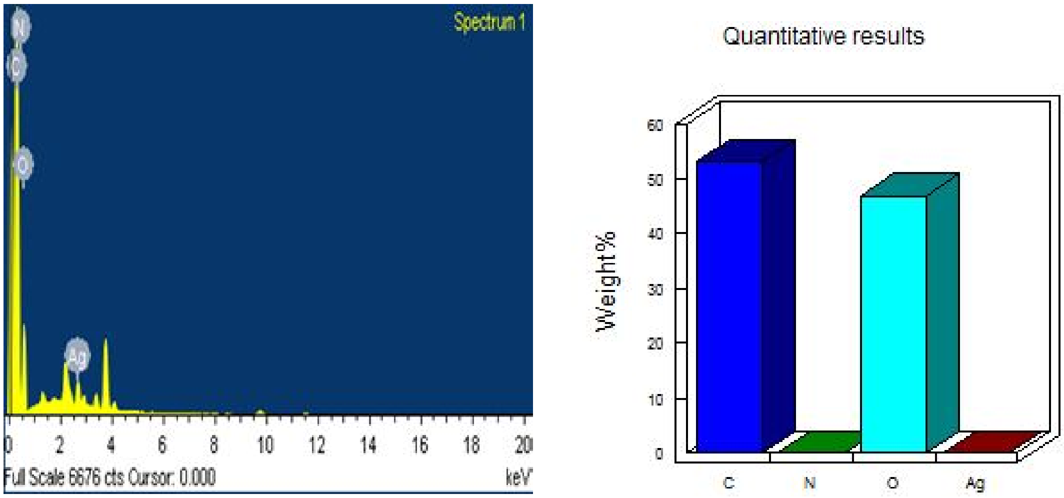
In the present study, after the synthesis of the silver and copper nanoparticles, infused with *Leucaena leucocephala* leaves, the samples were subjected to SEM and EDX analysis. **SEM** images, revealed the size and shape of the nanoparticles. Fig. 1 a and b depict the size of the silver nanoparticle to be 1 µm and 2 µm and the shape of the silver nanoparticles was found to be plate like. **EDX** spectra showed a peak at 2.8kev, (Fig. 2 a and b) confirming the formation of silver nanoparticles. Similarly when the SEM images of copper nanoparticles were observed, it showed the size of the nanoparticles to be 100 nm and 200 nm, and the shape of copper nanoparticles was found to be flower like (Fig. 3 a and b). **EDX** spectral analysis of Copper nanoparticles showed a prominent peak at 1kev, confirming the synthesis of copper nanoparticles (Fig. 4 a and b).

In the present study, we could successfully synthesize silver and copper nanoparticles, using *Leucaena* leaves. To confirm the shape and size of the nanoparticles, the samples were subjected to SEM analysis (Scanning Electron Microscope). SEM studies have distinctly confirmed the shape and size of nanoparticles as evident in the figures I and II in the results section. The electron micrographs of the two samples revealed the sizes to be slightly different from each other. The size of silver nanoparticles was found to 1µm and copper nanoparticles were found to be 100 and 200nm in size. Though the size of silver nanoparticles was 1 µm, it can still be considered as nanoparticle as suggested by Makio Naito *et.al,*[9], who have investigated in-depth about different properties and measurements of nanoparticles. According to these authors, the nano particles which range between 1 nm to 1 µm can also be called as nanoparticles. The shape of silver nanoparticles may be described as plate like as depicted in the Fig I a and b. When the EDX spectra (Fig- II a and b) of silver nanoparticles was observed it showed peak at 2.8Kev which is the binding energy of silver (Ag) in silver nanoparticles, confirming the green synthesis of silver nanoparticles. Similarly the copper nanoparticles, which were synthesized using leaves of *Leucaena* precisely showed the formation of nanoparticles, which were of the size of 100 nm and 200nm. The shape was resembling that of a flower (Fig. 3, a and b). These nanoparticles, may be described as "large nanoparticles" as suggested by Loredana *et.al,*[8] who have described about large nanoparticles made of zinc oxide and titanium oxide in their study, which were 200nm in size. The EDX spectra of copper nanoparticles, depicted a peak at 1 Kev, (Fig. 4 a and b) suggesting the binding energy of copper in copper nanoparticles, which is affirmatory of the production of copper nanoparticles blended with *Leucaena leaf* extract. This study was very successful in synthesizing green nanoparticles using *Leucaena* leaves infused with two different metals, i.e. silver and copper, which was a launching step in the study, where these particles will be further tested for their anthelminthic abilities *in vitro*. Studies by various authors like Mohamed *et.al,*[10]; Ruba Munir *et.al*,[13]; suggest the anticancer and antifungal properties of *Leucaena leucocephala* by synthesizing them as nanoparticles, using metals, like silver, copper, zinc etc. Similar kind of investigations, where in antimicrobial, anticancer properties and also wound healing qualities of various plant infused metal nanoparticles were reported by authors like Ananda Murthy *et.al*,[2]; Nilanjan Chakraborty *et. al*,[11]; Bhavyasree, Xavier[4]. Based upon these studies, it is very much evident that plant based compounds are endowed with immense therapeutic properties, and these can be further enhanced when they are downsized to Nano extent due to the synergistic effect of metal ion and the plant extract.



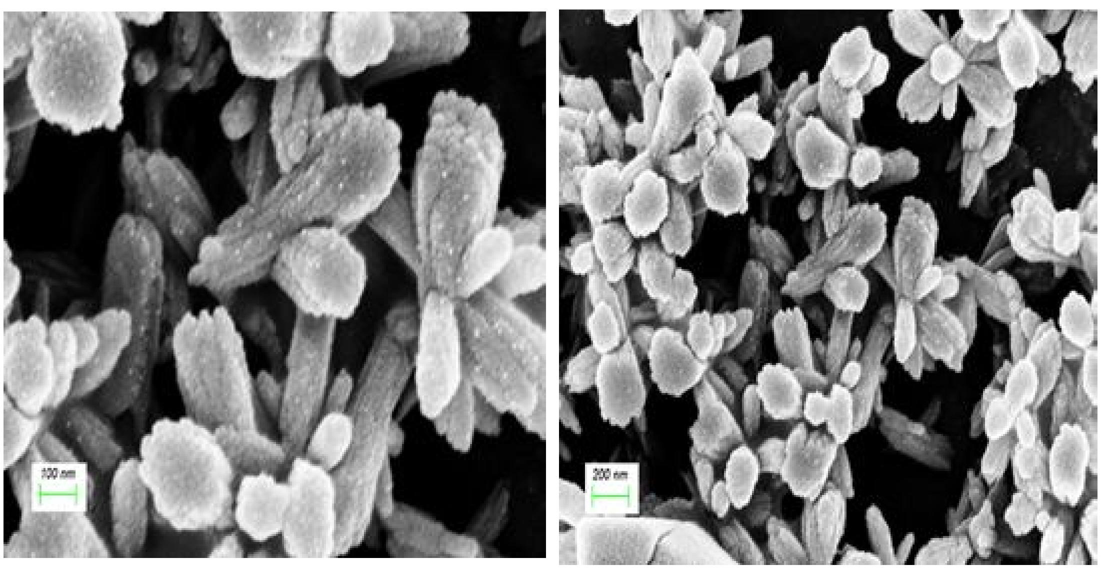
a Fig. 1 b

Silver nanoparticles (1µm) infused with leaves of *Leucaena leucocephala.*



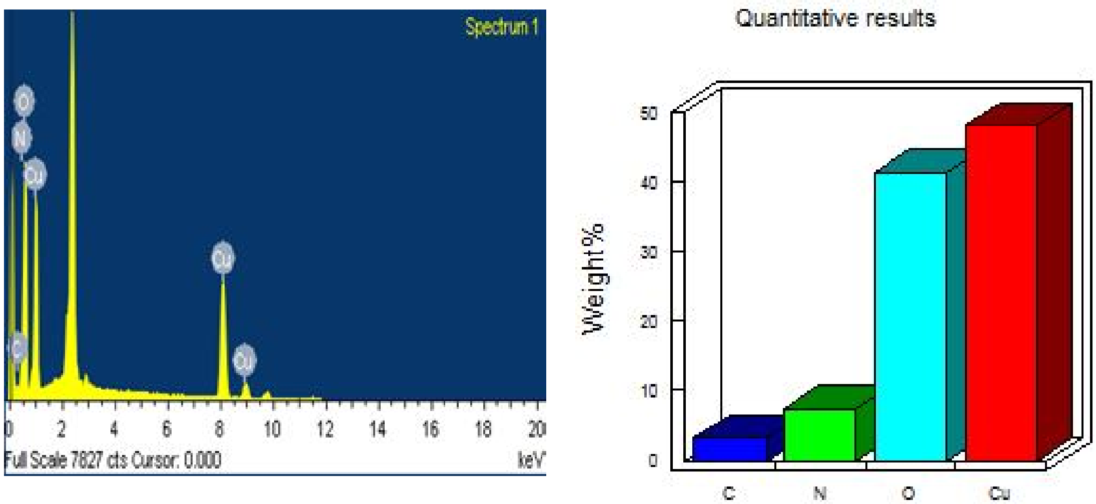
a Fig. 2 b

EDX spectra of Silver nanoparticles (AgNps)



a (100nm) b (200nm)

Fig. 3
Copper nanoparticles) infused with Leaves of *Leucaena*



a b

Fig. 4
EDX spectra of Copper nanoparticles (AgNps)

In summary it may be said, that we were successful in green synthesis of both silver and copper nanoparticles, infused with *Leucaena leucocephala*, which will be further tested for their anthelminthic efficacy upon *Ascardia galli in vitro followed* by *in vivo*.

Very much thankful to my Supervisor and staff of Department of zoology, TMV for providing me with all the facilities needed for the completion of this project.

Conflict of Interest

Authors declare that they don't have any conflict of interest.

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