SYNTHESIS OF PLANT MEDIATED GOLD NANOPARTICLES USING FLOWER EXTRACTS OF *CARTHAMUS TINCTORIUS* L. (SAFFLOWER) AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES

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The ecofriendly synthesis of nanoparticles through various biological sources helps in exploring various herbs**.** Generally, nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. In this report we use aqueous extracts of *Carthamus Tinctorius* L. (Safflower) flowers for the synthesis of gold (Au) nanoparticles. A rapid and convenient method was considered for the synthesis of gold nanoparticles by reduction with auric chloride. UV-visible spectroscopy studies were carried out to assess the formation Au nanoparticles. Transmission electron microscopy (TEM) was used to characterize the Au nanoparticles. TEM image divulges that nano triangle and spherical shape gold nanoparticles are formed with polydispersed size, and the sizes are in the range of 40 nm to 200 nm. The antimicrobial activity of gold nanoparticles was performed on various gram negative bacteria and fungus. The gold nanoparticles showed more inhibitory activity on pathogenic gram negative bacteria than fungus.

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1. Introduction

Nanotechnology concerns with the development of experimental processes for the synthesis of nanoparticles of different sizes, shapes and controlled dispersity [1-6]. This provides an efficient control over many of the physical and chemical properties [2-11] and their potential application in medicine [3,4,5-19]. Synthesis of nanoparticles using biological entities has great interest due to their unusual optical [6-24], chemical [25], photoelectro-chemical [26] and electronic properties [27].

Nanoparticles exhibit completely new or improved properties compared to larger particles of the bulk material and these novel properties are derived due to the variation in specific characteristics such as size, distribution and morphology of the particles. Nanoparticles present a higher surface area-to volume ratio with decrease in the size of the particles.

The synthesis & assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable 'green chemistry' procedure, probably involving organisms ranging from bacteria to fungi and even plants [28-29]. Large-scale production by chemical and physical methods usually results in particles larger than several micrometers while

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the biological synthesis can be successfully used for production of small nanoparticles in large-scale operations [30].

We herein repot the synthesis of gold nanoparticles by the reduction of aqueous $AuCl_4$- with the flower extract of *Carthamus Tinctorius* L. (Safflower). The *Carthamus Tinctorius* L. comes under the Kingdom- *Plantae*, Family – *Asteraceae*, Genus- *Carthamus,* and Species- *C. Tinctorius*

Safflower is widely distributed in eastern and western Asia. The flower of Safflower is used in folk medicine as an analgesic, antithrombotic and antihypertensive crude drug as well as a source of natural colorants [31]. Safflower has long been grown for the dye extracted from the flowers. Depending on the dyeing procedure and the addition of other colorants and mordant's, it imparts a yellow, red, brown or purple color to cloth. With the introduction of cheap synthetic dyes, its importance as a dye source has greatly declined. However, dyes are still produced on a small scale for traditional and religious purposes.

As a natural food colorant it is a substitute or adulterant for true saffron and flowers are commonly mixed with rice, bread, pickles and other food to give them an attractive orange color. The seed cake is used as animal feed. The cake from undecorticated seeds (botanically the fruit) containing matairesinol-glucoside is only suitable for ruminants. After removal of the bitter compounds, the cake from decorticated seed would be excellent feed for monogastric animals too, but decortication is generally too costly. Safflower meal and flour from decorticated seed are used in the production of high-protein human diet supplements. The flour can be added to wheat flour to make breads and pies.

Fig. 1. Safflower

2. Materials and methods

Flower extraction

The fresh flowers (20g) of Safflower samples were collected from Siddaganga Mutt, Tumkur, during the agricultural exhibition. Collected fresh flowers were washed, finely cut and soaked in 100ml boiling distilled water for 5-10 min and then it was filtered through Whatman filter paper no.1.

Synthesis of gold nanoparticles:

In a typical experiment gold nanoparticles were synthesized by taking 5ml of flower extract was added into 45ml 0.002M $AuCl_4$ obtained from Loba Chemie Pvt. Ltd. Mumbai and kept in dark for 3-4 hours. Within an hour yellow solution was obtained. The gold nanoparticles solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min.

Supernatant is discarded and the pellet is dissolved in deionized water. The bioreduction of Au^{3+} in aqueous solution was monitored by periodic sampling of aliquots of the suspension. The synthesized nanoparticles were screened for its antibacterial and antifungal activity by disc method.

Characterization

The gold nanoparticles characterized by Elico SL 164 double beam UV-Visible Soectrophotometer [32] .The morphology of the samples was studied by high-resolution transmission electron microscopy (HRTEM; JEOL JEM-2010F).

Antifungal and antibacterial activity

Aspergillus niger, *Aspergillus flavus*, *E.coli* and *Streptobacillus sp.* collected from authenticated stock culture of our college itself.

Culturing of Potato Dextrose Media

2.4 g of potato dextrose broth and 2g of agar are dissolved in 100ml of distilled water. The contents are subjected to autoclaving at 121^{o} C for 20 min at 15 lbs pressure. Potato dextrose agar media plate is prepared by pouring the nutrient agar media into the petriplates. The microbial suspension of *Aspergillus niger* and *Aspergillus flavus* was spread over the media. The standard antibiotic disc was also placed in one side of the petriplates which is the control and the pretreated antibiotic discs with the synthesized nanoparticles in another side. The inoculated petriplates is covered and it is kept for incubation at room temperature.

3. Results and discussion

Gold nanoparticles were synthesized from Hydrogen tetra chloroaurate solution containing Au^{+} ions by treating with the *Carthamus Tinctorius* L. flower extracts. The color of the solution changed to deep brownish color within 30 min of reaction with the Au^{+} ions. The appearance of the deep brownish color indicated formation of gold nanoparticles (Fig 2). They turned brown and the intensity of color was increased with the time of incubation.

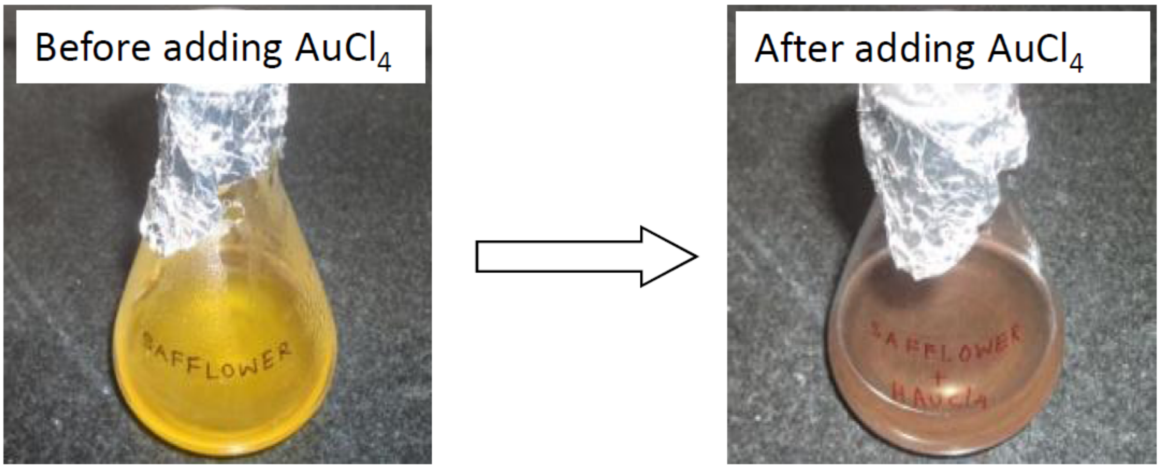
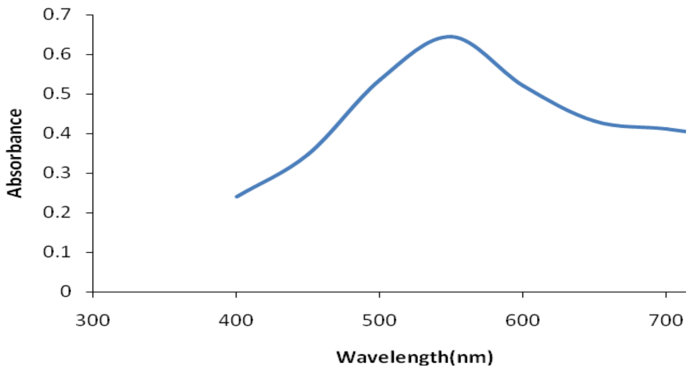


Fig. 2. Test for Safflower to show the synthesis of gold nanoparticles

The formation of gold nanoparticles was confirmed by color changes followed by UV-Visible spectrophotometer analysis. The UV- Visible spectrophotometer has proved to be very useful technique for the analysis of some metal nanoparticles. The UV- visible spectra (shown in Graph 1) indicate a strong Plasmon resonance that is located at ~560 nm. Presence of this strong broad plasmon peak has been well documented for various Me- NPs, with sizes ranging all the way from 2 to 100 nm [33].



Graph 1. UV-Visible spectrum of gold nanoparticles synthesized by Safflower flower extract

The microstructures and size of the biosynthesized gold nanoparticles were studied by TEM (Transmission Electron Microscopy) analysis. The typical TEM images of the gold nanoparticles synthesized by Safflower extract as reducing agent is shown in Fig. 3. The micrograph shows formation of nanotriangles and spherical like morphology. The size of the nanotriangles about 200 nm, while, the spherical like particles are smaller than the triangles with size ranging between 40-60 nm. The high resolution TEM images (Figure 3c and 3d) shows the well defined lattice fringes indicating that both type of particles are highly crystalline in nature.

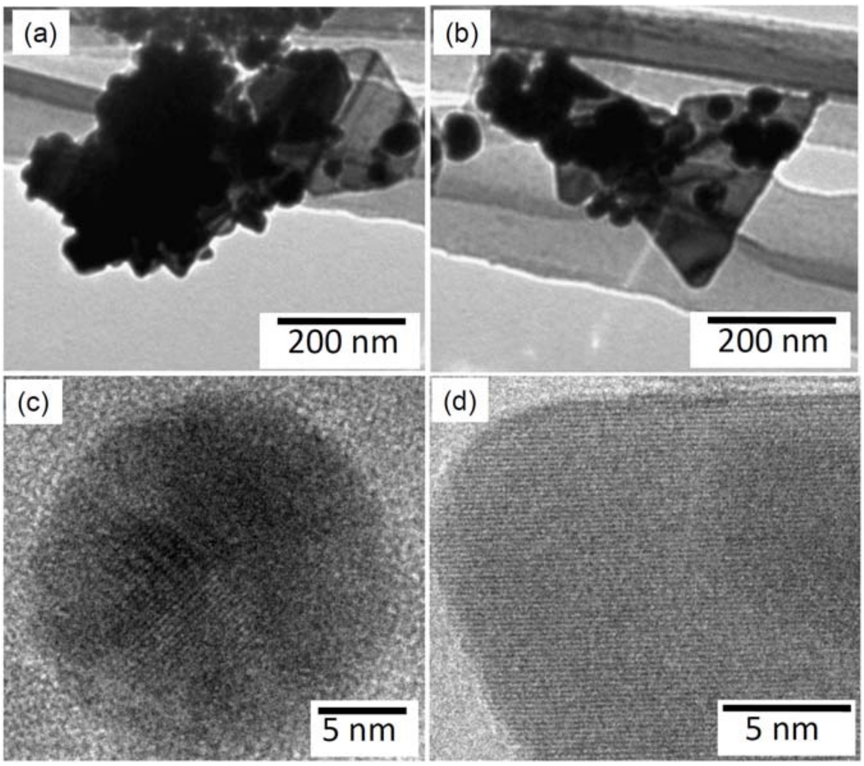
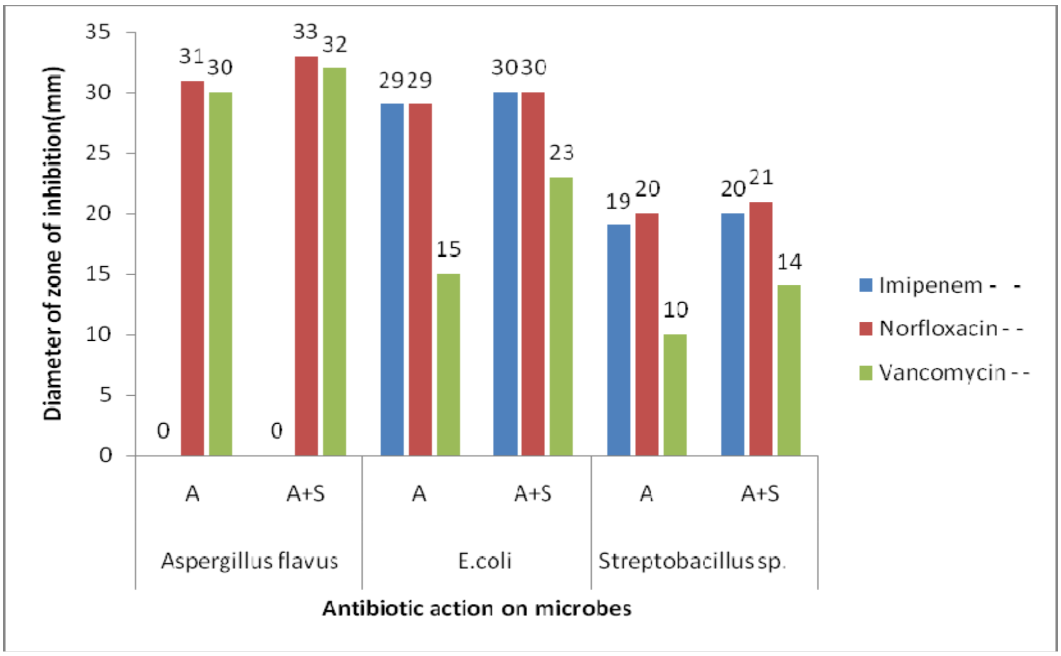


Fig. 3 TEM images of gold nanoparticles synthesized from Safflower with triangle (left) and spherical like (right) nanoparticles.

The gold nanoparticles obtained from flowers of *Carthamus Tinctorius* L. were tested against set of microorganisms in order to estimate their antimicrobial potentials. Anti bacterial study indicates that antibiotic with gold nanoparticles extracted from Safflower (A+S) exhibit more zone of inhibition compared to standard antibiotics (A) used (Table 1 and Graph 2). The zone of inhibition against *E.coli* is more when compared with the zone of inhibition exhibited on *Streptobacillus sp.* for gold nanoparticles with antibiotics like Imipenem, Norfloxacin and Vancomycin (Fig. 3). Vancomycin with gold nanoparticles has less zone of inhibition against *E.coli* and *Streptobacillus sp.* (23mm and 14mm respectively), where as gold nanoparticles with Norfloxacin and Imipenem shows more inhibition against *E.coli* and *Streptobacillus sp.* (30mm and 20mm respectively). Compare to antibiotics (A), antibiotics with gold nanoparticles (A+S) shows more zone of inhibition against *E.coli* and *Streptobacillus sp.*(30mm and 21mm respectively).

Table 1. Antibacterial activity of the gold nanoparticles synthesized from Safflower

Antibiotics	**Diameter of zone of inhibition in mm**							
	Organisms							
	Aspergillus niger		***Aspergillus flavus***		***E.coli***		***Streptobacillus sp.***	
	A	**A+S**	**A**	**A+S**	**A**	**A+S**	**A**	**A+S**
Imipenem	-	-	-	-	29	30	19	20
Norfloxacin	-	-	31	33	29	30	20	21
Vancomycin	-	-	30	32	15	23	10	14



Graph 2: Antimicrobial activity of extracted gold nanoparticles from Safflower

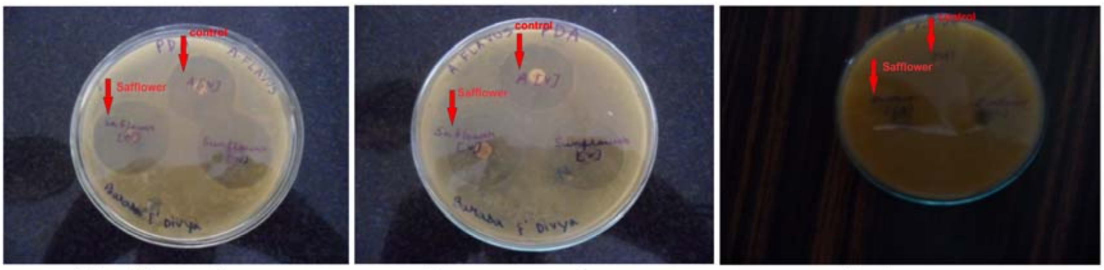


Fig 4.Antimicrobial activity of the gold nanoparticles synthesized from Safflower against Aspergillus flavus

Regardind the antibacterial activity, compare to *Aspergillus niger*, *Aspergillus flavus* shows more zone of inhibition for the antibiotics Norfloxacin and Vancomycin in both antibiotics (A) and antibiotics with Safflower extract (A+S). Imipenem with gold nanoparticles does not show any reaction against *Aspergillus niger* and *Aspergillus flavus*(Fig.4). Both Norfloxacin and Vancomycin shows more zone of inhibition against *Aspergillus niger* and *Aspergillus flavus* (33mm and 32mm). Compare to antibiotics (A), antibiotics with gold nanoparticles (A+S) shows more zone of inhibition against *Aspergillus flavu* (Table 1 and Graph 2).

Some authours studied the antihyperglycemic activity of leaves of Carthamus tinctorius L. [34] and they found good results. The antibacterial activity of the ethanolic and aqueous extract of carthamus tinctorius L on some bacteria was observed by Paramesh et al [35]. Zhang et al isolated some antioxidative compunds from the safflower oil cake [36]. These studies have established importance of Carthamus tinctorius flower exctract in the antimicrobial and antibacterial activities. Therefore, the gold nanoparticles obtained by using this flower extract has further proved the significance of using these flower extract in bio-synthesis and application of gold nanoparticles. Good results regarding antibacterial activity were obtained also for gold nanoparticles from Sunflower extracts as it can bee seen in the figure 4. These results are going to be the object of another scientific paper.

4. Conclusion

In conclusion, our study can be considered as the first report for the synthesis of gold nanoparticles using extracts of *Carthamus Tinctorius* L. flower samples. Gold nanoparticles were confirmed by color changes and were characterized by UV-visible spectrophotometer; the UV-visible spectra showed a broad peak located at 560 nm for gold nanoparticles. The TEM micrograph shows formation of nano triangle and spherical shape particles. The sizes of the nanoparticles were in the range of 40-200 nm, showing a broad size distribution. C*arthamus Tinctorius* L. appears to have significant antimicrobial capacity resembling a broad spectrum antibiotic against the common uro-gastro pathogenic *Escherichia coli*, one of the common bacteria with pathogenic strains and is relatively resistant towards synthetic drugs.

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