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Green synthesis and characterization of Silver nanoparticles using the flower extracts of *Tecoma stans* (L.) Juss.ex Kunth (Yellowtrumpet bush) and *Tagetes erecta* L. (African marigold)

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Abstract

The Nanoparticle is defined as a particle of matter between 1 and 100 nanometers in diameter. High quality colloidal silver nanoparticles were synthesised via a green approach by using the flower extracts of *Tecoma stans* and *Tagetes erecta*. Silver nitrate was used as a substrate ion while the plant extract successfully played the role of reducing and stabilising agents. The bio-reduction of aqueous Ag+ ions by *Tecoma stans* and *Tagetes erecta* extracts were identified by its colour change. During the synthesis of silver nanoparticle, the colour of the solution changed from yellowish-brown to dark brown indicating the formation of silver nanoparticles. The synthesised nanoparticles were characterised by Fourier Transform Infrared Spectroscopy and UV-vis Spectroscopy. The peak obtained at **450 nm** confirmed the presence of synthesised silver nanoparticles. The antibacterial activity of the synthesised nanoparticles was studied on *Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes* using disk diffusion method. The recent approach of green synthesis is quite impressive due to its eco-friendly, economical, feasible and non-toxic nature.

Nanotechnology is one of the most promising technologies of the 21st century. It is the ability to convert the nanoscience theory to useful applications by observing, measuring, manipulating, assembling, controlling and manufacturing matter at the nanoscale. There have been tremendous developments in the field of Nanotechnology in the recent past with numerous technologies formulated to synthesize nanoparticles with specific characteristics on Morphology (*ie*., shape and size) and distribution[1]. Although, there are several methods for the synthesis of pure, welldefined nanoparticles, they are very expensive and the use of toxic and hazardous chemicals which cause danger to Environment, Human and Biological means[8]. Many methods have been used for the synthesis of silver nanoparticles, like chemical and photochemical reduction[3] electrochemical techniques[6] and radiolysis methods[4]. Biosynthesis of silver nanoparticles is a single step process and it offers several

advantages such as time reducing, cost effective and Non-toxic. Nanocrystalline silver is a known Noble metal and they have tremendous applications in the field of Detection, Diagnostics, Therapeutics and Antimicrobial activity[2]. The current study is to develop a protocol for eco-friendly synthesis of silver nanoparticles using flower extract of *Tecoma stans & Tagetes erecta* and their characterization using UV visible spectroscopy, FTIR (Fourier Transform Infrared spectroscopy) and their antimicrobial analysis by using various microbes were studied.

Collection of fresh flowers*:* The flowers were washed thoroughly with tap water and followed by distilled water to remove the adhered material. The flowers are cut into small pieces. 10 g of cut flowers are mixed in ultrapure water and an extract is prepared. The extract is filtrated with the help of Whatman No. 1 filter paper and the filtrate was collected. This filtrate is used for further processes.

Biogenic synthesis of silver nanoparticles : It includes preparation of flower extract and synthesis of silver nanoparticles. The method involves 100 ml of 0.05 M aqueous $AgNO_3$ solution with 10 ml of the flowers extract for 24 hours. A marked colour change has been observed, as the colourless aqueous $AgNO_3$ which changes to yellowish brown and to dark brown and thereby indicating the formation of silver nanoparticles. The synthesized nanoparticles were extracted from mixture by removing water from the mixture by continuous heating.

UV-VIS Spectra analysis: The reduction of pure Ag + ions was monitored by measuring the UV-Vis spectrum of the reaction medium. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer. Observe the absorbance at multiple wavelengths. (Table-1).

Fourier Transforms Infrared Spectroscopy (FTIR) measurements : To identify the possible biomolecules responsible for the reduction of the Ag +ions and capping of the bio reduced Ag-NPs synthesized by flower extract, FTIR spectroscopy measurements were carried out (Fig. 2 & 3). Ag-NPs powder sample was prepared by heating the solution in an oven. Melt the agar by boiling in a water bath and recheck and adjust the pH. Sterilize the medium at 121^{o}C for 15 minutes in an autoclave. After sterilisation, cool the medium to 500^{o}C and pour the medium into sterile petri dishes. Allow the medium to set at ambient temperature.

Microbial analysis :

Procedure : From a pure bacterial culture, colonies were obtained with an inoculation loop and was transferred to a clean test tube containing 1ml water. A sterile cotton swab dipped into the bacterial suspension was used for inoculation into the agar by streaking. The surface of the medium was allowed to dry for 3-5 minutes. Well was made in the agar with a well puncture into which the sample was added and finally incubated at 37^{o}C.

As shown in table-1, for structural characterization of silver nanoparticles, UV-vis spectroscopy is used for preliminary characterization and examining the reduction of silver ions from aqueous $AgNO_3$ solution to silver nanoparticles. The resonance of the synthesized silver nanoparticles is analysed with the UV-vis double-beam bio-spectropho-

tometer using the software in the range of 350 to 1000 nm. The table 1 shows the UV spectra for $AgNO_3$ solution with 30 ml *Tecoma stans* flower extract in 300 ml of $AgNO_3$ precursor after 24 hrs. The intensity and UV absorbance are high and the peak is obtained at the wavelength of around **450** nm which confirmed the presence of silver nanoparticles in the flower extracts.

FTIR provides the information of changes in functional groups of chemicals, basically found in given flower extracts which were further utilized for bio reduction of silver nanoparticles. The spectra indicates that the molecules present in the *Tecoma stans* & *Tagetes erecta* extracts perform formation and stabilization of silver nanoparticles. In Fig. 2, FTIR spectra of *Tecoma stans* extracts clearly show 13 peaks from 1500-400 cm^{-1}. Around 1284 cm^{-1}, there is a broad peak which attributes to stretching vibration of C-N amide between 819.79^{-1} and 798.09 cm^{-1} & there is abundance of bending inorganic carbonates. The number of c=c conjugated are seen in between 2381.92 cm^{-1} and 2324.03 cm^{-1}. In Fig. 3, FTIR spectra of Tagetes extracts clearly shows 9 peaks from 1500-400 cm^{-1}. Around 1741 cm^{-1} -1715^{-1} there is a broad peak which attributes to -c=o str of ester, str of carbonic acid and nucleic acid. At 1515^{-1} tyrosine bond is present. At 1250^{-1}-1200^{-1} there are larger number of P=0 str of $P0_2$ phospho-diester bonds are observed.

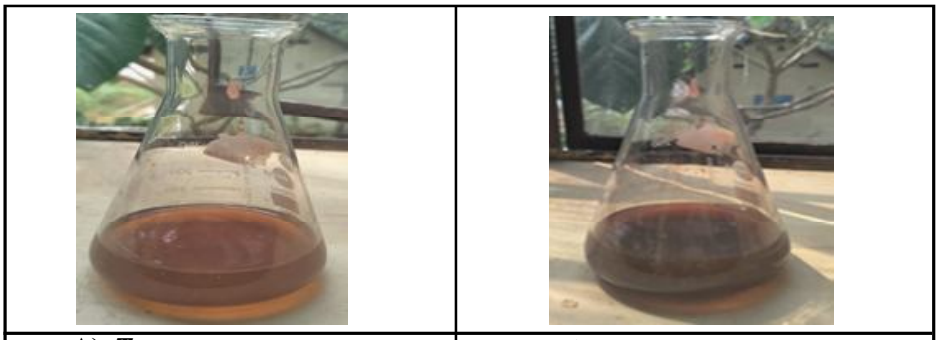
Antibacterial activity is done to detect the effect of these synthesised nanoparticles on various bacteria. For this well diffusion technique is used where the synthesised nanoparticles are placed in wells. By the action of these nanoparticles, the growth of bacteria is inhibited in the region around the well. Nanoparticle synthesis using *Tecoma stans & Tagetes erecta* showed a zone of inhibition against various bacteria (Fig. 4). Among them the nanoparticles synthesised using *Tecoma* on *Streptococccus pyogenes* showed a good zone of inhibition as shown in Table-2. Diameter of zone inhibition obtained for *Tecoma* against various bacteria such as *E. coli*, *Staphylococcus aureus and Streptococcus pyogenes* are 3.1, 4.2, **4.4** cm whereas in *Tagetes erecta* it is **2.6**, 3.3, 2.4 cm. Therefore, these results indicate that the nanoparticles synthesised using these flower extracts is effective against these three bacteria-*Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes.*

Kavitha *et al.*,[5] have performed the synthesis and characterization of silver nanoparticles using the flower extracts of *Tecoma stans & Tagetes erecta* which showed the UV vis spectra at 450 nm which was similar to works done here. V.V et al have synthesized truncated triangular silver nanoparticles using a simple one step chemical reduction method. The reduction of silver ions by sodium borohydride was performed in the presence of poly (vinyl pyrrolidone) as a stabilizing agent. The UV–vis spectrum showed three plasmon peaks located at 340, 412, and 700 nm confirmed the anisotropic Ag-NPs which was contradictory to peaks obtained at 450 nm by using the extracts of *Tecoma stans & Tagetes erecta.*

Hemali Padali *et al.*, (2015) have synthesised nanoparticles from Marigold flower which showed maximum peak at 430 nm. Synergistic antimicrobial potential of silver nanoparticles was evaluated with various commercial antibiotics against Gram positive (*Staphylococcus aureus* and *Bacillus cereus*),

Colour change :

Fig. 1 Solution becomes yellowish brown by the addition of 0.05M aqueous AgNO3 solution. After 1hour yellowish brown turns to reddish brown due to reduction of silver ion by A) *Tecoma stans* and B) *Tagetes erecta.*



A) *Tecoma stans* **B) *Tagetes erecta***

Table 1. Absorbance of silver nanoparticle synthesis using *Tecoma stans &Tagetes erecta* at multiple wavelengths(350-1000nm)

UV spectral analysis :

Wavelength	*Tecoma*	*Tagetes*
350	1.123	1.126
400	1.17	1.131
450	**1.325**	**1.271**
500	1.238	1.125
550	0.814	1.05
600	0.543	0.926
650	0.444	0.806
700	0.404	0.699
750	0.385	0.610
800	0.36	0.533
850	0.337	0.471
900	0.315	0.419
950	0.294	0.377
1000	0.274	0.342

Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. The antibacterial activity of AgNPs with antibiotics was better than antibiotics alone against the tested Gram negative bacteria.

The synthesis of silver nanoparticles using the *Tecoma stans* and *Tagetes erecta* flower extracts are presented. The reduction and stabilization of silver ions to silver nanoparticles are obtained due to the presence of water-soluble organic compounds in the given flower extracts. The results show that, these flower extracts were one among the best agents for the synthesis of silver nanoparticles. This biological method is potentially attractive

Ftir Measurements :

A) Tecoma stans

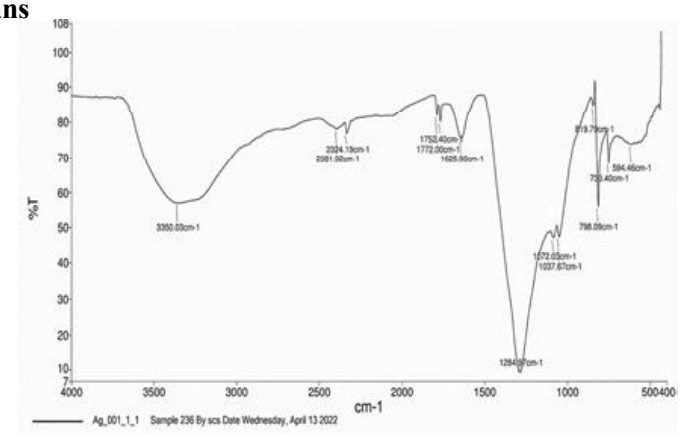


Fig. 2: FTIR images of silver nanoparticles present in the *Tecoma stans* flower extracts.

B) Tagetes erecta

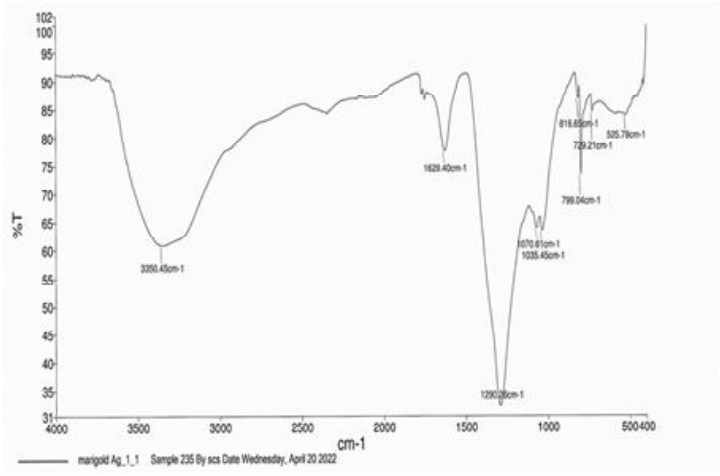


Fig. 3: FTIR images of silver nanoparticles present in the *Tagetes erecta* flower extracts.

Table. 2: Diameter of zone of inhibition of different AgNPs synthesis using *Tecoma stans*, *Tagetes erecta* against *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes.*

Bacteria	**Zone Of Inhibition**	
	Tecoma stans (sample **A***)* cm	*Tagetes erecta (*sample **B**) cm
Escherichia coli	3.1	2.6
Staphylococcus aureus	4.2	**3.3**
Streptococcus pyogenes	**4.4**	2.4

Fig. 4: Antibacterial activity of AgNPs synthesised using *Tecoma stans* and *Tagetes erecta*. Fig. A shows the activity of synthesised nanoparticles against *Escherichia coli.* Fig. B shows the activity of synthesised AgNPs against *Staphylococcus aureus*. Fig. C shows the activity of synthesised AgNPs against *Streptococcus pyogenes*.

for the largescale synthesis and also very useful for environmental remediation because of its antibacterial property against *Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes*. The silver nanoparticles produced in the above proposed method can be used in solar cells as well as in the optical communications.

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Conflict of interest :

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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