International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 01-01-2022, Accepted: 16-01-2022, Published: 01-02-2022
Volume 7, Issue 2, 2022, Page No. 5-9

Anti-microbial activities of green synthesized *Tagetes erecta* L. leaf broth

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Abstract
A simple, rapid, low cost and eco-friendly green synthesis of silver nanoparticles using the leaf broth of *Tagetes erecta L.* as a reducing agent has been investigated. The resulting silver nanoparticles were characterized using UV-Vis spectrophotometer, X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM) and Fourier Transformations Infra Red (FT1R) spectroscopic analysis. The identification of the crystallinity of silver nanoparticles was characterized using an XPert Pro X-ray diffractometer and the morphology of the synthesized silver nanoparticles by the Scanning Electron Microscope (FEI Quanta 250). XRD and SEM studies confirmed the formation of metallic silver nanoparticles with average crystallite size of 15.4 nm. The FTIR spectrum analysis of the synthesized silver nanoparticles reveals that biomolecules with carbonyl, hydroxyl and amine functional groups have the potential for metal ion reduction and for capping the newly formed nanoparticles. The antimicrobial activity was assayed by agar well diffusion method using 20 ml each of sterile Nutrient Agar (NA) (Hi-Media) and Potato-Dextrose Agar (PDA) (Hi- Media) for testing the bacterial and filamentous fungal activity respectively. Amphotericin-B (Hi-Media) for fungi and Ciprofloxacin (Hi- Media) for bacteria were used as control. The resulting silver nanoparticles exhibited moderate antimicrobial activities against the tested bacterial pathogens but not in fungal pathogens.

Keywords: silver nanoparticles, green synthesis, *Tagetes erecta L.*, antibacterial activity, minimum inhibitory concentrations

Introduction
Nanoparticles research is currently a great influencing area of intense scientific research community due to a wide variety of potential applications in biomedical, optical, and electronic fields. Out of all kinds of nanoparticles, silver nanoparticles have been attracting more and more attention owing to their intriguing electrical, thermal and optical properties. Metal nanoparticles can be synthesized by conventional chemical and physical methods (Balantrapu K and Goia V.D, Rodriguez-Sanchez, L *et al*, and Shenava Aashrith). However, most of the current chemical synthetic processes are regarded as having a relatively high environmental cost. Recently biosynthetic methods employing plant extracts have been emerged as environmentally sustainable alternatives to chemical synthetic procedures (S.P Dubey *et al*., D. Philip and Shankar, S.S, Rai *et al*). Biosynthesis of silver nanoparticles using medicinal plants extract is a very quiet novel method which provides advancement over chemical and physical methods. It is a simple, rapid, low cost, eco friendly and a single step method and easily scaled up for large scale biosynthesis process. In the present study, we report the biosynthesis of silver nanoparticles by the reaction of aqueous solution of silver nitrate with *Tagetes erecta L.* leaf broth at room temperature. *Tagetes erecta L.* has known medicinal properties forming an important component of ayurvedic medicine and has been used since ages to treat several ailments like rheumatism, inflammation, cold, bronchitis, renal problems etc. Moreover, It has been reported that more than 70% of the developing world's population still depends on the traditional medicine. (Kaliyaperumal et. al) owing to its natural origin and lesser side effects.

Materials and Methods
Preparation of *Tagetes erecta L.* leaf broth
Fresh leaves of *Tagetes erecta L.* were collected from the local areas and washed thrice in distilled water and dried on paper toweling. 20 gram each of the leaves were cut into fine pieces and boiled at 100°C with 100ml of sterile distilled water for about 15 minutes. The crude extract was filtered through a Whatman filter paper (No. 40) to prepare the aqueous leaf extract. 1mM aqueous solution of Silver nitrate ($AgNO_3$, AR grade) was prepared and used in the synthesis of silver nanoparticles. 10 ml of the aqueous *Tagetes erecta L.* leaves extract was added to 190 ml of 1 mM aqueous $AgNO_3$ solution in a beaker separately and kept at room temperature for 48 hours for reduction.

Characterization of synthesized silver nanoparticles.

UV-Visible Spectrum analysis

The UV-Vis spectrum for the reaction solution of silver nanoparticles was measured with UV-Vis Spectrophotometer (Model: HR Ocean Optics 4000).

XRD analysis

The XRD measurement was carried out for the identification of the crystallinity of silver nanoparticles using an XPert Pro X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a 2θ configuration.

SEM analysis

The morphology of the synthesized silver nanoparticles was characterized by the Scanning Electron Microscope (FEI Quanta 250).

FTIR analysis

FTIR spectrum of the synthesized silver nanoparticles was recorded with a Shimadzu spectrometer (Model FTIR- 8400S).

Antimicrobial evaluation

The antimicrobial activity was assayed by agar well diffusion method using 20 ml each of sterile Nutrient Agar (NA) (Hi-Media) and Potato-Dextrose Agar (PDA) (Hi- Media) for testing the bacterial and filamentous fungal activity respectively (Kaliyaperumal *et al*).

Results and Discussion

The UV-Vis spectrum analysis of the synthesized silver nanoparticles is presented in Fig.1. It shows the appearance of a single but strong surface plasmon resonance band absorption peak centered at 420 nm which indicates the formation of silver nanoparticles (Reeves DS et.al.].

The prepared silver nanoparticles were also purified by repeated centrifugation at 5000 rpm for 10 minutes followed by re-dispersion in 10 ml of distilled water. Fig. 2 shows the XRD pattern of silver nanoparticles. The peaks at 2θ= 38.16°, 44.54°, 64.58° and 77.61°

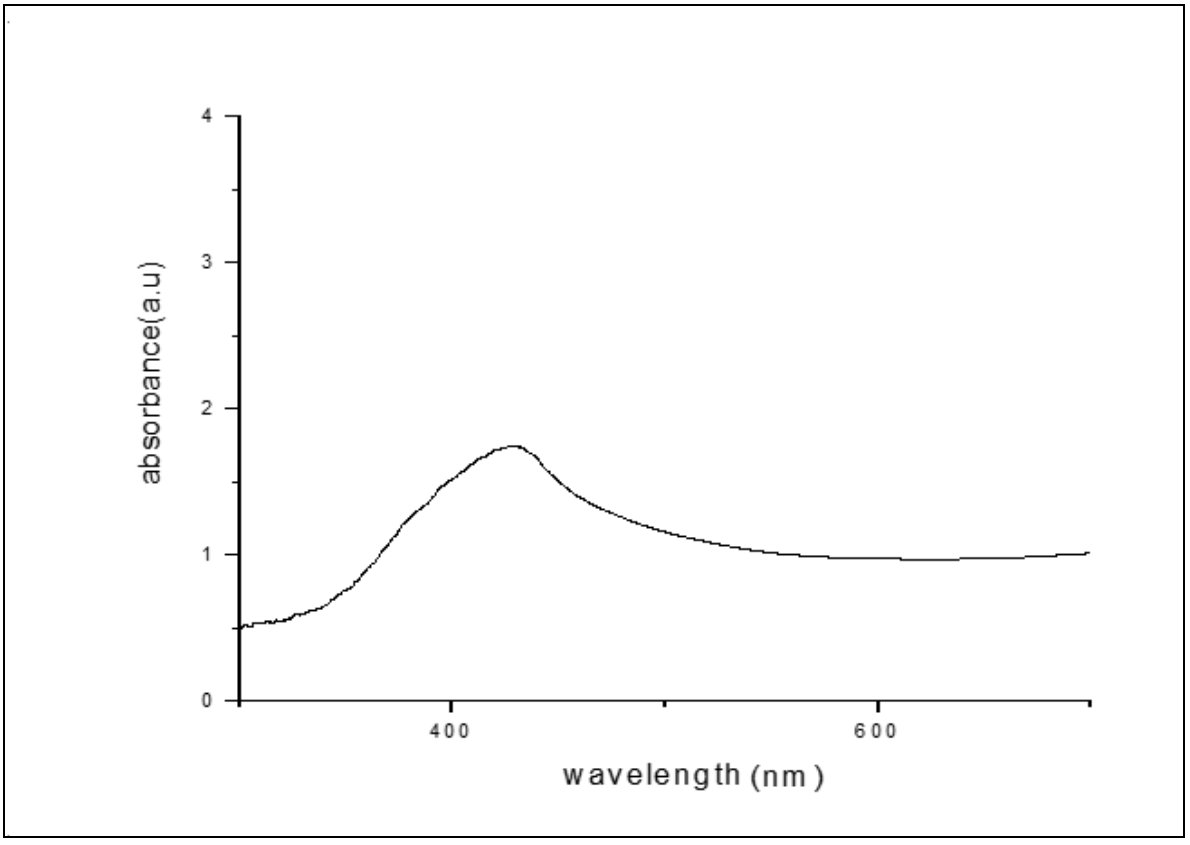

Fig 1: UV- Vis spectrum of synthesized silver nanoparticles

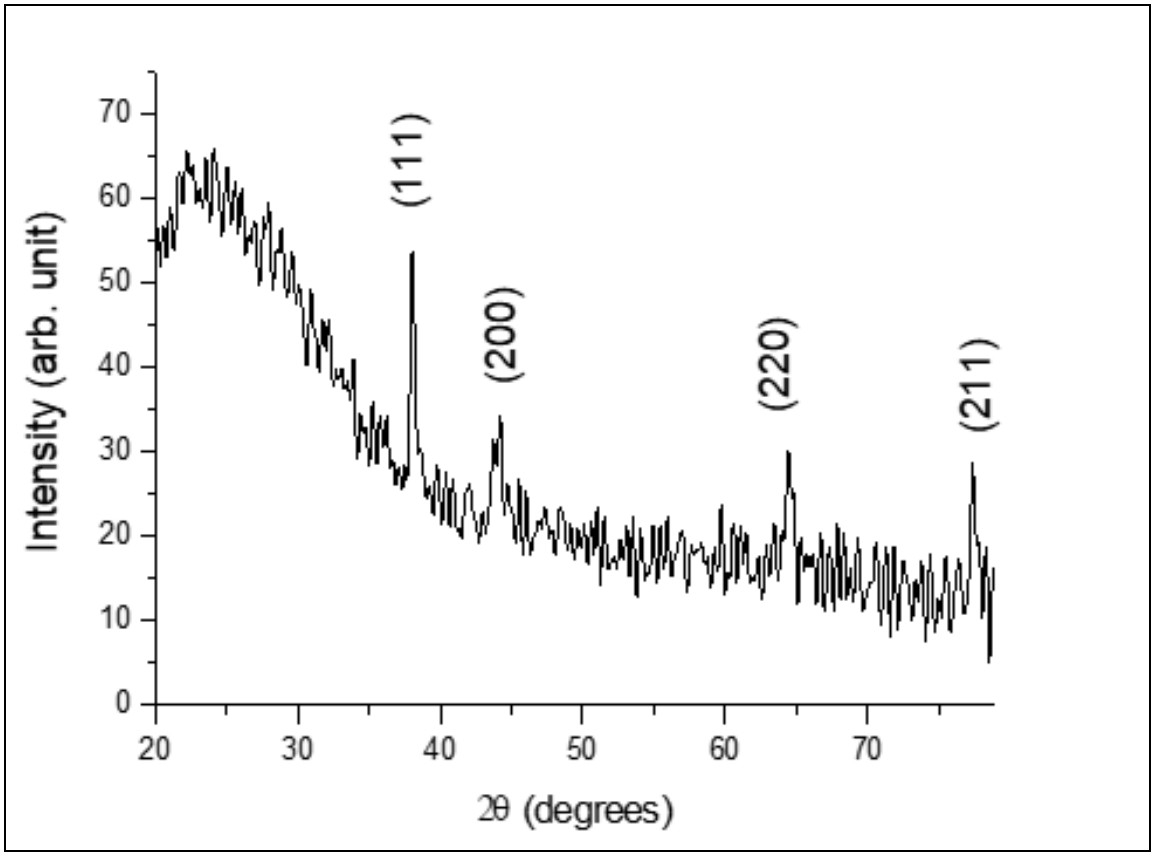

Fig 2: XRD pattern of synthesized silver nanoparticles

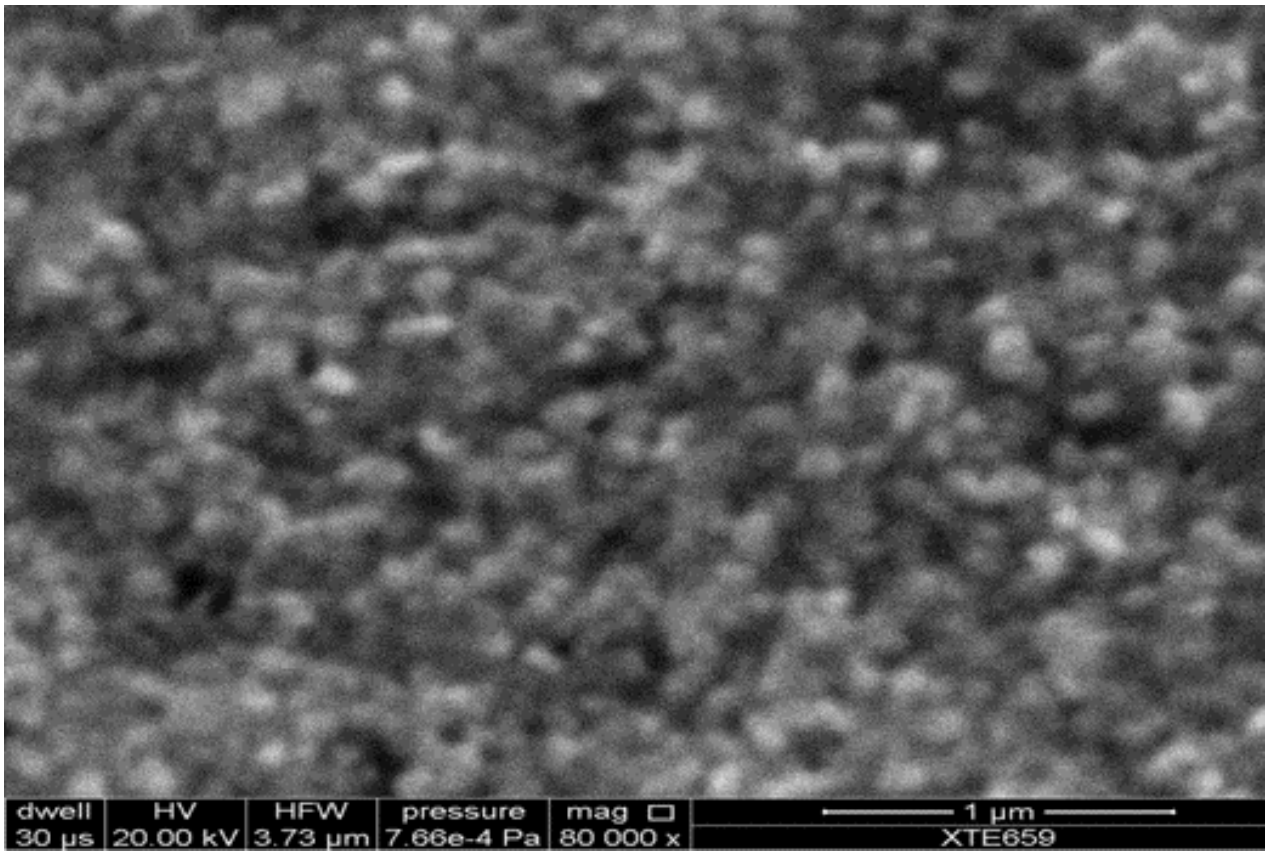

Fig 3: Typical SEM image of the synthesized silver nanoparticles.

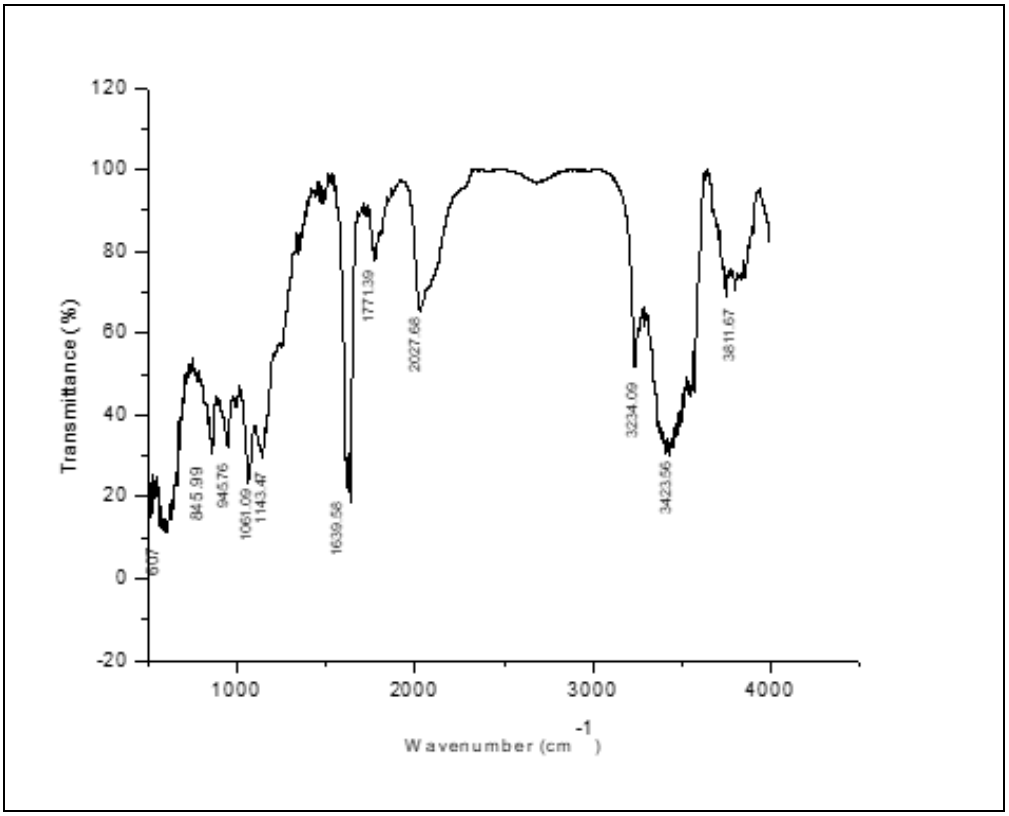

Fig 4: FTIR spectrum of the synthesized silver nanoparticles.

Table 1: Antifungal Activity (Zone of inhibition)

	Zone of inhibitions (mm)									
	Proteus mirabilis		***Klebsiella pneumoniae***		***Escherichia coli***		***Salmonella paratyphi***		***Pseudomonas aeruginosa***	
	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5
Silver nanoparticles	18	15	20	18	24	20	20	17	12	10
Ciprofloxacin **(**16µg/ml)	32		34		36		36		34	

Table 2: Antibacterial Activity (Zone of inhibition)

	Zone of inhibitions (mm)									
	Aspergillus flavus		***Aspergillus fumigatus***		***Aspergillus niger***		***Candida albicans***		***Candida krusei***	
	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5
Silver nanoparticles	-	-	-	-	-	-	-	-	-	-
Amphotericin-B (16µg/ml)	32		34		38		38		40	

Table 3: MIC for antifungal activity

	Fungal	**MIC**
Silver nanoparticles	*Aspergillus flavus*	>1
	Aspergillus fumigatus	>1
	Aspergillus niger	>1
	Candida albicans	>1
	Candida krusei	>1

Table 4: MIC for Antibacterial activity

	Bacteria	**MIC**
Silver nanoparticles	*Proteus mirabilis*	= 0.0625
	Klebsiella pneumoniae	< 0.0625
	Escherichia coli	= 0.015625
	Salmonella paratyphi	< 0.125
	Pseudomonas aeruginosa	= 0.125

Correspond to the (111), (200), (220) and (311) planes respectively of face centred cubic structure of metallic silver ions. No impurities were detected from this pattern within the resolution limit of XRD. The average crystallite domain size was found to be 15.4 nm and it was calculated from the width of the XRD peaks using the Debye Scherrer formula.

$$D = 0.94 \lambda / \beta \cos\theta.$$

Where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the Full Width at Half Maximum (FWHM) and θ is the diffraction angle.

A typical SEM image of the synthesized silver nanoparticles is presented in Fig.3. It was clearly seen that the prepared samples consisted of an abundance of spherical nanoparticles. The average diameter of the nanoparticles is about 30-55 nm on average.

The FTIR spectrum analysis of synthesized silver nanoparticles is shown in Fig. 4. The medium intense band at 1061.09 cm^{-1} is assigned to the C-N stretching mode of amine group. The sharp band at 1639.58 cm^{-1} arises from C=O (amide I band). The absorption bands located at 3234.09 cm^{-1} and 3423.56 cm^{-1}may be attributed to O-H stretching mode of alcohols and phenols. The presence of these active functional groups in leaf extract results in the swift reduction of silver ions into silver nanoparticles.

For the antimicrobial activity evaluation of synthesized silver nanoparticles, the test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Sterile 6mm diameter cork borers were pierced in the agar at equidistant spots. 20µl of the diluted solution (16µg/ml) was deposited on the inoculated well and left for 10 min at room temperature for the compound diffusion. Amphotericin-B (Hi-Media) for fungi and Ciprofloxacin (Hi- Media) for bacteria were used as control. The plates inoculated with bacteria were incubated at 37°C for 24 hr and for fungal cultures at 30°C for 24-48 hr. The experiment was repeated thrice and the average results were recorded. The antimicrobial activity was determined by measuring the diameter of the inhibition zone (mm) around the well [Table 1 and Table 2]. In the present investigation, the biologically synthesized silver nanoparticles are found to be moderate toxic against the tested bacterial pathogens but not in fungal pathogens.

The susceptibility of microbial was determined by minimum inhibitory concentration determination method (Bannoth Reddya Naik et. al.). The minimum inhibitory concentrations (MICs) of the prepared silver nanoparticles were determined by serial dilution against the micro-organisms. The minimum concentrations at which no visible growth was observed were defined as the MICs, which were expressed in mg/ml. The prepared *Tagetes ercta L.* silver nanoparticles showed the best inhibitory activities against *Escherichia coli* among the tested bacterial pathogens.[Table 3 and Table 4].

Conclusion

The present study confirmed a simple, efficient biological method at room temperature for the synthesis of spherical nanoparticles with diameters in the range of 30- 55 nm using *Tagetes erecta L.* leaf broth and the testing of their antimicrobial activities. Biomolecules with carbonyl, hydroxyl and amine functional groups have the potential for metal ion reduction and for capping the newly formed nanoparticles. Antimicrobial activities were assayed and exhibited a moderate antibacterial activity against the tested bacterial pathogens but not in fungal pathogens.

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