

# Biosynthesis of Silver Nanoparticles from *Tridax procumbens* Linn Plant Extract and to study its Antifungal Activity

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**Abstract:** The present investigation explores a plant-mediated approach for the synthesis of silver nanoparticles (AgNPs) using an aqueous extract of *Tridax procumbens* Linn. and examine its in vitro antifungal activity against *Aspergillus niger*. The synthesis was carried out by combining the plant extract with 0.1 M silver nitrate and allowing the reaction to proceed for 48 h. A visible colour transition from yellow to brown provided an initial indication of nanoparticle formation. The resulting *T. procumbens*-mediated silver nanoparticles (TpAgNPs) were characterized using UV-Visible spectroscopy, X-ray diffraction (XRD), and Fourier-transform infrared (FTIR) spectroscopy. UV-Visible analysis revealed a broad absorption maximum at 430 nm. XRD analysis exhibited five observed peaks at 14.7°, 15.4°, 16.2°, 18.5°, and 14.0°, with the reported particle size being less than 50 nm. FTIR analysis displayed characteristic bands at 680, 787, 1084, 1388, and 1559 cm<sup>-1</sup>, attributed to benzene, C-H, C-O, phenolic O-H, and amine N-H functional groups, respectively. The antifungal efficacy of TpAgNPs was evaluated against *A. niger* by the well-diffusion method at TpAgNPs demonstrated a concentration-dependent antifungal activity, with inhibition zones increasing from 10.67 ± 0.47 to 16.07 ± 2.07 mm at 48 h and from 10.27 ± 0.25 to 22.67 ± 0.47 mm at 72 h across 250–1000 ppm. At 96 h, the corresponding zones were 9.67 ± 1.25, 11.33 ± 2.49, 12.33 ± 1.70, and 14.67 ± 3.30 mm, with fluconazole as the reference drug and 10% DMSO as the control. The findings indicate an invitro inhibitory effect of TpAgNPs against *A. niger*, highlighting the potential of *T. procumbens* extract as a biological route for silver nanoparticle synthesis.

**Keywords:** *Tridax procumbens* Linn.; silver nanoparticles; green synthesis; TpAgNPs; *Aspergillus niger*; antifungal activity; UV-Visible spectroscopy; XRD; FTIR

## I. INTRODUCTION

Nanotechnology encompasses the preparation and application of materials whose characteristic dimensions are commonly within the nanoscale range. Silver nanoparticles (AgNPs) have received sustained scientific attention because nanoscale silver displays physicochemical and biological properties that differ from those of bulk silver. Their optical response, high surface-to-volume ratio and interaction with biological systems have supported investigations in antimicrobial materials, sensing, catalysis and biomedical applications (Ahmed et al., 2016; Zhang et al., 2016). AgNPs can be produced through physical, chemical and biological approaches. Conventional chemical reduction can be effective for nanoparticle production, but the process may involve reducing and stabilizing chemicals that increase environmental and processing concerns. Biological synthesis, particularly plant-mediated synthesis, offers a route in which naturally occurring biomolecules in plant extracts can participate in the reduction of metal ions and stabilization of the resulting nanoparticles (Ahmed et al., 2016; Kuppusamy et al., 2016).



Plant extracts contain diverse secondary metabolites and other biomolecules. Their composition depends on plant species, tissue, geographical conditions and extraction method. Consequently, reaction conditions such as precursor concentration, extract concentration, temperature, pH and reaction time can influence the characteristics of the synthesized nanoparticles. Characterization is therefore necessary to complement the visual indication of nanoparticle formation. UV–Visible spectroscopy is commonly used to observe surface plasmon resonance, XRD provides information on crystalline characteristics, and FTIR can indicate functional groups associated with surface capping or stabilization (Ahmed et al., 2016; Zhang et al., 2016).

*Tridax procumbens* Linn. is a member of the Asteraceae family and is widely distributed in India. The supplied project report describes flavonoids, tannins and other phytochemical constituents in the plant and notes its traditional medicinal importance especially for jaundice and liver treatment (Wagh & Shinde 2018, 2011, 2010). Previous work has also demonstrated that *T. procumbens* extracts can be used in the biosynthesis of silver nanoparticles and that such products can exhibit antimicrobial activity (Bhati-Kushwaha, 2014).

Accordingly, the investigation was undertaken to biosynthesize silver nanoparticles using an aqueous extract of *T. procumbens* Linn., characterize the synthesized  $\text{TpAgNPs}$  using UV–Visible spectroscopy, XRD and FTIR, and assess their *in vitro* antifungal activity against *A. niger* by a well-diffusion assay.

## **II. RESEARCH METHODOLOGY**

### **2.1 Collection and preparation of plant material**

Whole *T. procumbens* Linn. plant material was collected from the local region of Nagpur, Maharashtra, India, during January–February 2024. The plant material was washed with distilled water to remove adhering soil and other surface contaminants, shade-dried, chopped and milled to a fine powder using an electrical grinder. The powdered material was stored for subsequent extraction.

### **2.2 Preparation of aqueous extract**

A 20% aqueous mixture of the powdered plant material was heated for 1 hr at 60°C in a conical flask. After cooling, the mixture was filtered through Whatman No. 1 filter paper. The filtrate was collected in a dark bottle and stored at 4°C until use.

### **2.3 Biosynthesis of silver nanoparticles**

The preparation of silver nanoparticles is taken from (Gardea-torresdery et al.2002,2003) (Svithramma et. al.,2011) and (Ravindra B. Malabdi et. al.2011) with slight modification.

For  $\text{AgNPs}$  synthesis 0.1M aq.  $\text{AgNO}_3$  solution was prepared, and stored in an amber color bottle. In 250 ml conical flask, 10 ml plant extract was mixed with 100ml of  $\text{AgNO}_3$  solution by adding drop wise with constant stirring at 650c-750c and colour was observed. Mixture was mixed thoroughly and incubate the flask at room temperature for next 40-48 hrs. After 48 hrs., change in colour from yellow to brown was observed. The color shift indicated the formation and presence of silver nanoparticles. Thereafter this solution was centrifuged at 10,000 rpm for 20 min in cooling centrifuge (Remi-C-24). The pellet was recovered by discarding supernatant. These collected pallets were dried in a desiccator at room temperature and stored at 40c until further use.

### **2.4 UV–Visible spectroscopy**

UV–Visible analysis was performed over 200–900 nm using a double-beam UV–Visible spectrophotometer. 3ml of the reaction solution before centrifugation was placed in a quartz cuvette and scanned across the stated wavelength range. The absorption maximum was used as an optical indicator of the synthesized nanoparticle preparation.

### **2.5 XRD and FTIR characterization**

For XRD analysis, the synthesized nanoparticle sample was submitted to the Maharashtra State Information Commission (CIC/SIC), Amravati.

### **2.6 Preparation of *A. niger* inoculum**

An *Aspergillus niger* culture was isolated from black mould on onion and subsequently transferred to Potato Dextrose Agar (PDA). The culture was incubated in a moist chamber approximately for 24 hours. Spores were harvested using



sterile water and quantified with a Neubauer chamber. The resulting colonies of *A. niger* were standardized to a concentration of  $1 \times 10^8$  cells/mL based on CFU (Colony Forming Unit) counts (Karaman, Rafik et al., 2016), yielding a calculated density of 12,000 spores/ $\mu$ L prior to a 1:10 dilution in sterile water.

### 2.7 Well-diffusion antifungal assay

A 0.05ml of suspension of fungal spore at the concentration of  $1 \times 10^5$  CFU/ml was applied to the center of the petri dish and spread with the help of sterile spreader on solidified PDA (Potato Dextrose) Agar plates. Four wells were prepared in each plate. The experiment was performed in triplicate for TpAgNPs and fluconazole, with a separate 10% DMSO control. TpAgNP stock was prepared in 10% DMSO and the wells received 100, 75, 50 and 25  $\mu$ L corresponding to nominal concentrations of 1000, 750, 500 and 250 ppm, respectively. Fluconazole was prepared from a commercially available tablet using sterile distilled water to obtain a reported stock concentration of 1000 ppm in 100  $\mu$ L, followed by the same concentration series. The plates were incubated at 37°C in a moist chamber and inhibition zones were recorded after 48, 72 and 96 hrs.

## III. RESULTS AND DISCUSSION

Silver nanoparticles (AgNPs) represent one of the most explored categories of nanomaterials for new and improved biomaterials and biotechnologies, with impressive use in the pharmaceutical and cosmetic industry, anti-infective therapy and wound care, food and the textile industry. Their extensive and versatile applicability relies on the genuine and easy-tunable properties of nano silver, including remarkable physicochemical behavior, exceptional antimicrobial efficiency, anti-inflammatory action and antitumor activity. Besides commercially available and clinically safe AgNPs-based products, a substantial number of recent studies assessed the applicability of nano silver as therapeutic agents in augmented and alternative strategies for cancer therapy, sensing and diagnosis platforms, restorative and regenerative biomaterials. Given the beneficial interactions of AgNPs with living structures and their nontoxic effects on healthy human cells, they represent an accurate candidate for various biomedical products.

The mechanism of silver nanoparticle toxicity depends on nanoparticle's intrinsic and optical properties and the type of bacterial species. Nanoparticles may cause cell membrane disruption or produce free radicals that induce oxidative stress leading to mitochondrial damage, DNA damage and oxidation of cellular components, ultimately destroying an entire cell (Hajipour, et al., 2012). Pathogenic microbial species are found everywhere in our environment posing high risks in hospitals and community environments (Lara et al., 2010) and specifically, the drug-resistant strains that have created a menace around the globe. With great research it has been established that AgNPs are efficient antimicrobial (Nanda et al., 2015; Tang and Zheng, 2018).

Conventionally silver nanoparticles are synthesized by chemical method using chemicals as reducing agents which later on become accountable for various biological risks due to their general toxicity; engendering the serious concern to develop environment friendly processes. Thus, to solve the objective; biological approaches are coming up to fill the void; for instance, green syntheses using biological molecules derived from plant sources in the form of extracts exhibiting superiority over chemical and/or biological methods. This plant based biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticle syntheses reported by Ahmed S., et. al., (2016).

Inconsideration of vast applications and safety of plant extract-based silver nanoparticles this present research work was an attempt to synthesized silver nanoparticle using one of the most useful medicinal plants *Tridax procumbens* Linn. This plant belongs to family Asteraceae which itself is a biggest family in plant kingdom. We have successfully synthesized silver nanoparticles by following the procedure described by Ponarulselvam et al. 2012. The change in color of plant extract mixed with 0.1M AgNO<sub>3</sub> after 48hrs was an indicative of formation of nanoparticles

### 3.1 Visual observation and UV-Visible spectrum

UV-Visible analysis showed in fig.-1, where a broad absorption peak at 430 nm can be seen. This absorption feature was interpreted as a surface plasmon resonance response associated with the synthesized TpAgNPs. The result provides instrumental support for the visual indication of nanoparticle formation.



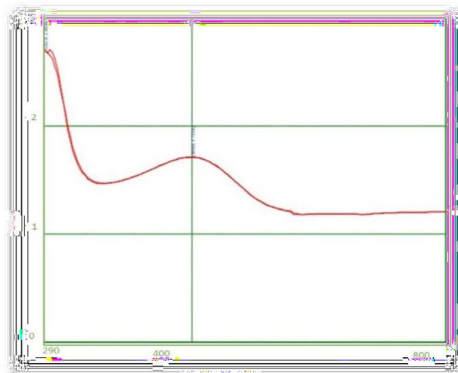


Fig-1: Surface plasmon resonance analysis of synthesized T. procumbens linn SNPs with UV-V is Spectroscopy, show a typical broad peak at 430nm

### 3.2 XRD observations

The crystallinity of the synthesized silver nanoparticle using T. procumbens linn leaf extract was examined through X-ray diffraction (XRD) (Fig no.-2). The size of the nanoparticles was calculated based on the Debye-Scherrer equation: ( $D = k\lambda/\beta\cos\theta$ ).

We observed 5 peaks of XRD Pattern at 14.7, 15.4, 16.2, 18.5, 14.0 respectively in (Table no.1) This result show that the particles synthesized from Tridax procumbens linn was small at size i.e. less than 50nm which show that the nanoparticles were synthesized.

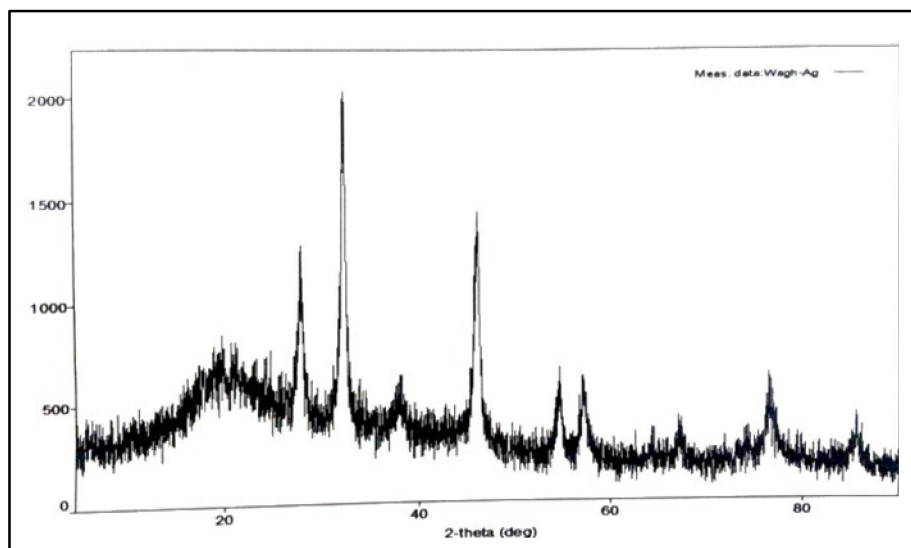


Fig-2: XRD crystallographic studies of synthesized T. Procumbens linn SNPs show Five intensive peaks with 50nm size



| Measurement Condition |                    |                              |                   |  |  |  |  |
|-----------------------|--------------------|------------------------------|-------------------|--|--|--|--|
| X-ray                 | 40 kV , 15 mA      | Scan speed/<br>Duration Time | 10.0000 deg/min   |  |  |  |  |
| Goniometer            | Mini flex 300/600  | Step width                   | 0.0200deg         |  |  |  |  |
| Attachment            | Std. sample Holder | Scan Axis                    | Theta/2-Theta     |  |  |  |  |
| Filter                | K-Beta             | Scan range                   | 5.0000-90.0000deg |  |  |  |  |
| CBO selection slit    | -                  | Incident Slit                | 1.250deg          |  |  |  |  |
| Diff. beam mono.      | None               | Length Limiting Slit         | 10.0mm            |  |  |  |  |
| Detector              | SC-70              | Receiving Slit # 1           | 1.250deg          |  |  |  |  |
| Scan mode             | CONTINUOUS         | Receiving Slit # 2           | 0.3mm             |  |  |  |  |

| No. | 2-<br>Theta(Deg) | d (ang)    | Height(cps ) | FWHM( deg) | Int.I(cps deg) | Int.W(deg) | Size (ang) |
|-----|------------------|------------|--------------|------------|----------------|------------|------------|
| 1   | 27.70(4)         | 3.218(5)   | 447(61)      | 0.58(4)    | 352(16)        | 0.79(14)   | 147(11)    |
| 2   | 31.99(2)         | 2.7957(19) | 1040(93)     | 0.56(2)    | 810(17)        | 0.78(9)    | 154(6)     |
| 3   | 54.75(6)         | 1.6752(16) | 233(44)      | 0.58(10)   | 222(12)        | 1.0(2)     | 162(28)    |
| 4   | 67.2(2)          | 1.392(4)   | 59(22)       | 0.54(17)   | 39(10)         | 0.7(4)     | 185(59)    |
| 5.  | 76.65(5)         | 1.2421(7)  | 185(39)      | 0.76(10)   | 232(12)        | 1.3(3)     | 140(19)    |

Table-1: Observed XRD five peaks Pattern at 14.7, 15.4, 16.2, 18.5, 14.0 of T. procumbens linn SNPs

### 3.3 FTIR observations

T. procumbence linn silver nanoparticles sample was analyzed in the scan range from 4000cm<sup>-1</sup> to 500cm<sup>-1</sup> of near IR spectra by FT-IR. The figure-3 shows result of broad peaks obtained at 680, 787, 1084, 1388 and 1559 cm<sup>-1</sup> in graph were assigned for Benzene, C-H bond of Carbon hydrogen, C-O bond of Carbon oxygen, O-H bond of phenol and N-H bond of Amine respectively based on their standard reference values as per mentioned in table-2 .

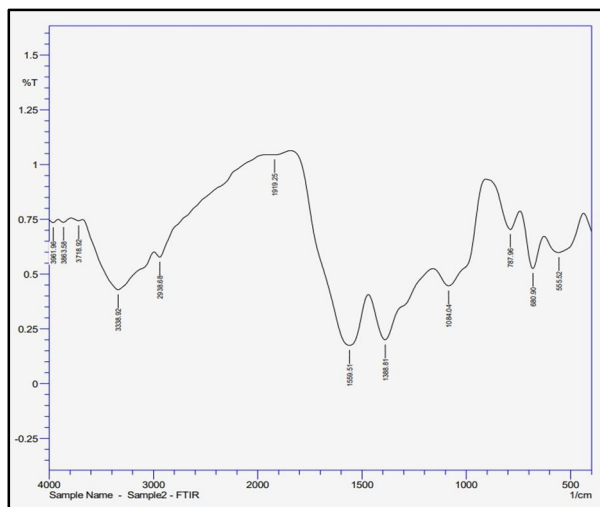


Fig-3: FTIR of synthesized T. procumbens linn SNPs showing transmittance at different bond

| Sr.no | Name of compound      | Reference Value  | Observed value |
|-------|-----------------------|------------------|----------------|
| 1.    | Benzene               | 680 - 720 cm-1   | 680 cm-1       |
| 2.    | Carbon hydrogen (C-H) | 760 - 800 cm-1   | 787cm-1        |
| 3.    | Carbon oxygen (C-O)   | 1050 – 1085 cm-1 | 1084cm-1       |
| 4.    | Phenol (O-H)          | 1330 - 1420 cm-1 | 1388cm-1       |



|    |             |   |          |
|----|-------------|---|----------|
| 5. | Amine (N-H) | - | 1559cm-1 |
|----|-------------|---|----------|

Table-2: FTIR analysis of T. procumbens linn SNPs of different bonds

### 3.4 Antifungal activity against A. niger



After 48 hours

After 72 hours

After 96 hours

Fig-4: Effect of Experimental drug Fluconazole (Control) on Aspergillus Niger after 48, 72, 96 hours



After 48 hours

After 72 hours

After 96 hours

Fig-5: Effect of Tridax procumbens Linn SNPs on Aspergillus Niger after 48, 72 and 96 hours

The antifungal activity of AgNPs synthesized using T. procumbens Linn extract was tested against Aspergillus niger spp. Which was isolated from onion and compared its potency against a reference drug fluconazole. Different concentrations of AgNPs were used (1000, 750, 500 and 250ppm). In addition to this a separate control (10%DMSO) was tested. The results of the inhibition zones of the AgNPs at different concentrations are demonstrated in (Table no.3). As expected, control pate exposed with 10% DMSO up to 96 hrs. does not show any inhibitory effect this result supports the choice of solvent for preparing TpAgNP solution was perfect.

The inhibition zones of different concentrations TpAgNPs ranged from  $10.67 \pm 0.47$ mm to  $10.13 \pm 0.66$ mm against a reference drug fluconazole  $8.27 \pm 0.52$  mm to  $10.13 \pm 0.66$  mm after 48hrs of incubation. However, inhibition zone of a reference drug fluconazole was significantly reducing from  $8.27 \pm 0.52$  mm to 0.00mm in well with concentration of 250ppm,  $10.33 \pm 2.62$  to  $7.77 \pm 0.56$  in 500ppm,  $10.33 \pm 0.47$  to  $7.73 \pm 0.38$  in 750ppm and  $10.13 \pm 0.66$  to  $9.33 \pm 0.47$  in 1000ppm concentration after 72hrs. On the other hand, TpAgNPs showed reduction of diameter from  $10.67 \pm 0.47$  to  $10.27 \pm 0.25$  in concentration of 250ppm. But surprisingly zone of inhibition in rest of the concentration i.e. 500, 750 and 1000ppm was significantly increased from  $11.67 \pm 0.47$  to  $12.13 \pm 0.12$  in 500ppm,  $13.33 \pm 0.94$  to  $14.33 \pm 0.29$  in 750ppm and  $16.07 \pm 2.07$  to  $22.67 \pm 0.47$  in 1000ppm concentration. After 96hrs inhibition diameter of all four concentrations of a reference drug fluconazole was reduced to 0.00mm in contrast with a reference drug fluconazole plate added with TpAgNPs were still retain significant inhibition diameter i.e.  $9.67 \pm 1.25$  in 250ppm,  $11.33 \pm 2.49$  in



500ppm,  $12.33 \pm 1.70$  in 750ppm and  $14.67 \pm 3.30$  1000ppm, we observed that the inhibition diameters decreased with a decrease in the concentration of the AgNPs, which may indicate that the synthesized AgNPs are dose-dependent. Moreover, it is well known that the well or disk diffusion tests are used as the preliminary study in screening the antimicrobial activity of an antimicrobial agent (Loo Y.Y et. al., 2018) therefore, further investigation in determining the long-term antifungal activity of TpAgNPs using MIC and MFC is needed.

| Sr. No | Concentration      | 250ppm           | 500ppm           | 750ppm           | 1000ppm          |
|--------|--------------------|------------------|------------------|------------------|------------------|
|        | Time of Incubation |                  |                  |                  |                  |
| 1      | Testdrug48hrs      | $8.27 \pm 0.52$  | $10.33 \pm 2.62$ | $10.33 \pm 0.47$ | $10.13 \pm 0.66$ |
| 2      | TpAgNPs48hrs       | $10.67 \pm 0.47$ | $11.67 \pm 0.47$ | $13.33 \pm 0.94$ | $16.07 \pm 2.07$ |
| 3      | Testdrug72hrs      | $0.00 \pm 0.00$  | $7.77 \pm 0.56$  | $7.73 \pm 0.38$  | $9.33 \pm 0.47$  |
| 4      | TpAgNPs72hrs       | $10.27 \pm 0.25$ | $12.13 \pm 0.12$ | $14.33 \pm 0.29$ | $22.67 \pm 0.47$ |
| 5      | Testdrug96hrs      | N.A.             | N.A.             | N.A.             | N.A.             |
| 6      | TpAgNPs96hrs       | $9.67 \pm 1.25$  | $11.33 \pm 2.49$ | $12.33 \pm 1.70$ | $14.67 \pm 3.30$ |

Table no.3: Effect of different concentrations of TpAgNP growth of *A. Niger* against test drug (Fluconazole) at different time interval. (mean  $\pm$  SD (N=3))

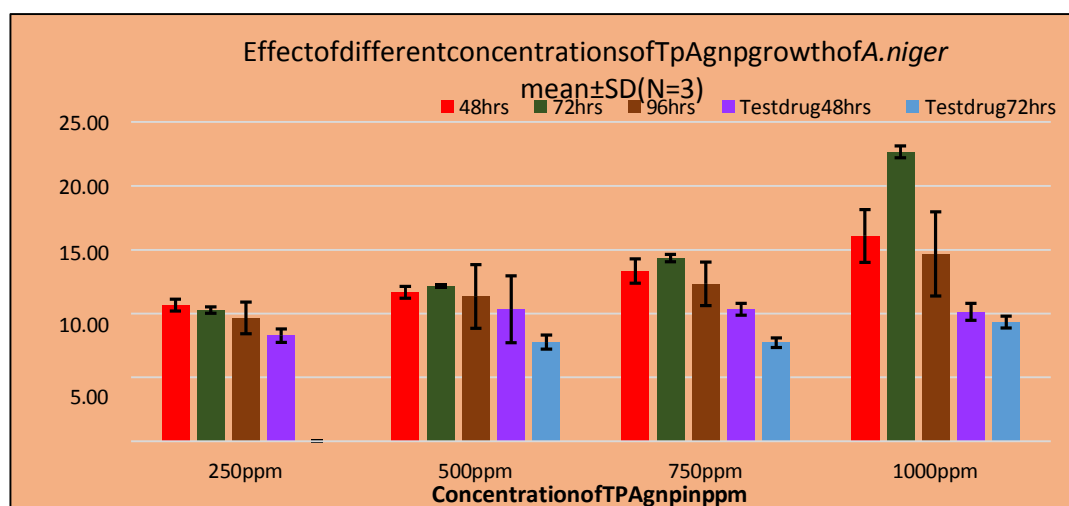


Fig-6. Graph showing zone of inhibition at different concentrations of TPAgNP (well diffusion method)

### 3.5 Relation to previous studies

The present observations agree in general with literature describing antimicrobial activity of plant-mediated AgNPs. Ahmed et al. (2016) emphasized the role of plant biomolecules in green synthesis and the broad antimicrobial relevance of AgNPs. Bhati-Kushwaha (2014) specifically reported *T. procumbens*-mediated AgNP synthesis and antimicrobial testing that included *Aspergillus* species. Pungle et al. (2022) also demonstrated antimicrobial properties of *T. procumbens*-derived AgNPs, although their synthesis and biological models differed from those of the present work. The current study therefore contributes a separate dataset focused on *A. niger* under the described well-diffusion conditions.

The diffusion assay is a preliminary screening method. The persistence of measurable TpAgNP inhibition through 96 hrs is notable within this experimental model, but additional quantitative testing is required to establish MIC and MFC values. Likewise, detailed particle morphology, elemental composition, surface chemistry, toxicity and mechanism of antifungal action were not fully established by the experiments reported in the supplied project.



#### IV. CONCLUSION

The study established a plant-mediated route for synthesis of silver nanoparticles using an aqueous extract of *T. procumbens* Linn. The yellow-to-brown colour transition after 48 hrs, the broad UV-Visible absorption peak at 430 nm, the reported XRD observations and FTIR bands together provided characterization evidence for the synthesized TpAgNP preparation. A particle size of less than 50 nm and identifies FTIR bands associated with several functional groups that may participate in nanoparticle surface stabilization.

TpAgNPs showed measurable in vitro antifungal activity against *A. niger* at 250–1000 ppm. The largest recorded inhibition zone was  $22.67 \pm 0.47$  mm at 1000 ppm after 72 hrs. The 10% DMSO control showed no inhibitory effect under the reported conditions. The results support further investigation of *T. procumbens*-mediated AgNPs as a source of antifungal activity. However, MIC/MFC determination, detailed structural and morphological characterization, toxicity evaluation, mechanistic studies and appropriately designed in vivo investigations are required before any therapeutic implication can be considered.

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