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Characterisation and synthesis of agnps from *Aristolochia bracteolata* leaves

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Abstract
Objective: The objective of this research was to present the antimicrobial potential of AgNP's synthesised from *Aristolochia bracteolata* leaves extract.
Methods: Evaluation of antimicrobial activity of the leaves extract was tested using agar well diffusion method along with the commercial control antibiotics (Ciprofloxacin) and the synthesised AgNPs were also tested against several microbial cultures (MTCC 10619, MTCC 0424, MTCC 872 & MTCC 1755). Further phytochemical analysis and characterisation of the synthesised nanoparticles was done to determine the shape and size of synthesised nanoparticles.
Results: Prepared methanolic extract of *Aristolochia bracteolata* showed significant antibacterial activity against chosen microbial cultures (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Fusarium oxysporum* and *Aspergillus niger*) showing gradual increase in inhibition zone as the extract concentration increased from 20µl to 80µl. *Bacillus subtilis* among the bacterial strain and *Aspergillus niger* among the chosen fungal strain showed the maximum inhibition at 80 µl 12.7 ±1.729 mm and 15.7 ±1.729 mm respectively. But in case of AgNP's Bacillus subtilis showed 14 ±1.132 mm at 80µl as compared to *Pseudomonas aeruginosa* 8 ±1.132 mm at 80µl. In case of fungal strains Aspergillus niger showed max. Inhibition 15.7 ±1.729 mm for methanolic extract of leaves and for AgNP's solution the max. Inhibition was showed by *Fusarium oxysporum* 9 ±1.132 mm at 80µl. In phytochemical analysis the presence of tannin and phenol was noted whereas alkaloids were absent. SEM revealed the smooth and spherical structure of the nanoparticles.
Conclusion: *Aristolochia bracteolata*-derived AgNP's presented an effective approach for antimicrobial applications, demonstrating efficiency against a range of pathogens.

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Keywords: *Aristolochia bracteolata,* antimicrobial activity, phytochemicals, Silver nanoparticles (AgNP's)

Introduction

The geographical distribution of India, extends from the Himalayas in the north to the Western Ghats in the south, and from the deserts of Rajasthan to the rainforests of the Northeast, maintains a vast array of plant species with medicinal properties. This abundance of plants has long been the foundation of traditional medicine and recently attracted attention from modern science and global pharmaceutical industries. (Bharathi, A., & Singh, R. H; 2022) [14].

Recent progress in medicine have increasingly highlighted the therapeutic potential of medicinal plants, bringing together traditional knowledge and modern scientific research including the phytochemical profiling of plants, bioactivity screening of extracts and compounds, targeted drug discovery, biotechnological approaches for enhanced production, standardization and quality control measures, evidence-based clinical trials, personalized medicine applications, development of combination therapies, and the integration of herbal medicine into integrative healthcare practices.

(Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016) [9]; Atanasov, A. G., *et. al.*, 2015 [10]; Wink, M. (2015) [11]; Newman, D. J., & Cragg, G. M. (2020) [12]; Efferth, T., & Koch, E. (2011) [13].

In the 21st century we have seen immense progress in the medicinal field, now scientists are focused on combining more and more traditional medicine with nanotechnology. Biology offers nanotechnology based models and bio-assembled nanotechnology tools, while nanotechnology provides the instruments and technological platform for the exploration and transformation of biological processes. Nano-biotechnology is defined as a field that uses biological principles and materials to produce new devices and systems that are integrated from the nanoscale and that applies nanoscale concepts and techniques to understand and modify bio systems (both living and non-living) The ability to measure at the subcellular level and the knowledge of the cell as a highly organised, self-repairing, self-replicating, information-rich molecular machine have both seen significant advancements, Smalley divided nanotechnologies into wet and dry categories, with the first category describing living biological systems and the second focusing on nanoscale structures built by humans (D. Mubarak Ali *et.al.*).

Aristolochia bracteolata, commonly known as "Indian birthwort," holds significant medicinal importance in traditional systems of medicine due to its wide range of healing properties (Singh, S. K., & Pandey, V. B.; 2018) [4]. It is distributed throughout the India, this plant species has been used for centuries in Ayurveda to treat anti-inflammatory, analgesic, antimicrobial, and antioxidant effects (Joshi, V., & Jain, A.; 2019) [5]. The medicinal activities of *A. bracteolata* is due to its rich phytochemical composition which includes alkaloids, flavonoids, terpenoids, and phenolic compounds (Pillaiyar, T., *et. al.*, 2017) [6]. Although its traditional uses are well documented, recent scientific research has provided valuable information of its pharmacokinetics and therapeutic potential, opening the way for further research and the development of new drugs (Ahsan, W., *et.al.*, 2021) [7]. However, it is important to identify the potential toxicity associated with some aristolochic acid compounds found in some Aristolochia species, which calls for caution in pharmacokinetics (Vanherweghem, J. L., & Depierreux, M.; 1995) [8].

Silver nanoparticles (AgNPs) have gained attention in recent years due to their unique physical and chemical properties and broad range of applications in different fields which includes medicine, electronics, catalysis, environmental remediation and many more. These nanoparticles are usually synthesized through physical, chemical, or biological methods, with each approach offering advantages in terms of particle size, shape, stability, and scalability (Suresh, A. K. *et.al.*,). In medicine, AgNPs has shown promising results of antimicrobial properties against a wide variety of pathogens, including bacteria, viruses, and fungi, making them potential substance to be used for the development of novel antimicrobial agents and wound dressings (Morones, J. R. *et.al.*). AgNPs have shown potential in cancer therapy as they can specifically target tumor cells while minimizing the damage to the healthy tissues, primarily through mechanisms such as induction of oxidative stress, DNA damage, and apoptosis (Xiu, Z. M. *et.al.*). Additionally, AgNPs have been explored for their anti-inflammatory, antioxidant, and wound healing properties, suggesting their potential utility in the treatment of various inflammatory and degenerative diseases (Zhang, X. F. *et.al.*). However, concerns regarding the potential cytotoxicity, environmental impact, and development of antimicrobial resistance associated with AgNPs necessitate further research to elucidate their safety profile and optimize their therapeutic applications (Jena, P., & Mohanty, S.; 2012).

Fig 1: *Aristolochia bracteolata*

Material and Methods

The current study, "Characterisation and synthesis of AgNPs from *Aristolochia bracteolata*," was carried out at the Department of Botany at Career Point University in Kota (Rajasthan). The materials utilised, methodology used, experiments, microbial culture used and techniques are detailed below.

Chemicals and solvents

The information of all materials and chemicals used in the present study are tabulated in Table below-

Table 1: List of the chemicals used during the experiment

Chemicals	Used for
Silver nitrate ($AgNO_3$)	Silver nanoparticles formation
PBS (phosphate buffer solution)	For preparing of suspension of fungal spores
Mueller Hinton agar no. 2	Determination of Antibacterial property
Sabouraud's dextrose agar, (SDA)(sub culturing)	Determination of Antifungal property
Dimethyl Sulphoxide (DMSO)	Dilution of plant extract
Ciprofloxacin	Antibacterial drug
Ketoconazole	Antifungal drug
Whatman's filter paper	$AgNO_3$ solution filtration

Preparation of plant extract

Plant leaves were washed thoroughly with tap water 2-3 times then the leaves were again washed with distilled water. The washed leaves were then air dried. The powdered leaves were thoroughly blended with 100 millilitres of distilled water. After that solution was filtered by using (Whatman Number one) filtered paper.

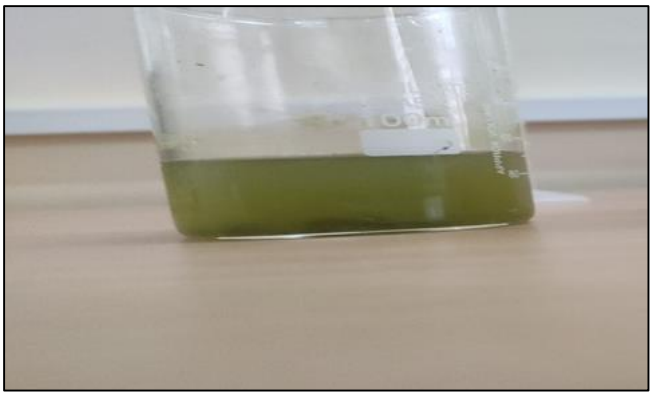

Fig 2: Leaves extract

The basic information of the plant samples used in the present study are tabulated in Table below-

Table 2: General characteristics of the plants used in experiment Antibacterial assessment

Characteristics	*Aristolochia bracteolata*
Location	Mali to Arabian Peninsula and Tanzania, Indian Subcontinent to Myanmar.
Temperature	20 and 29 °C (68 and 84 °F)
Soil type	Sandy or lava soil
Propagation	trap-and-release mechanisms
Plant type	herb
Life span	perennial
Benefits	used in the treatment of colic, amenorrhoea, dysmenorrhoea, intermittent fever and worms
Common pests and diseases	Leaf blight is observed in the plantation during the winter season.

The *in vitro* antibacterial activity of the sample against *Bacillus subtilis (MTCC 10619), Pseudomonas aeruginosa (MTCC 0424)*, was evaluated using the agar well diffusion method (Perez *et.al.*, 1990). Mueller Hinton agar no. 2 (Hi-Media, India) was used as the bacteriological medium. The extracts were diluted in Dimethylsulphoxide (DMSO) at 10 mg/mL concentrations. A standardized inoculum (1.5×108 CFU/mL, 0.5 McFarland) was added aseptically to the molten agar and poured into sterile Petri dishes to form solid plates. Wells were created in the seeded agar plates. The test compound (20, 40, 60, and 80 µl) was placed in the wells (6 mm). The plates were then incubated overnight at 37ºC. The antimicrobial spectrum of the extract was determined by measuring the zone sizes around each well for the bacterial species. The diameters of the inhibition zones produced by the extract were compared with those of the commercial control antibiotics (Ciprofloxacin). To establish the Minimum Inhibitory Concentration (MIC), the lowest concentration of the sample in µl showing inhibitory activity against pathogenic bacterial strains was determined. The experiment was repeated three times to minimize errors, and the average values were recorded.

Antifungal assessment

Anti-fungal activity of the experimental sample was investigated by agar well diffusion method (Bonjar *et.al.*, 2005). The fungi were sub-cultured onto Sabouraud's dextrose agar, (SDA) and respectively incubated at 37°C for 24 h and 25°C for 2-5 days. Suspensions of fungal spores were prepared in sterile PBS (phosphate buffer solution) and adjusted to a concentration of 106 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. 6 mm well were punctured in the culture media using gel puncture. 20, 40, 60, 80 µL of several dilutions of sample was administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in mm). Ketoconazole is used as antifungal drug (control). To identify MIC, minimum concentration of sample in µl were used that show inhibitory activity against pathogenic fungal strains - *Aspergillus niger (MTCC 872)*, and *Fusarium moniliforme (MTCC 1755).*

Nanoparticle formation

Plant part mediated AgNPs was synthesized by using Loo *et.al.*, 2012 method. 12 millilitres of filtrate was stirred properly with 100 millilitres of 1mM $AgNO_3$, and was cautiously heated for 60 seconds. Then continuously stirred with the help of magnetic stirrer. Colour changes of the solution were observed.

Preliminary phytochemical screening of secondary metabolites

1. Tannin

Fecl3 test: 2 ml plant extract was taken in a test tube. Add few drops of 0.1% FeCl3 solution. Formation of blue green, blackish green color or precipitate indicated the presence of tannin (Trease and Evans, 1989).

2. Alkaloids

Mayer's reagent: Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5 g) in water (100 ml).

Mayer test: 2ml of plant extract was taken in a test tube. Add 5 ml of 1% aqueous HCl and 100µl or 4-5 drops freshly prepared Mayer reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids (Sofowora, 1993).

3. Phenol

A few drops of 10% ferric chloride solution were added into 2ml of the extract; the appearance of bluish green/black colour indicated the presence of phenol (Shalini and Sampathkumar, 2012).

Characterisation of nanoparticles

Characterization of nanoparticles is essential to understand their properties, behaviour, and potential applications.

Scanning electron microscope

The sample was in the powdered form and the voltage used was 5.0Kv and was carefully placed on the SEM stub along with the copper grid. A working distance of 8.1 mm means that the final lens of the SEM is positioned 8.1 millimetres away from the surface of the sample. This distance can vary depending on the specific parameters set for the SEM imaging, such as the magnification level and the desired depth of focus. The image was magnified 80,000 times

compared to the actual size of the sample

Result

Table 3: Anti-bacterial results of *Aristolochia bracteoata* leave methanol extract

S. No	Bacterial Strains	Code	Inhibition Zone(std.)	20µl	40µl	60µl	80µl
1	*B.subtilis*	(MTCC 10619)	41	9.3333 ±1.729	9.6667 ±1.729	12.3333 ±1.729	12.6667 ±1.729
2	*P. aureginosa*	(MTCC 0424)	35	0	0	0	8 ±1.132

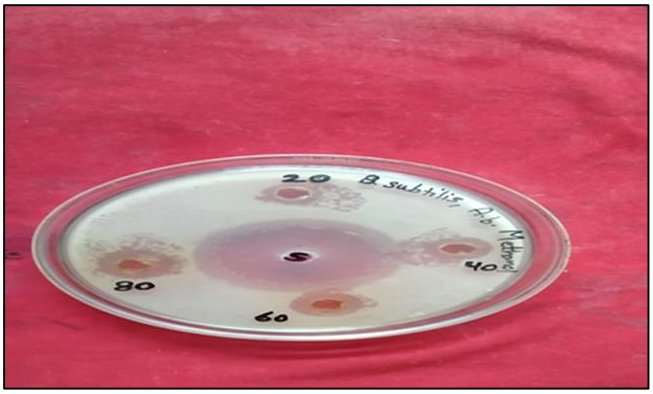

Fig 3: Effect of methanolic leaves extract of *Aristolochia bracteolata* on *B. subtilis*

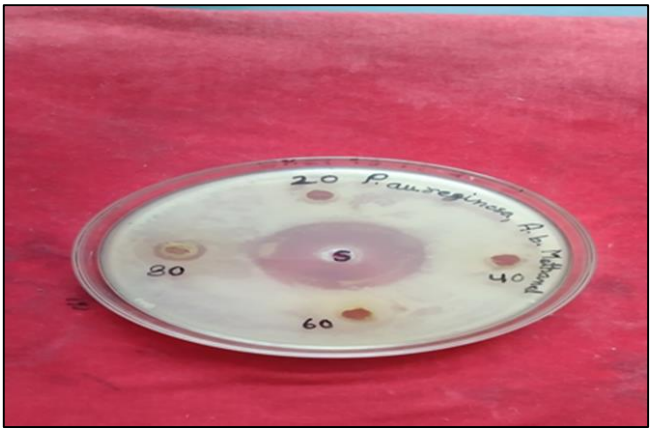

Fig 4: Effect of methanolic leaves extract of *Aristolochia bracteolata* on *P. aureginosa*

Table 4: Anti-bacterial results of AgNP's of *Aristolochia bracteoata*

S. No	Bacterial Strains	Code	Inhibition Zone(std.)	20µl	40µl	60µl	80µl
1	*B.subtilis*	(MTCC 10619)	41	9.3±0.653	10.7 ±0.653	12.7 ±1.729	14 ±1.132
2	*P. aureginosa*	(MTCC 0424)	35	0	0	0	8±1.132

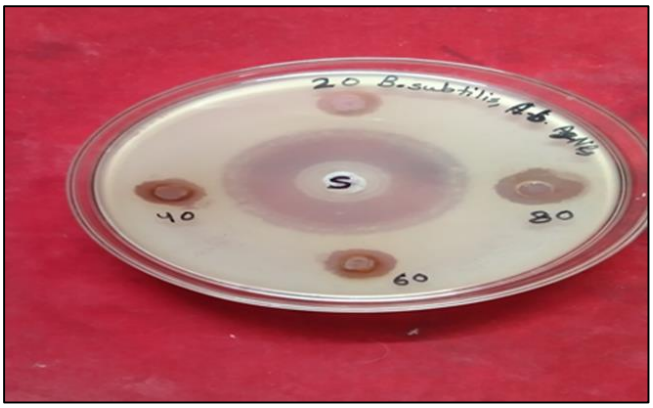

Fig 5: Effect of AgNP's solution of *Aristolochia bracteolata* on *B. subtilis*

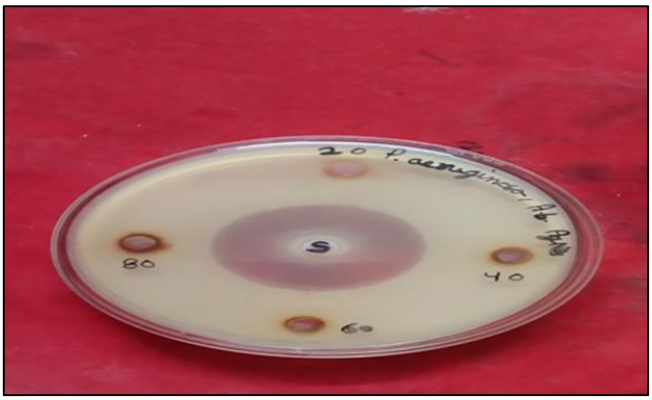

Fig 6: Effect of AgNP's solution of *Aristolochia bracteolata* on P.aureginosa

Table 5: Anti-fungal results of *Aristolochia bracteoata* leave methanol extract

S. No	Fungal Strains	Code	Inhibition Zone(std.)	20µl	40µl	60µl	80µl
1	*Fusarium oxysporum*	(MTCC 1755)	22	0	0	0	10 ±1.132
2	*Aspergillus niger*	(MTCC 872)	23	0	0	0	15.7 ±1.729

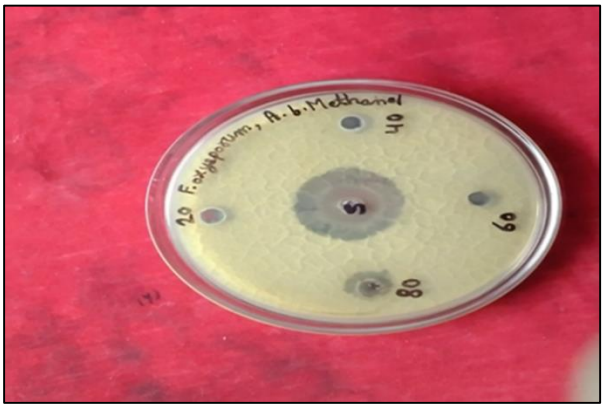

Fig 7: Effect of methanolic leaves extract of *Aristolochia bracteolata* on *F. oxysporum*

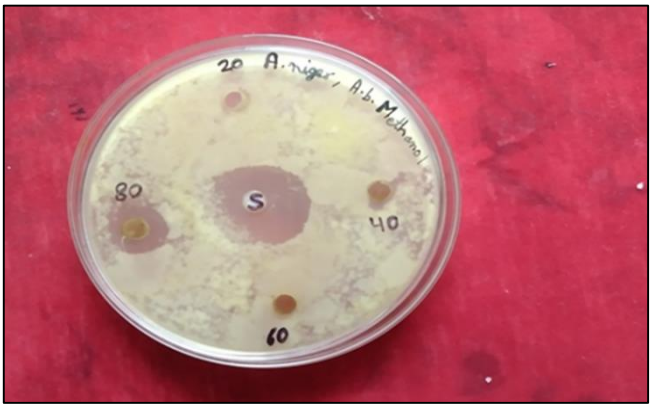

Fig 8: Effect of methanolic leaves extract of *Aristolochia brateolata* on *A. niger*

Table 6: Anti-fungal results of AgNP's of *Aristolochia bracteoata*

S. No	Fungal Strains	Code	Inhibition Zone(std.)	20µl	40µl	60µl	80µl
1	*Fusarium oxysporum*	(MTCC 1755)	22	0	7±2.263	7.7 ±2.356	9 ±1.132
2	*Aspergillus niger*	(MTCC 872)	21	0	7.3 ±0.653	7.9 ±1.246	8.6 ±0.623

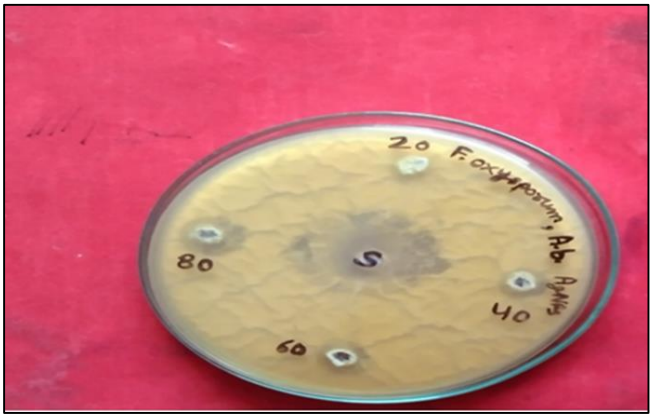

Fig 9: Effect of AgNP's solution of *Aristolochia bracteolata* on *F. oxysporum*

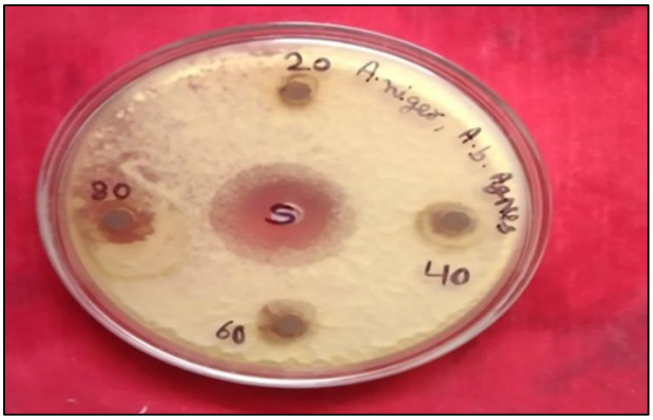

Fig 10: Effect of AgNP's solution of *Aristolochia bracteolata* on *A. niger*

Table 7: Preliminary phytochemical screening of secondary metabolites

Phytochemical compounds	*Aristolochia bracteolata*
Tannin	+
Alkaloids	-
Phenol	+

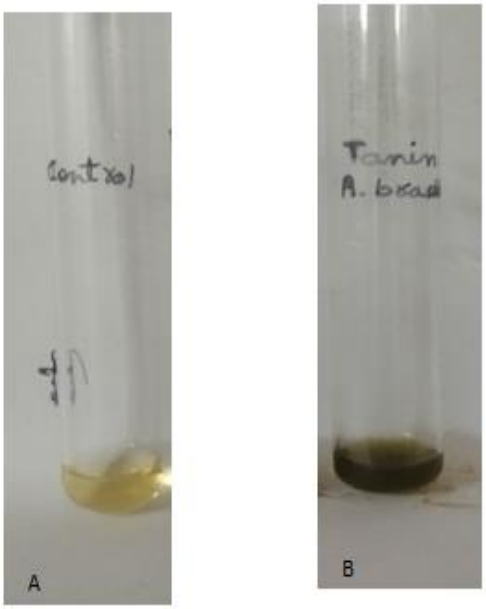

Fig 11: A) Tannin test control, B) *Aristolochia bracteolata*

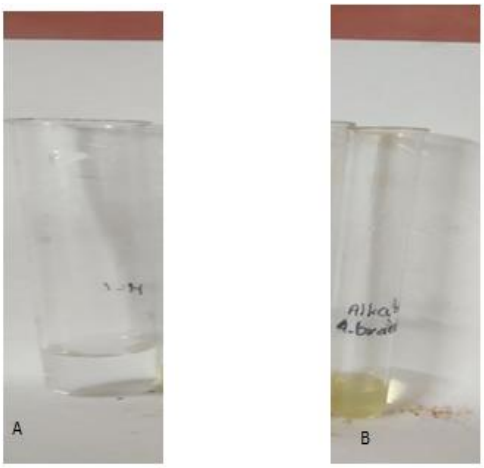

Fig 12: A) Alkaloid control, B) *Aristolochia bracteolata*

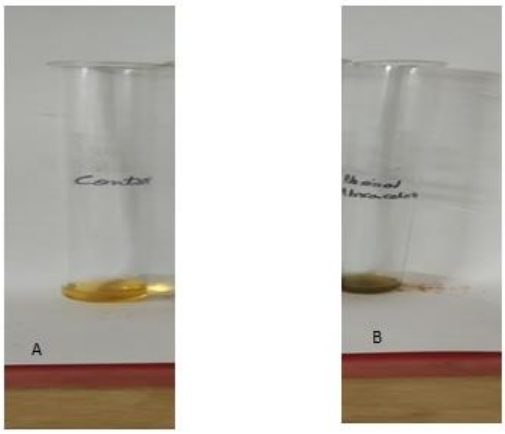

Fig 13: A) Phenol test control, B) Aristolochia bracteolate

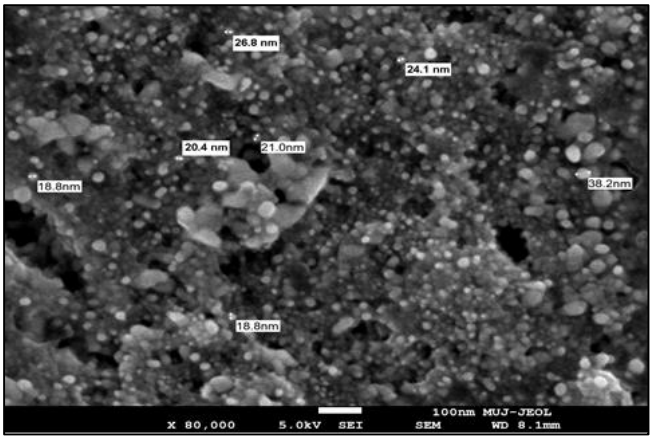

Fig 14: SEM image of spherical Ag nanoparticles from *Aristolochia bracteolata* leaves

Discussion

The phytochemical analysis revealed the presence of phenol and tannins and absence of phenol in leaf extract of *Aristolochia bracteolata*. Previous investigations corroborated these findings, highlighting the existence of alkaloids, tannins, phenols, flavonoids, glycosides, lignin, and saponins in the aqueous leaf extract (Angala Parameswari, *et.al.*, 2011).

In vitro antibacterial assays conducted with the aqueous extracts from *A. bracteolata* leaves exhibited significant inhibitory effects against *B.subtilis and P. aeruginosa* compared to Ciprofloxacin, antibacterial drug used as a positive control. *In-vitro* antifungal assay was tested against *F. oxysporum* and *A. niger* compared to Ketoconazole antifungal drug used as a positive control.

Among the tested microorganisms, *A. niger* exhibited heightened sensitivity to the extracts. Specifically, *A. niger* displayed the highest susceptibility, with inhibition zones measuring 15.7 ±1.729 for methanolic leaf extract (0.5g/10ml).

The presence of secondary metabolites such as tannins, phenols, is implicated in the observed antibacterial and antifungal activity, consistent with their known bioactive properties.

Conclusion

We can conclude that, the data showed that both the concentration of antibacterial agents and the specific bacterial strain being targeted have a significant impact on the size of inhibition zones. This suggests that adjusting the concentration of antibacterial agents as well as antifungal agents can enhance their effectiveness against certain bacterial and fungal strains. It was reported earlier as well that this property of the leaves extract is due to the presence of phytochemicals.

Conflicts of Interests

All authors have none to declare

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References

1. Shirwakar A, *et al*. Wound healing studies of *Aristolochia bracteolata* Lam. with supportive action of antioxidant enzymes. *Phytomedicine*. 2003;10(6-7):558-62. doi: 10.1078/094471103322331548.
2. Abdelgadir AA, Ahmed EM, Eltohami MS. Isolation, characterization and quantity determination of aristolochic acids, toxic compounds in *Aristolochia bracteolata* L. *Environmental Health Insights*. 2011;5:1-8. https://doi.org/10.4137/EHI.S6292.
3. Parameswari A, Madhusudhanachetty C, Chandrasekar KB. International Journal of Research in Phytochemistry & Pharmacology. 2011;1(2):83-87.
4. Singh SK, Pandey VB. *Aristolochia bracteolata*: Traditional uses, phytochemistry and pharmacology. Chinese Journal of Natural Medicines. 2018;16(7):503-511.
5. Joshi V, Jain A. Evaluation of anti-inflammatory and analgesic activities of *Aristolochia bracteolata* Lam. leaves. Pharmacognosy Research. 2019;11(4):331-334.
6. Pillaiyar T, Manickam M, Jung SH, Jung SH. Recent development of signaling pathways inhibitors of aromatase and lipoxygenase to overcome multidrug resistance in cancer treatment. Bioorganic & Medicinal Chemistry Letters. 2017;27(6):1172-1185.
7. Ahsan W, Arif M, Ejaz A, Alhazmi HA, Asif M. *Aristolochia bracteolata*: A review on its ethnobotanical, phytochemical, and pharmacological aspects. Acta Scientifica Naturalis. 2021;8(1):27-38.
8. Vanherweghem JL, Depierreux M. [Pathological aspects of a newly described nephropathy related to the prolonged use of Chinese herbs]. Annales de Pathologie. 1995;15(4):265-269.
9. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. Journal of Nutritional Science; 2016:5.
10. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, *et al*. Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnology Advances.

2015;33(8):1582-1614.
11. Wink M. Modes of action of herbal medicines and plant secondary metabolites. *Medicines*. 2015;2(3):251-286.
12. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products. 2020;83(3):770-803.
13. Efferth T, Koch E. Complex interactions between phytochemicals: the multi-target therapeutic concept of phytotherapy. Current Drug Targets. 2011;12(1):122-132.
14. Bharathi A, Singh RH. Biodiversity and conservation of medicinal plants in India. Current Science. 2022;122(5):619-627.