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Anti Cariogenic and Anti Inflammatory Activity of *Achyranthes Aspera* Mediated Silver Nanoparticles

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ABSTRACT

Introduction: Nanotechnology is considered the sector of research and innovation that aims at building things, systems, devices, materials at a scale of atoms and molecules. Nanotechnology is an incredibly growing field in the era of modern science. Silver nanoparticles are known to have good pharmacological properties. Green synthesis of silver nanoparticles eliminates toxicity and is also without side effects. On the other hand *Achyranthes aspera* is an annual herb that belongs to the family of *Amaranthaceae*. Every part of the plant has medicinal values. Our study deals with the green synthesis of *Achyranthes aspera* mediated silver nano particles and assessment of their anti cariogenic and anti inflammatory properties.

Aim: The current study aims at assessing the anti cariogenic and anti inflammatory properties of *Achyranthes aspera* mediated silver nano particles and investigating the efficacy of *Achyranthes aspera* mediated Silver nano particles.

Materials and Methods: The methodology includes Green synthesis of *Achyranthes aspera* mediated Silver nano particle synthesis followed by tests for anti cariogenic and anti inflammatory properties.

Results: Both the anti cariogenic and anti inflammatory activities of the*Achyranthes aspera* mediated silver nano particles had shown a proportionate increase in activity with the increase in µl concentration.

Conclusion: *Achyranthes aspera* mediated silver nano particles have shown significant anti inflammatory and anti cariogenic activity and they are considered as potent anti inflammatory and anti cariogenic agents.

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INTRODUCTION

Nanotechnology is considered the sector of research and innovation that aims at building things, systems ,devices, materials at a scale of atoms and molecules (Rajeshkumar *et al.*, 2018). Nanotechnology is an incredibly growing field in the era of modern science.. It aims at the design, characterization, manufacture of materials, devices and systems, by controlled manipulation of size and shape at the scale of the nanometer, producing structures that have one or more novel or superior properties (Nandhini, Rajeshkumar and Mythili, 2019). Nanotechnology is a booming research industry that forms an important component of each and every aspect of life ranging from cosmetics to advanced biotechnological approaches(Vairavel, Devaraj and Shanmugam, 2020) . The number of nano tech industries are growing every year that aim at producing better products by repairing or manufacturing the smallest components of the product at low costs (M. Gomathi *et al.*, 2020). It synthesizes and manufactures materials at the nanoscale level which is 1 to 100 nanometers. At the nanoscale level the particles, atoms and molecules exhibit unusual and unexpected properties that cannot be explained by the classical laws of physics and chemistry (Rajasekaran *et al.*, 2020). Of different types of nanoparticles, Noble metal nanoparticles are of great importance in the field of medicine as they help in formulation of newer drugs of pharmacological value (Santhoshkumar *et al.*, 2019). There are different types of noble metal nanoparticles known such as gold, silver, platinum, palladium,selenium, zinc oxide, iron and copper nanoparticles (R *et al.*, 2020).

KEYWORDS:
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In recent times silver nanoparticles are becoming the focus of interest of researchers as they have got exemplary optical, electrical, thermal, chemical and biological properties (Saravanan *et al.*, 2018). These unique properties make them eligible for use in material, optical, biomedical, anti microbial and nanotoxicological studies(Gheena and Ezhilarasan, 2019). Basically, silver nanoparticles are made up of silver oxide. They usually range between 1 to 100 nm size(Christopher, Roy and Rajeshkumar, 2021).They have a large surface to bulk silver atom ratio. The increased surface area permits its coordination with various other ligands. They are known to be used in diagnosis, drug delivery and treatment of various diseases (Ezhilarasan, Sokal and Najimi, 2018). Its well documented antimicrobial properties makes it the choice of nanoparticle used in the field of medicine and pharmacology (Rajeshkumar, 2016). Its mechanism of action at various levels of cellular attachment is noted (Ezhilarasan, 2018). Silver atom when attaches to the cell wall of bacteria, simply blocks the transport of materials in and out of the cell(Vanaja *et al.*, 2014; Niyas *et al.*, 2017; Rj *et al.*, 2017). They then cause unwinding of DNA and further alter the hydrogen bonding causing interference to bacterial cell wall synthesis and cell division (A. C. Gomathi *et al.*, 2020) . Though silver nanoparticles possess great pharmacological properties, their toxic nature, short longevity caused by particle aggregation and high cost of manufacture serve as obstacles in the large-scale chemical production of the nanoparticles(Sivaji, Asha and Rajeshkumar, 2017). This has paved way for the evolution of green synthesis of silver nanoparticles (Dua *et al.*, 2019). The green method sustains non toxicity by eliminating the production of hazardous materials (Rajeshkumar, 2017). In our study, the green process of silver nanoparticle synthesis is mediated by aqueous leaf extracts of the plant called *Achyranthes aspera(Ezhilarasan, Apoorva and Ashok, 2019)* (Danda, Krishna, *et al.*, 2010) (Ramadurai *et al.*, 2019) (Sathivel *et al.*, 2008) (Panda *et al.*, 2016) (Neelakantan *et al.*, 2012) (Govindaraju, Neelakantan and Gutmann, 2017) (Sekhar, Narayanan and Baig, 2001) (DeSouza *et al.*, 2014) (Nasim *et al.*, 2010) (Danda, Muthusekhar, *et al.*, 2010) ('Molecular structure and vibrational spectra of 2,6-bis(benzylidene)cyclohexanone: A density functional theoretical study', 2011) (Putchala *et al.*, 2013) (Neelakantan, Grotra and Sharma, 2013) (Suresh *et al.*, 2014). *Achyranthes aspera* is an annual herb that belongs to the family of *Amaranthaceae (Ramesh et al., 2018)*. It is commonly called as Indian prickly chaff flower (Arumugam, George and Jayaseelan, 2021). The plant itself has been used as folk medicine in countries like India, Australia and Kenya (Joseph and Prasanth, 2021). All the parts of the plant, its seed, root, shoot and leaf are of medicinal value (Ezhilarasan, Apoorva and Ashok, 2019). They are found everywhere in lands of tropical and subtropical regions (Duraisamy *et al.*, 2019). In ancient times, the roots of the plant were ground and used as tooth powder and the stems and roots themselves were used as mechanical toothbrushes. The plant juice was used in relieving toothache (Gnanavel, Roopan and Rajeshkumar, 2019).

In our study, the anti cariogenic and anti inflammatory properties of *Achyranthes aspera* mediated silver nanoparticles are studied. Dental caries and Periodontitis are the most prevalent diseases of the oral cavity. Dental caries are most often caused by microorganisms such as *Streptococcus mutans* and *Lactobacillus* species (Markov *et al.*, 2021). In our study, the silver nanoparticles synthesised with aqueous leaf extracts of *Achyranthes aspera* which are greatly known for their antimicrobial properties are tested against the caries causing bacteria. As periodontitis is an inflammatory process, the anti-inflammatory properties of *Achyranthes aspera* mediated silver nanoparticle solution is also evaluated. Therefore, the current study aims at assessing the anti cariogenic and anti inflammatory properties of *Achyranthes aspera* mediated silver nano particles and investigating the efficacy of *Achyranthes aspera* mediated silver nanoparticles.

MATERIALS AND METHODOLOGY

Synthesis of Nanoparticles

1 gram of *Achyranthes aspera* leaf extract was added to 100 ml of distilled water. The solution was heated for 2 minutes. Then 2 milli molar Silver nitrate was added to the solution which was kept in the shaker for nanoparticle synthesis.

Anti cariogenic activity

Anti cariogenic activity of the *Achyranthes aspera* mediated silver nanoparticles were tested against the microorganisms mentioned. The microorganisms used in our study include *S.mutans, E. fecalis, Lactobacillus species* and *C.albicans*. Muller hinton agar plates were utilized for this purpose. They were used in determining the zones of inhibition. The plates were prepared and sterilized for 45 minutes at 120 lbs. Media was poured into the sterilized plates and were let stable for solidification. The wells were cut with the help of a well cutter and the test organisms were swabbed. 25, 50 and 100 μL concentrations of Achyranthes aspera mediated silver nanoparticle solution were incorporated into the wells and the plates were incubated at 37°C for 24h.The zones of inhibition were then measured after the incubation time.

Anti inflammatory activity

2ml of 1% bovine albumin was taken and mixed with the *Achyranthes aspera* extract of different concentrations ranging from 10-50μg/ml. Then distilled water (390-350μl) had been added to the varying concentrations of *Achyranthes aspera* extract (10-50μg/ml). It is then incubated at room temperature for about 10 minutes. The mixture was then heated at 55 degree celsius for 20 minutes in a water bath and was cooled to room temperature. The absorbance value was recorded at 660 nm. An equal amount of *Achyranthes aspera* extract was replaced with DMSO for control. Diclofenac sodium was used as a standard. All the tests were done in triplicate.

RESULTS AND DISCUSSION

Anti cariogenic activity

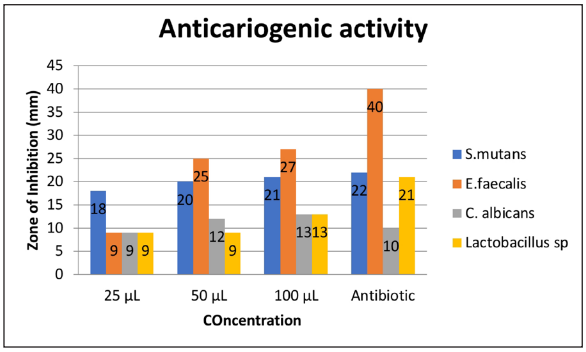


Figure 1: The anti cariogenic activity of Achyranthes aspera mediated silver nanoparticles against S.mutans, E. fecalis, Lactobacillus species and C.albicans, compared against the standard drugs.

Fig. 2: The zone of inhibition of *Achyranthes aspera* mediated silver nanoparticles and the standard drug against *S.mutans*

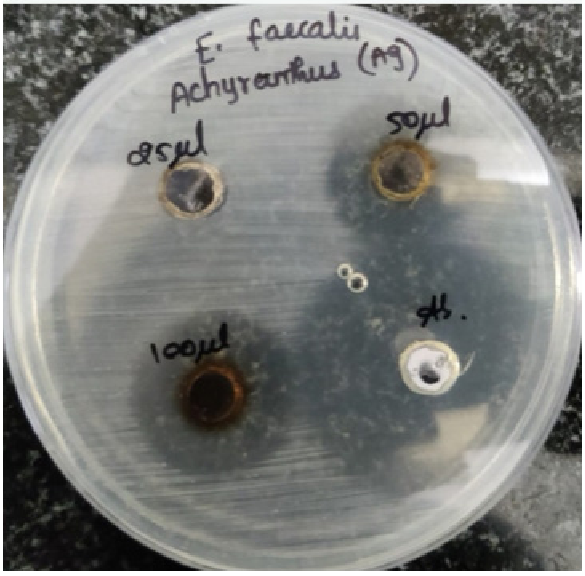


Fig. 3: The zone of inhibition of *Achyranthes aspera* mediated silver nanoparticles and the standard drug against *E.fecalis*

Figure 4: The zone of inhibition of *Achyranthes aspera* mediated silver nanoparticles and the standard drug against *C.albicans*

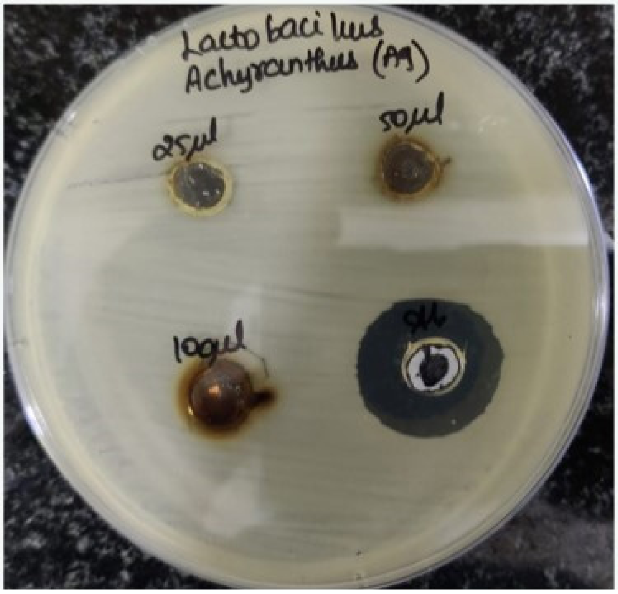


Figure 5: The zone of inhibition of *Achyranthes aspera* mediated silver nanoparticles and the standard drug against *Lactobacillus species*

The figure 1 shows the Anti cariogenic activity of *Achyranthes aspera* mediated silver nanoparticles against the test microorganisms namely *S.mutans, E. fecalis, Lactobacillus species* and *C.albicans*. The zone of inhibition of the nanoparticle solution against *S.mutans* at 25µl concentration is 18mm, at 50µl concentration is 20mm and at 100µl concentration is 21mm. The standard drug for the same had exhibited 22mm zone of inhibition (Figure 2). Therefore at the highest concentration, at 100µl concentration, the difference between the zone of inhibitions of the standard drug and *Achyranthes aspera* mediated silver nanoparticles is just 1mm. Similarly, The zone of inhibition of the nanoparticle solution against *E. fecalis* at 25µl concentration is 9mm, at 50µl concentration is 25mm and at 100µl concentration is 27mm (Figure 3). The standard for the same had exhibited 40mm zone of inhibition. Therefore at the highest concentration, at 100µl concentration, the difference between the zone of inhibitions of the standard drug and *Achyranthes aspera* mediated silver nanoparticles is found to be 13mm.

The zone of inhibition of *Achyranthes aspera* mediated silver nanoparticles against *C.albicans* has been listed. The zone of inhibition of the nanoparticle solution against *C.albicans* at 25µl concentration is 9mm, at 50µl concentration is 12mm and at 100µl concentration is 13mm. The standard drug for the same had exhibited 10mm zone of inhibition (Figure 4). Therefore at the highest concentration, at 100µl concentration, *Achyranthes aspera* mediated silver nanoparticles have shown greater zone of inhibition than the standard used and the difference is found to be about 3mm. Similarly, the zone of inhibition of the nanoparticle solution against *Lactobacillus species at* 25µl concentration is 9mm, at 50µl concentration is 9mm and at 100µl concentration is 13mm (Figure 5). The standard drug for the same had exhibited 21mm zone of inhibition.Therefore at the highest concentration, at 100µl concentration, the difference between the zone of inhibitions of the standard drug and *Achyranthes aspera* mediated silver nanoparticles is found to be about 8mm.

From this we state that *Achyranthes aspera* mediated silver nanoparticles are more effective against *S.mutans* and *C.albicans* than on *E.fecalis* and *Lactobacillus species*. Similar studies of silver nanoparticles with other plant extracts have also shown good results (Saquib, no date; 'ECLIPTA PROSTRATA MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AND IT'S ANTI-BACTERIAL ACTIVITY IN URINARYTRACT INFECTION', 2020; Samadi *et al.*, 2021)

Anti inflammatory activity

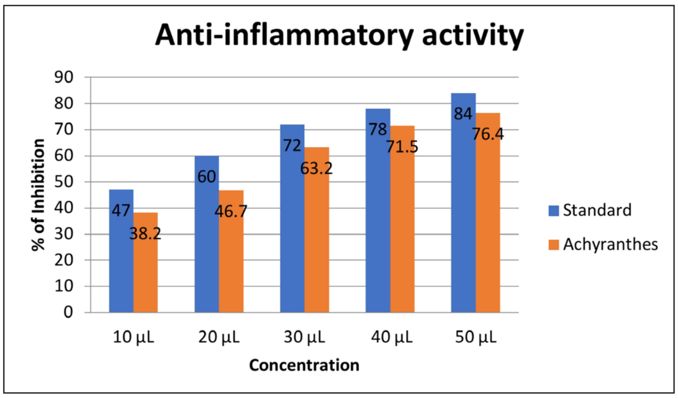
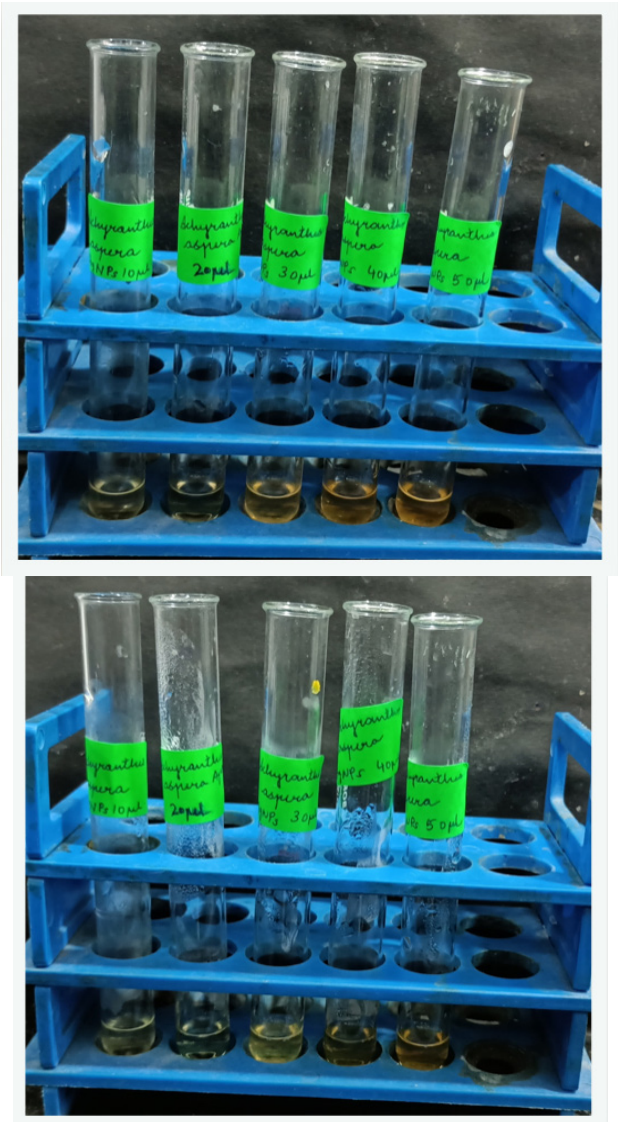


Fig. 6: The anti inflammatory activity of *Achyranthes aspera* mediated silver nanoparticles compared against the standard drug.



Figs 7 and 8: The color change before and after the heating process

Our results show that the *Achyranthes aspera* mediated silver nano particles showed about 38.2% protein denaturation inhibition at 10µl concentration, 46.7% inhibition at 20µl concentration, 63,2% inhibition at 30µl concentration, 71.5% inhibition at 40µl concentration and 76.4% inhibition at 50µl concentration (Figure 6).Figure 7 shows the colour of the nanoparticle solution before heating and Figure 8 shows the same after the heating process.

Similarly. The standard drug, Diclofenac sodium had showed about 47% protein denaturation inhibition at 10µl concentration, 60% inhibition at 20µl concentration, 72% inhibition at 30µl concentration, 78% inhibition at 40µl concentration and 84% inhibition at 50µl concentration.

From this we can infer that the anti-inflammatory activity of *Achyranthes aspera* mediated silver nanoparticles had increased proportionately with the increase in concentration of the solution. The margin of difference between the *Achyranthes aspera* mediated silver nanoparticles and the standard drug is found to be about 8.8%, 13.3%, 8.8%, 6.5% and 7.6% respectively for the increase in concentration of the solutions between 10µl to 50µl concentrations.At the 50µl concentration i.e the highest concentration used in our study, the margin of difference of protein denaturation between the *Achyranthes aspera* mediated silver nanoparticles and the standard drug is found to be about 7.6% and the average difference is found to be 9%. Therefore, *Achyranthes aspera* mediated silver nanoparticles prove to be a good anti-inflammatory agent. The silver nano particles synthesized using herbal extracts and herbal formulations show very good anti-inflammatory and anti cariogenic activities using biochemical procedures (Saquib, no date).

CONCLUSION

From our study, We state that Achyranthes aspera mediated silver nanoparticles have significant anti cariogenic and anti inflammatory agents. So, we can conclude that Achyranthes aspera mediated silver nanoparticles to be good anti cariogenic and anti inflammatory agents. Because of the pronounced cumulative and irreversible reactions of modern pharmaco-logical drugs, the world is shifting towards herbal medicines. (Rajendran *et al.*, 2019) (Ashok, Ajith and Sivanesan, 2017) (Malli *et al.*, 2019) (Mohan and Jagannathan, 2014) (Menon *et al.*, 2018) (Samuel, Acharya and Rao, 2020) (Praveen *et al.*, 2001) (Neelakantan *et al.*, 2011)

('Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases', 2019) (Kumar *et al.*, 2006). The maintenance of good health of the people in most of the developing countries forms the normative basis of the usage of traditional medicines.Our study paves the way for future research and development of a new drug that can be used as a good alternative to the already existing therapeutic drugs.

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