# EVALUATION OF *IN VITRO* ANTI- INFLAMMATORY ACTIVITY OF SILVER NANOPARTICLES AND *Hygrophila auriculata* LEAF EXTRACT

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## *ABSTRACT*

*This paper presents an empirical analysis on the use of aqueous extract of Hygrophila auriculata for the production of silver nanoparticles (AgNPs) from aqueous silver nitrate. The Hygrophila auriculata leaf extract, AgNPs and Diclofenac sodium at different concentrations was incubated with egg and Bovine serum albumin in controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present findings exhibited a concentration dependent inhibition of protein denaturation by Hygrophila auriculata extract and AgNPs. The effect of AgNPs was found to be high when compared with the Hygrophila auriculata extract and near to the diclofenac sodium. From the present study it can be concluded that AgNPs possessed marked in vitro anti-inflammatory effect against the denaturation of protein.*

***Key words:*** *Silver nanoparticles, Hygrophila auriculata extract, Diclofenac sodium, Anti-inflammatory effect*

## INTRODUCTION

Inflammation is the complex response of the immune system to infection and injury that leads to removal of ending factors and restoration of tissue structure and physiological function (Ricciotti E, FitzGerald, 2011). The symptoms of inflammation are characterized by pain, heat, redness, swelling and loss of function. It can be classified into two major types either acute or chronic, based on the duration of the inflammatory reaction. Though initiated as a protective phenomenon, loss of regulation of this complex process can lead to the development of various inflammatory disorders.

Herbs and herbal extracts have been used to treat various ailments since ages. Their derivatives have attracted tremendous attention therapeutically and are promising as remedies to treat diseases of diversified origin. Herbs especially have fallen into limelight, anticipating their replacement with sophisticated drugs. More than 50% of modern drugs existing in clinical use today are derived from plants. Metal nanoparticles have proved to be of significance due to their lesser volume to surface area ratio along with their catalytic, optical, electrical and magnetic characteristics (Nelson *et al*., 2010), that are extensively used owing to their antimicrobial properties. Silver nanoparticles are highly conductive, chemically stable and highly economical (Niraimathi *et al.,* 2014). The plant extract was used for the preparation of silver nanoparticles owing to its least toxicity and lesser need for

elaborate purification as compared to the chemical methods. The present work essentially deals with increasing therapeutic efficacy of the selected drug in its nanoparticle form. The present study aims to synthesis silver nanoparticles using the aqueous leaf extract of *Hygrophila auriculata* and evaluation of its anti- inflammatory activity.

## MATERIALS AND METHODS

### Preparation of leaf extract

The dried leafs were pulverized well with mortar and pestle to make powder. Twenty grams of powder sample was mixed with 100 ml of deionized water and the mixture was boiled for 10 min. After cooling the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4◦C for further use.

### Synthesis of Ag nanoparticles using leaf extracts

For the Ag nanoparticles synthesis, 5 ml of *Hygrophila auriculata* leaf extract was added to 45 ml of 1 mM aqueous $AgNO_3$ solution in a 250 ml Erlenmeyer flask. The flask was then incubated in dark for 5hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without leaf extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Ag nanoparticles were freeze dried (Arunachalam *et al*., 2012).

### *IN VITRO* ANTI-INFLAMMATORY ACTIVITY

Anti-inflammatory activity of the *Hygrophila auriculata* leaves extract and SNPs was evaluated by protein denaturation method as described by Padmanabhan and Jangle (2012).

## RESULTS AND DISCUSSION:

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of *Hygrophila auriculata* leaf extract and AgNPs. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins (Egg albumin and Bovine serum albumin) *in vivo* (Opie, 1962; Umapathy *et al*., 2010). Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development.

The use of nano-herbal-technology to synthesize compounds with improved anti-inflammatory properties is an area of current research by many scientists. In our study, we report the non-toxic, practical and environmentally benevolent approach for the synthesis of silver nanoparticles using the aqueous leaf extract of *Hygrophila auriculata* with potent anti-inflammatory activity. The synthesize and characterization of AgNPs from *Hygrophila auriculata* leaf extract showed the particle size between 10-60nm as well the cubic structure of the nanoparticles was reported in our earlier report (Ravishankar and Christibai Juliet Ester, 2018).

The increments in absorbances of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein denaturation by *Hygrophila auriculata* leaf extract, AgNPs and reference drug diclofenac sodium. The present findings exhibited a concentration dependent inhibition of protein denaturation by the *Hygrophila auriculata* leaf extract and AgNPs. The lowest activity of *Hygrophila auriculata* leaf extract, AgNPs and Diclofenac sodium were 11.84%, 14.47% and 23.68% in the concentration of 100µg/ml respectively while the highest activity of *Hygrophila auriculata* leaf extract, AgNPs andDiclofenac sodium were 57.89%, 71.05% and 80.26% in the concentration of 500µg/ml respectively. The greatest effect of AgNPs (500 µg/ml) was found to be near to standard diclofenac sodium. The half inhibition concentration ($IC_{50}$) of *Hygrophila auriculata* leaf extract, AgNPs and diclofenac sodium were 426.74, 346.37 and 294.63µg/ml$^{-1}$ respectively. From the present study it can be concluded that AgNPs showed marked *in vi*tro anti-inflammatory effect against the denaturation of protein (Table 1 and Figure 1). Our results was in good agreement with the earlier reports (Aparna Mani *et al*., 2015; Giridharan *et al*., 2014).

**Table 1: Effect of *Hygrophila auriculata,* AgNPs and Diclofenac sodium on protein denaturation (Fresh egg albumin)**

| Groups | Concentrations | % of inhibition | | |
|---|---|---|---|---|
| | | *Hygrophila auriculata* | AgNPs | Diclofenac sodium (Standard) |
| Group I | 100µg/ml | 11.84 ± 0.82 | 14.47 ± 1.01 | 23.68 ± 1.65 |
| Group II | 200µg/ml | 21.05 ± 1.47 | 25.00 ± 1.75 | 34.21 ± 2.39 |
| Group III | 300µg/ml | 36.84 ± 2.57 | 46.05 ± 3.22 | 50.00 ± 3.50 |
| Group IV | 400µg/ml | 47.36 ± 3.31 | 59.21 ± 4.14 | 65.78 ± 4.60 |
| Group V | 500µg/ml | 57.89 ± 4.05 | 71.05 ± 4.97 | 80.26 ± 5.61 |
| **$IC_{50}$ (µg/ml)** | | 426.74 | 346.37 | 294.63 |

Values are expressed as Mean ± SD for triplicates

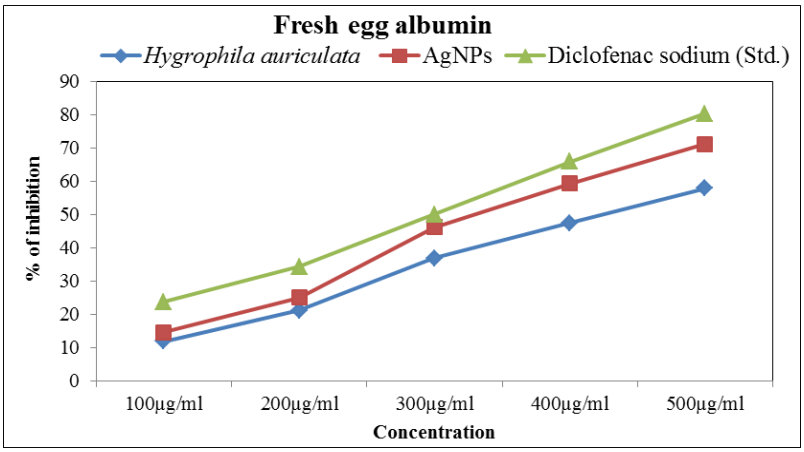


**Figure 1: Effect of *Hygrophila auriculata,* AgNPs and Diclofenac sodium on protein denaturation (Fresh egg albumin)**

The present findings exhibited a concentration dependent inhibition of protein (Bovine serum albumin) denaturation by the *Hygrophila auriculata* leaf extract and AgNPs. The lowest activity of *Hygrophila auriculata* leaf extract, AgNPs and Diclofenac sodium were 22.42%, 25.57% and 26.31% in the concentration of 100µg/ml respectively while the highest activity of *Hygrophila auriculata* leaf extract, AgNPs and Diclofenac sodium were 65.15%, 72.31% and 78.73% in the concentration of 500µg/ml respectively. The half inhibition concentration ($IC_{50}$) of *Hygrophila auriculata* leaf extract, AgNPs and ascorbic acid were 352.64, 297.54 and 251.50µg/ml$^{-1}$ respectively. The greatest effect of AgNPs (500 µg/ml) was found to be near to standard diclofenac sodium. From the present study it can be concluded that AgNPs showed marked *in vitro* anti-inflammatory effect against the denaturation of protein (Table 2 and Figure 2). Our result agrees with the earlier report (Aparna Mani *et al.*, 2015; Giridharan *et al*., 2014)

**Table 2: Effect of *Hygrophila auriculata,* AgNPs and Diclofenac sodium on protein denaturation (Bovine serum albumin)**

| Groups | Concentrations | % of inhibition | | |
|---|---|---|---|---|
| | | *Hygrophila auriculata* | AgNPs | Diclofenac sodium(Standard) |
| Group I | 100µg/ml | 22.42 ± 1.56 | 25.57 ± 1.78 | 26.31 ± 1.84 |
| Group II | 200µg/ml | 35.68 ± 2.49 | 40.10 ± 2.80 | 48.84 ± 3.41 |
| Group III | 300µg/ml | 46.31 ± 3.24 | 52.42 ± 3.66 | 59.26 ± 4.14 |
| Group IV | 400µg/ml | 53.36 ± 3.73 | 61.05 ± 4.27 | 66.63 ± 4.66 |
| Group V | 500µg/ml | 65.15 ± 4.56 | 72.31 ± 5.06 | 78.73 ± 5.51 |
| **$IC_{50}$ (µg/ml)** | | 352.64 | 297.54 | 251.50 |

Values are expressed as Mean ± SD for triplicates

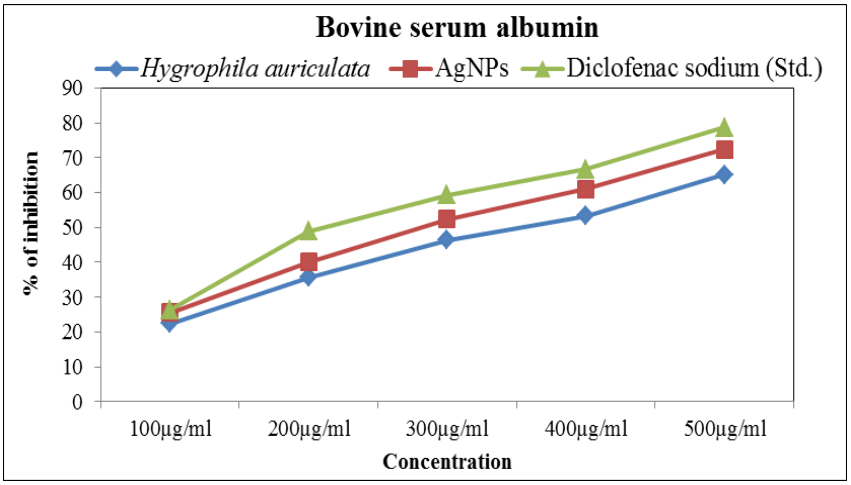


**Figure 2: Effect of *Hygrophila auriculata,* AgNPs and Diclofenac sodium on protein denaturation (Bovine serum albumin)**

## CONCLUSION

The synthesised silver nanoparticles are capped by the phytochemicals of *Hygrophila auriculata* leaf extract especially flavonoids and show significant anti-inflammatory effects. In conclusion combining the benefits of phytomedicine with nanomedicne can result in the formation of more efficient silver nanoparticles. This finding suggests that the synthesis of AgNPs using *Hygrophila auriculata* leaf extract could be a good source for developing green nano-medicine for the management of inflammation.

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