

## 5. SUMMARY AND CONCLUSION

The present study focussed on the green synthesis of silver and gold nanobioconjugates from the leaf extracts of *Clitoria ternatea* bearing blue and white flowers and their bioactivities namely antibacterial and anti-inflammatory *in vitro* and their anti-inflammatory activities were confirmed using *in vivo* model. Methods of synthesis were optimized, characterized and their biocompatibility was also checked.

In the first phase, leaves, seeds and roots of two varieties of *Clitoria ternatea* were examined for their antioxidant and antibacterial activity with solvents of varying polarity. The antioxidant potential of the extracts of *Clitoria ternatea* leaves, prepared in solvent of varying polarity, was tested using a battery of radical and oxidants namely DPPH, ABTS, hydroxyl and hydrogen peroxide. The methanolic extracts of the leaves of both the varieties of *Clitoria ternatea* bearing blue and white flowers exhibited better oxidant-scavenging activity than the other plant parts and other solvent extracts studied against all the radicals tested. Following this, all the extracts were screened for their antibacterial activity against a Gram negative (*E.coli*) and a Gram positive (*S.aureus*) organism, using turbidity method in order to find out the best plant part and the best solvent for bioactivity analysis. The methanolic leaf extracts of both the varieties tested showed better antibacterial effect. Therefore, only the methanolic leaf extracts of both the varieties were taken for further analysis.

Having identified the best part and the best extract that exhibits maximum bioactivity, an attempt was made to increase the efficacy of the extracts by preparing nanobioconjugates. Two metal nanobioconjugates, namely those from silver and gold, were synthesized using green synthesis approaches.

Various methods namely bright sunlight, water bath heating and microwave heating with varying time periods of exposure were explored for the effective synthesis of silver and gold nanobioconjugates. The synthesis

was followed by the rapid colour change and was qualified by a change in the absorbance. The silver and gold nanobioconjugates gave characteristic peaks revealing that effective synthesis of nanobioconjugates occurred. Exposure to bright sunlight for 20 minutes gave the maximum yield when compared with the other methods, namely water bath and microwave and other incubation periods tested. Therefore, the exposure to bright sunlight for 20 minutes was fixed as the method of choice for synthesizing the nanobioconjugates of silver and gold from *Clitoria ternatea* leaf extracts.

The nanobioconjugates synthesized from the methanolic leaf extracts of both the varieties of *C. ternatea*, were characterized using a series of physicochemical parameters, namely TEM, SEM, EDX, XRD, FTIR and Zeta potential.

The TEM and SEM images of the silver and gold nanobioconjugates of both the varieties of *Clitoria ternatea* leaf extracts revealed that the size of the synthesized nanobioconjugates were well within the range of nanoparticles (<100nm). This observation implies that they will have easier penetrating power into the cell. Both the silver and gold nanobioconjugates exhibited a spherical shape, as revealed by the TEM and SEM images.

The EDX profile of the four nanobioconjugates studied showed typical peak patterns of silver and gold peaks, with additional peaks that corresponded to the chemical elements of organic matter like carbon and oxygen, demonstrating the effective conjugation between silver/gold and the biomaterial, resulting in the synthesis of bioactive nanobioconjugates. This confirmed the presence of silver, gold and organic material and the crystalline nature of the nanobioconjugates.

The comparison of the XRD spectra of silver and gold nanobioconjugates synthesized from blue variety and white variety, with the respective spectrum of standard silver and gold, matched with the peaks of standard silver and gold, showing the successful conjugation of silver and gold with the compounds of the methanolic leaf extracts of *Clitoria ternatea*.

Fourier transform infrared (FTIR) spectroscopy analysis of the extracts and their nanobioconjugates were also compared to identify the functional groups involved in the synthesis of nanoparticles. This revealed the characteristic functional groups contributing to the formation of absorption bands at specific wavenumbers. Phenolic (OH) group and polysaccharides of the plant extracts were observed to be involved in the nanobioconjugate formation. This also indicates that these functional groups are involved in the interaction and stabilized the nanobioconjugates.

The long term stability of the nanobioconjugates can be predicted through its zeta potential. The zeta potential values of the all the four synthesized nanobioconjugates were well within the stable range, with a slight shift to the negative side, indicating an overall negative charge on the nanobioconjugates. Poly dispersity index of all the four nanobioconjugates were <0.4, indicating that the nanobioconjugates are well-dispersed, without aggregation.

Thus, all the physicochemical parameters studied confirmed the successful conjugation of the leaf extracts with silver and with gold, forming spherical, well-dispersed and stable particles, that had a dimension in the nanoscale that is highly desirable for drug delivery systems. In the next phase of the study, the bioactivity of the synthesized nanobioconjugates was analyzed *in vitro*, as the antibacterial and anti-inflammatory effects.

The antibacterial activity was assessed using clinical isolates of a Gram negative (*E. coli*) and a Gram positive (*S. aureus*). Along with the four different nanobioconjugates synthesized [silver nanobioconjugates from blue flower bearing leaves (AgB) and white flower bearing leaves (AgW), and gold nanobioconjugates from blue (AuB) and white flower bearing leaves (AuW)] and the unconjugated extracts of the leaves (BL, WL) were also analyzed for comparison.

The results showed that all the nanobioconjugates synthesized exhibited higher antibacterial effect, compared to their respective

unconjugated extracts. Among the two types of leaves, the white variety elicited a better response than the blue variety. Among the two types of metals used, gold nanobioconjugates showed higher antibacterial activity than the silver conjugates.

Following this, the *in vitro* anti-inflammatory activity was assessed using HRBC membrane stabilization, protein denaturation, heat induced hemolysis and proteinase inhibition activities and the results showed that the gold nanobioconjugates exhibit better protection against *in vitro* inflammation and both the nanobioconjugates are good sources of anti-inflammatory activity.

Thus, our results showed that the *in vitro* bioactivity of the extracts of *C. ternatea* leaves can be effectively enhanced by preparing silver and gold nanobioconjugates from them. As the ultimate aim of synthesizing such preparations is to administer them to humans to combat disease conditions, the next step of the study was to assess the safety of the preparations, as biocompatibility with human blood cells.

The nanobioconjugates were assessed for their biocompatibility using hemolysis, blood clotting time and morphological changes of RBC and blood lymphocytes. The kinetics of the drug release showed that all the four nanobioconjugates synthesized released their drug cargo steadily over a period of 16 hours. All the experimental results proved that the synthesized nanobioconjugates were safe. The results of the third phase, thus, showed that the silver and gold nanobioconjugates of *C. ternatea* leaf extracts were bioactive (in terms of *in vitro* antibacterial and anti-inflammatory activities) and also were non-toxic as well as biocompatible with human cells.

The final phase of the study was dedicated to the *in vivo* analysis of anti-inflammatory activity of nanobioconjugates and their unconjugated leaf extracts, to authenticate the results obtained *in vitro* as there are many biological factors that influence the pharmaceutical action of an entity *in vivo*.

Swiss albino mice were used to assess the *in vivo* anti-inflammatory activity against both acute and chronic inflammation.

The results showed that the administration of the synthesized silver and gold nanobioconjugates reduced carrageenan-induced paw edema, reduced the arthritis score, and reversed the weight loss of the complete Freund's adjuvant-injected mice than their unconjugated leaf extracts. These results proved the anti-inflammatory activity of the nanobioconjugates *in vivo*, against both acute and chronic inflammation. This bears a lot of significance, as most of the diseases and disorders afflicting man today, including diabetes, cancer and cardiovascular disorders, are associated with chronic inflammation. Our study provides evidence that the silver and gold nanobioconjugates of *C. ternatea* leaf extracts can eventually be developed into pharmaceutical preparations to help the humans affected by chronic inflammatory conditions and diseases.

Thus, the present study demonstrates that the methanolic extract of the *Clitoria ternatea* leaves showed significant antibacterial and anti-inflammatory activity. The results obtained *in vivo* confirmed the results obtained in the *in vitro* studies, validating the anti-inflammatory effects of the nanobioconjugates. This clearly proves that the leaves of *Clitoria ternatea* bearing blue and white flowers are a potential source of anti-inflammatory components. These components rendered better bioactivity when administered as silver and gold nanobioconjugates. The study, thus, proves that the synthesis of nanobioconjugates improves the bioactivity of the plant extracts. Gold nanobioconjugates were better than silver nanobioconjugates and the white variety was better than the blue variety.

*Clitoria ternatea*, which is a widely available ornamental and medicinal plant in India (and other parts of the world), can be developed into effective antibacterial and anti-inflammatory preparations, as evidenced by this study. More studies are needed to identify the exact component(s) involved in the process.

### **Suggestions for further research:**

The present study opens up a number of avenues for further research. Some of them are listed below:

- The molecular mechanism of the anti-inflammatory effect can be studied using the signaling pathways involved.
- The interaction of silver and gold nanobioconjugates to the biological targets can be determined using *in silico* analysis.
- *In vivo* studies for the antibacterial activity can be tested to validate the *in vitro* results.
- As the antioxidant, anti-inflammatory properties are related to apoptosis and cancer, the synthesized silver and gold nanobioconjugates can be assessed for their anti-apoptotic and anti-cancer activity.