Section A-Research paper

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Biosynthesis Of Silver Nanoparticles From The Leaf Extracts Of *Euphorbia Hirta* And Evaluation Of Their Antidiabetic Activity

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ABSTRACT:

Euphorbia hirta belongs to the Euphorbiaceae family and is highly valued for its medicinal properties. The plant's leaves are consumed as a vegetable by tribal communities, and it is widely used in traditional Indian medicine to treat diabetes. Nanotechnology is a field that focuses on producing nanoparticles of varying sizes, shapes, and chemical compositions to benefit human health. Plant-mediated biological synthesis of nanoparticles is becoming increasingly popular due to its simplicity and eco-friendliness. This present study involved using the leaf extract of *Euphorbia hirta* to carry out green synthesis of silver nanoparticles at room temperature. The nanoparticles were characterized using UV-Vis Spectrophotometer and FTIR, and their antimicrobial activity was evaluated on gram-positive (*Staphylococcus aureus*) and gram-negative (*Escheria coli)* bacteria. The plant's *in vitro* antidiabetic potential was determined using

ISSN 2063-5346

alpha amylase, alpha glucosidase, and DPP-IV enzyme inhibition study. The nanoparticles of *E.hirta* show a strong antidiabetic potential in an *in vitro* model, according to the findings of the current investigations, and more research can be done in an *in vivo* model.

Key words: *Euphorbia hirta, silver nanoparticles, UV-Vis Spectrophotometer, FTIR, cell line antidiabetic activity.*

INTRODUCTION:

The Indian subcontinent has a diverse range of medicinal plant species, numbering over 2500, thanks to its tropical climate (Bennet Rohan, *et al.*, 2020). *Euphorbia hirta*, a plant from the Euphorbiaceae family, is one such herb used to cure various ailments such as tumours, gonorrhoea, dysentery, jaundice, acne, and more. These annual plants can grow up to 40 cm tall and have purple stems that bear 1-2 mm-long fruit. The plants feature elliptical leaves that are about 1-2.5 cm long and are oppositely oriented (Kumar, *et al.,* 2010). Fresh plant decoctions are used as gargles in Ayurvedic medicine, while dry leaf decoctions are used for treating skin conditions. Studies have shown that E. hirta (L) has a potent medicinal effect and established its analgesic, antipyretic, anti-inflammatory, antidiabetic, antidepressant, antihypertensive, sedative, and anxiolytic properties (Williamson, 2002). Nanobiotechnology is widely used in the field of nanomedicine, which is a new and developing area. This process is cost-effective, environmentally friendly, non-toxic, and can easily be scaled up. Silver nanoparticles are known to have antimicrobial properties, making them useful in medical and industrial processes (Mani *et al.,* 2012). The production of silver nanoparticles through green synthesis, which involves the use of natural sources such as plants and microbes, is a rapidly growing area of research. This approach offers numerous benefits, such as lower costs, reduced environmental impact, and the possibility of discovering new applications in medicine and industry. In particular, the use of plants to synthesize nanoparticles is becoming increasingly popular due to its simplicity and environmentally friendly nature(Elumalai., *et al,* 2010). Diabetes mellitus is a chronic metabolic disorder that occurs due to insufficient insulin secretion or insulin receptor insensitivity. It is characterized by a group of metabolic symptoms that result in elevated blood sugar levels, known as hyperglycemia. The alpha-glucosidase enzyme breaks down oligo and disaccharides

ISSN 2063-5346

into monosaccharides, which are absorbed through the mucosal border in the small intestine. Inhibiting this enzyme reduces blood glucose levels. Another effective method to control diabetes is by inhibiting the activity of alpha-amylase enzyme, which converts starch into simple sugars. This can be done by using alpha-amylase inhibitors that delay glucose absorption rate, thereby reducing blood glucose levels in hyperglycemic individuals(Shilpa., *et al,* 2019). Thus objective of the present study is to investigate the biosynthesis of sliver nanoparticles of *E.hirta* and find the *invitro* antibacterial, anti-diabetic activity and cytotoxicity study by using B-cell line.

MATERIALS AND METHOD:

Plant material and synthesis of Ag nanoparticles

Euphorbia hirta leaves were obtained from the locality of a college campus and Coimbatore city, Tamil Nadu, India. These leaves were air-dried for ten days and then subjected to a temperature of 60°C in a hot air oven for 24-48 hours. Subsequently, the dried leaves were ground to a fine powder. To prepare a final solution of 200 ml, 1 mM silver nitrate was added to the plant extract, which was then centrifuged at 18,000 rpm for 25 minutes. The collected pellets were stored at -40°C. Upon heating the supernatant at a temperature range of 50°C to 95°C, a change in the color of the solution was observed.

UV-VIS Spectra analysis

The reduction of pure Ag+ ions remained monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a little aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer UV-2450 (Shimadzu).

FTIR analysis of silver nanoparticles

For FTIR measurements, the synthesized silver nanoparticles solution was centrifuged at 10000 rpm for 30 minutes. The pellet was washed thrice with 5 ml of deionised water to get rid of the free proteins or enzymes that are not capping the silver nanoparticles. The pellet was dried by using vacuum drier. This was analyzed by FTIR.

ISSN 2063-5346

Antibacterial activity by agar well diffusion method:

The ability of the nanoparticle to interact with cells was studied by antibacterial activity against the test organism *E. coli* and *Staphylococcus aureus* (Bennet Rohan, et al., 2020).

Cytotoxicity analysis by MTT assay

Maintenance Of Cell Lines:

The Raw 264.7 (Mouse Monocyte/Macrophage cell line) is purchased from NCCS, Pune, India. The RAW 264.7 cells were maintained in DMEM High Glucose media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution and 1% L-Glutamine (200mM) in atmosphere of 5% CO_2, 18-20% O_2 at 37^0C temperature maintained in the CO_2 incubator and sub-cultured for every 2 days .

Anti diabetic activity

The anti-diabetic activity of different concentrations of *E.hirta* AgNPs was studied against HS1cells, the cells cytotoxicity also tested by MTT assay(Bahuguna, *et al.,* 2017) . The parameters such as inhibitory concentration 5(6.25,25,25,50,100ug/ml). The determination of alpha amylase, alpha glucosidase, and DPP-IV enzyme inhibition and the results were mentioned in the graphs ***(***Manikandan*, et al.,* 2016**).**

Concentrations Used For The Cytotoxicity Study:

In this study, given test compound was evaluated to measure the cytotoxicity study Raw 264.7 cells pre stimulated by LPS. The used concentrations of the compound to treat the cells as follows:

Sl.No	Condition	Cell line	Concentration treated to cells
1	Untreated	Raw 264.7	No treatment
2	LPS	Raw 264.7	1ug/ml
3	Blank	-	Only media without cells
4	LPS+HS1	Raw 264.7	LPS+6(3.125, 6.25, 12.5, 25,50,100µg/ml)

Table 1: Details of compound with concentrations treated on LPS induced Raw 264.7 cells used for the study.

ISSN 2063-5346

Concentrations Used For The Anti diabetic study:

In this study, test compound was used to measure the alpha amylase, alpha glucosidase and DPP-IV enzyme inhibition studies. The used concentrations of the compound for the study as follows:

Sl.No	Test Compounds	Concentration treated to cells
1	Untreated	No treatment
2	Standard (acarbose)	6.25, 12.5, 25, 50 and 100uM/ml
3	Sitagliptin	6.25, 12.5, 25, 50 and 100uM/ml
3	Blank	Only extract
4	HS1	6.25, 12.5, 25, 50 and 100ug/ml

Table 2: Details of Stds and HS1 concentrations used for the study.

RESULTS & DISCUSSION:

The Ag nanoparticles that were synthesized were observed visually and confirmed. The color changed to reddish brown as a result of the reduction of silver ions. It is a widely known fact that Ag nanoparticles display a reddish-brown color in aqueous solutions because of the excitation of surface plasmon vibrations. The confirmation of the nano synthesis was carried out by analyzing it through different instruments. The authentication was established by means of UV-Visible spectroscopy, which showed a peak at 300-700nm. As depicted in figure 1, the absorbance increased at different time intervals and peaks were observed at 370-617nm.According to Jain , *et al*., 2009, the mixture of leaf extracts and silver ion complex in an aqueous solution caused a change in color to reddish-brown. This change was due to the activation of surface plasmon vibrations and indicated the formation of Ag nanoparticles. Similarly Elumalai, 2020, discovered that Ag nanoparticles formed in the reaction media after 10 minutes had an absorption spectra with a broadened peak at 430 nm.

Fig-1 -A- Plant sample, B- before colour change, C- reddish brown colour.

FTIR analysis was conducted to determine the biomolecules that could be responsible for effectively stabilizing the metal nanoparticles produced by leaf broth. In *E.hirta*, the OH

ISSN 2063-5346

stretching peaks were observed near 3326.37cm-1, while the amide I band at 1630.71 cm-1 indicated the presence of carbonyl stretch in proteins. According to Basarkar, *et al.,* 2014, the OH stretching peaks were observed at 3462.37cm-1, while the weaker band at 1635.71 cm-1 was attributed to the carbonyl stretch in proteins known as amide I.

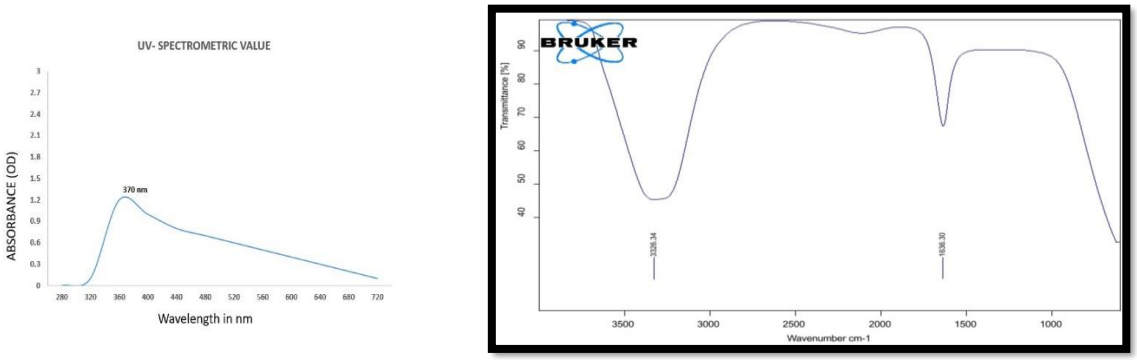

Fig-2 -UV-Analysis and FTIR analysis.

Antibacterial Activity:

The study examined the production of silver nanoparticles from *E.hirta* and their ability to combat pathogenic microorganisms. The plant extract's Ag nanoparticles demonstrated strong antimicrobial activity against *E. coli* (13mm)and *S. aureus (9mm)*, displaying a significant zone of inhibition. Silver nanoparticles interact preferentially with bacterial membrane proteins and DNA due to the presence of sulphur and phosphorus compounds which have a higher affinity for silver. This interaction leads to an antibacterial effect through a dual mechanism of antimicrobial activity. The effect is achieved through the bactericidal effect of Ag+ and the disruption of the membrane by the polymer subunits.(Saifuddin, *et al.,* 2009).

ISSN 2063-5346

Plate-1- Anti bacterial activity.

Table 3. Zone of inhibition using well diffusion method

Name of the plant extract	**Zone of inhibition(mm)**	
	E. coli	*S. aureus*
E.hirta	13	9

Cytotoxicity analysis by MTT assay

HS1 was cytoprotective in nature against the LPS induced Raw 264.7 cells with **greater than 80% cell viability** at highest concentration of 100ug/ml after the treatment of 24hours of incubation at 37^{o}C temperature. Based on the viability values, decided maximum concentration of the compound which showed greater than or equivalent to 80% as a optimum concentration for further studies.

Table 4. Table showed the % cell viability values of LPS induced Raw 264.7

Culture condition (ug/ml)	**% cell viability**	**Max non-toxic conc (ug/ml)**
Untreated	100.00	**100ug/ml**
LPS-1ug	54.54	
HS1-3.125	53.90	
HS1-6.25	55.01	
HS1-12.5	58.07	
HS1-25	67.20	
HS1-50	81.40	
HS1-100	88.92	

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cells treated with various concentrations of HS1.

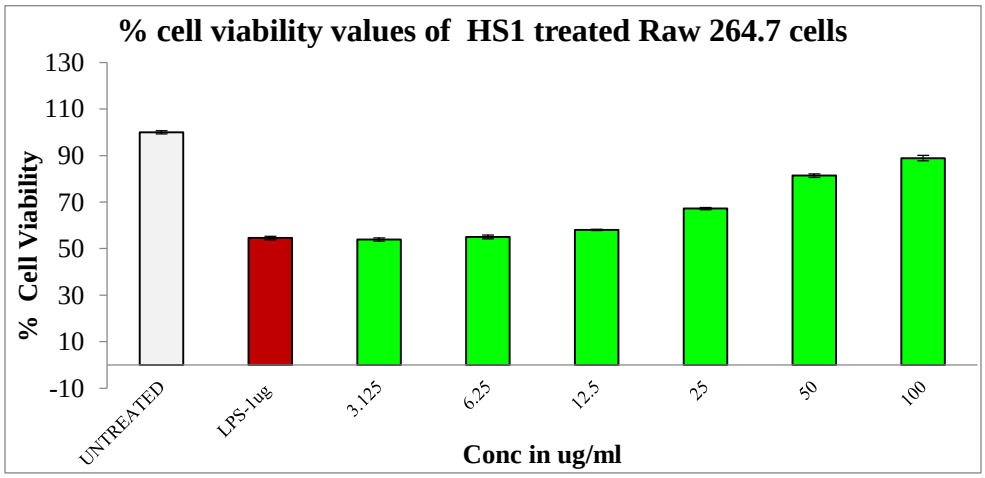

Fig-3- % cell viability values of LPS induced Raw 264.7

Anti diabetic activity:

Given Test compound, HS1 AgNPs showed the IC_{50} concentration (The Concentration of the compounds have the capacity to show the inhibition of alpha amylase enzyme or alpha glucosidase enzyme activity) of 47.46ug/ml (47) and 50.26ug/ml (50) compared to the std drug acarbose which showed the the IC_{50} concentration of 6.14uM and 48.47uM respectively. HS1 AgNps showed the IC_{50} concentrations (The Concentration of the compounds have the capacity to show the inhibition of DPP-IV enzyme activity) at 61.86ug/ml (62) compared to the std drug Sitagliptin which showed the the IC_{50} concentration of 1.02uM/ml respectively.

Alpha amylase enzyme inhibition study of the HS1:

Drug conc (ug/ml)	% amylase inhibition	IC50 conc (ug/ml)
0	0.00	**47.46**
6.25	9.75	
12.5	19.64	
25	34.47	
50	56.89	
100	92.01	

ISSN 2063-5346

Table 5: The results of *in vitro* Anti-diabetic activity of HS1 by the use of alpha amylase enzyme..

Alpha glucosidase enzyme inhibition study of the HS1:

Drug conc (ug/ml)	% glucosidase inhibition	IC50 conc (ug/ml)
0	0.00	**50.26**
6.25	8.32	
12.5	17.28	
25	26.24	
50	52.32	
100	93.84	

Table 6: The results of *in vitro* Anti-diabetic activity of HS1 by the use of alpha glucosidase enzyme.

DPP-IV enzyme inhibition study of the HS1:

Drug conc (ug/ml)	% glucosidase inhibition	IC50 conc (ug/ml)
0	0.00	**61.86**
6.25	4.09	
12.5	10.07	
25	19.07	
50	38.46	
100	79.36	

Table 7: The results of in vitro Anti-diabetic activity of HS1 by the use of DPP-IV enzyme.

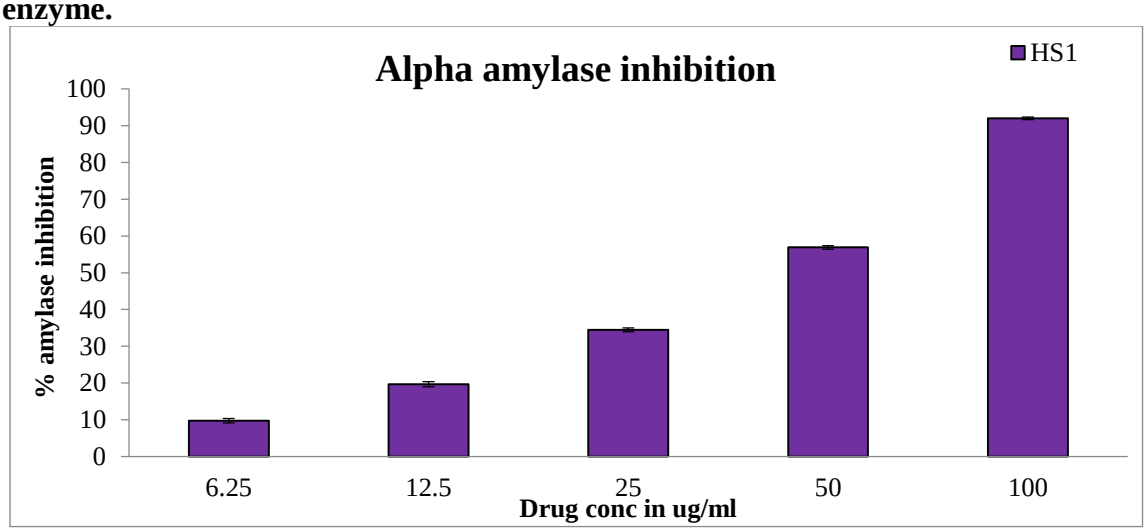

FIG-4 depicted the Alpha amylase inhibition capacity of HS1 with different concentrations used

ISSN 2063-5346

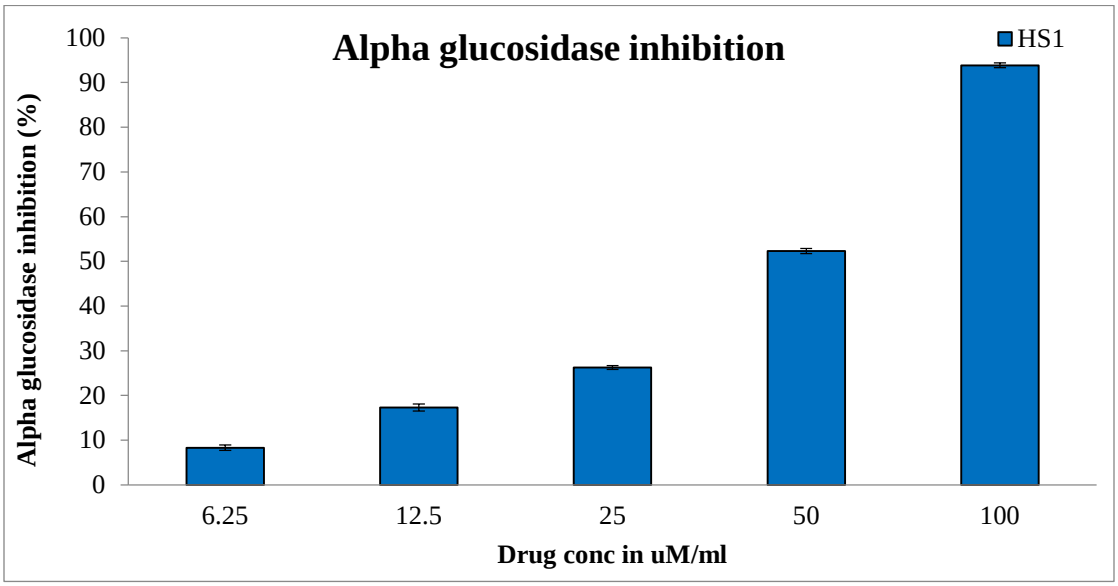

Fig-5-depicted the Alpha glucosidase inhibition capacity of HS1 with different concentrations

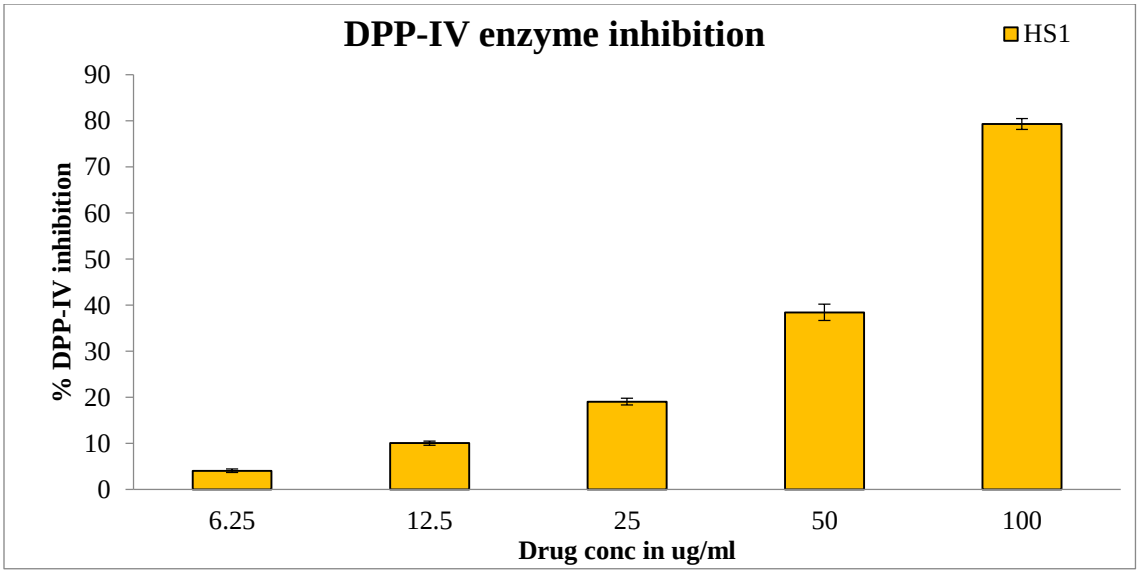

Fig-6- depicted the DPP-IV enzyme inhibition capacity of HS1 with different concentrations

CONCLUSION:

The Biosynthesis technique was active to create Ag nanoparticles by utilizing *Euphorbia hirta* leaf extract and characterization was done with UV-spectroscopy and FTIR. It was exposed that the resulting Ag nanoparticles had powerful antibacterial properties against *E. coli* and *S. aureus.*

The present study clearly proved that the extract *Euphorbia hirta* have an antidiabetic activity in an *in vitro* model. So further *in vivo* studies and compound isolation studies are needed.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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