

Development of wound healing hydrogels using biosynthesized silver nanoparticles and its antibacterial activity of Major Wound pathogens

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ABSTRACT

In this present study, biosynthesis of AgNp's from methanolic extracts of *H. Colorata* and its wound healing activity was documented. The synthesis of AgNp's was done by treating AgNO₃ solution with aqueous extract of *H. colorata*. The production of AgNp's were confirmed by a color change of the solution from clear to brown color. The reduced AgNp's were characterized by Scanning Electron Microscope (SEM), UV-vis spectroscopy. From UV analysis peak was observed at 415nm and spherical shaped AgNp's were observed. The antibacterial activity and Minimum inhibitory Concentration (MIC) of the silver nanoparticles were determined. The results suggest that biosynthesized AgNp's from aqueous extracts of *H. colorata* showed a significant antibacterial activity against wound pathogens.

Keywords: *Hemigraphis colorata*, Green synthesis, silver nanoparticles, Characterization, UV-Vis, SEM, Antibacterial activity, MIC

INTRODUCTION:

Wound is described as disruption of cellular, anatomical, and useful continuity of a dwelling tissue. It can be produced via way of means of physical, chemical, thermal, microbial, or immunological insult to the tissue. Optimum recovery of a cutaneous wound calls for a well-orchestrated integration of the complicated organic and molecular activities of molecular migration and proliferation and of extracellular matrix deposition and remodelling [1]. Inflammation is a complicated organic reaction of vascular tissue to dangerous stimuli, pathogens, irritants characterised through redness, warmth, swelling and pain[2]. Large institution of medicinal flora are used as conventional medicine, that have capacity to treatment numerous ailments. These medicinal flowers which include capacity resets of phytochemical compounds are used for plenty healing Purposes. Plants have the colossal capability for the control and remedy of wounds. A Large wide variety of vegetation are utilized by tribal and Folklore in many nations for the remedy of Wounds and burns. These herbal sellers Induce recuperation and regeneration of the misplaced Tissue through more than one mechanisms. These Phytomedicine aren't simplest reasonably-priced and Affordable however also are safe [3].

Wounding and wound recuperation take vicinity in all tissues and organs of the body. Many of those restore procedures are not unusualplace to all tissues. Although the technique of restoration is continuous, it's far arbitrarily divided into one-of-a-kind stages with the intention to knowledge of the physiological strategies which are taking region withinside the wound and surrounding tissue. Healing is a complicated technique related to co-ordinated interactions among various immunological and organic systems. It entails a cascade of cautiously and exactly regulated steps and activities that correlate with the advent of numerous mobileular kinds withinside the wound mattress at some stage in wonderful levels of the recovery method [4]. Separate components of a wound can be at unique ranges of restoration at anybody time. Timing and interactions among the additives taking element withinsresourceide the wound recovery manner vary for acute and continual wounds, despite the fact that the principle stages stay the same. The diverse tactics Of acute tissue restore, which might be brought on via way of means of tissue injury, can be united into a series of 4 time-structured stages: (i)coagulation and haemostasis, starting Immediately after injury; (ii) inflammation, which starts offevolved quickly thereafter; (iii)Proliferation, which begins offevolved inside days of the Injury and encompasses

the predominant restoration Processes; and (iv) wound remodelling, wherein scar tissue formation takes area, and which can also additionally last as long as a year or more[5].

Diabetes mellitus is one of the important individuals to continual wound restoration problems. When diabetic sufferers expand an ulcer, they emerge as at excessive threat for principal complications, together with contamination and amputation. The pathophysiologic dating among diabetes and impaired restoration is complicated. Vascular, neuropathic, immune function, and biochemical abnormalities every make contributions to the altered tissue restore. Despite remedy of those continual wounds, which includes tight glucose manipulate and meticulous wound care, the diagnosis for his or her recovery is pretty poor. Newer modalities that supply herbal or engineered boom elements display a outstanding deal of promise.[2]. Diabetes mellitus is maximum not unusualplace ailment of the altered glucose homeostasis. Diabetics have impaired wound recovery and impaired formation of coronary collaterals. The peculiar apoptosis or angiogenesis can also additionally purpose a few of the medical manifestations of diabetes. Silver has been recognised to have powerful bactericidal residences for centuries. Nowadays, silver-primarily based totally topical dressings were extensively used as a remedy for infections in burns, open wounds, and continual ulcers. Silver nanoparticles are novel nanosized and tremendously crystalline antibacterial agent which consists of Ag⁺ ions through ionexchanging. [6].

Nanoparticles are described as particulate dispersions or stable debris with a length withinside the variety of 10-1000nm. The drug is dissolved, entrapped, encapsulated or connected to a nanoparticle matrix. Depending upon the approach of preparation, nanoparticles, nanospheres or nano pills may be obtained. Nano pills are structures wherein the drug is constrained to a hollow space surrounded through a completely unique polymer membrane, whilst nano spheres are proteins, peptides and genes structures wherein the drug is bodily and uniformly dispersed. [7] In current years, biodegradable polymeric nanoparticles, especially the ones lined with hydrophilic polymer along with poly(ethylene glycol) (PEG) referred to as lengthy-circulating debris, were used as cappotential drug shipping gadgets due to their capacity to flow into for a extended length time goal a specific organ, as providers of DNA in gene therapy, and their capacity to supply proteins, peptides and genes .[4].

Hemigraphis colorata (Acanthaceae), an uncommon plant tailored to India, is a flexible tropical low-creeping perennial herb that reaches a top of 15 to 30 cm. It prostrates and spreads with rooting stems while grown on ground. The leaf has metal pink lustre on higher floor and a strong darkish crimson on ventral side. The leaves are opposite, ovate to cordate, serrate-crenate, approximately 2 to eight cm lengthy and four to six cm wide, bearing well-described veins. This plant is observed to have antibacterial anti-elastase pastime and antioxidant specific pharmacognostical and phytochemical look at of the plant turned into finished via way of means of Saravanan et al. The above stated plant life are utilized by tribal and folklore healers for the remedy of infection and wound withinside the Wayanad district of Kerala. [8].

The present study concentrates on green synthesis of silver nanoparticles using the leaf extracts of *H. colorata*. The nanoparticles were characterized using UV-VIS spectroscopy and SEM. Wound healing gel was prepared and antibacterial activity was determined against major wound pathogens.

MATERIALS AND METHODS

Collection of *Hemigraphis colorata* leaves and processing

Hemigraphis colorata leaves were collected from the areas of Palakkad, Kerala. The leaves were washed using distilled water and dried in shade under room temperature. The dried leaves were finely grounded to powders and stored in sterile container for extraction.

Extraction of bioactive compounds using Soxhlet apparatus

Powders of *Hemigraphis colorata* leaves were placed in a porous bag or “thimble” made from a strong filter paper or cellulose, which is placed, is in thimble chamber of the Soxhlet apparatus. Extraction solvent (methanol) is heated in the bottom flask, vaporizes into the sample thimble, and condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents is emptied into the bottom flask again and the process is continued. For the study, infusion method of Soxhlet Extraction had been adopted. The dried powder was filled in the thimble and placed in the soxhlet extractor. The extractor had been filled with solvent solution of methanol and the temperature of 60°C was set and left for 6 hours. The extracts were collected and the solvents were evaporated. The dried extracts were collected and stored in sterile containers.

Green synthesis of silver nanoparticles

10mg of leaf extracts were dissolved in 10ml of distilled water. In green synthesis of AgNPs, 0.1M (1.69g in 100ml distilled water) of AgNO₃ (99.99%) was used. 5mL of leaf extract was added to 45 ml of 0.01M AgNO₃ aqueous solution and allowed at ambient condition to react. After different time intervals, the color change of reaction mixture is observed from colourless to dark brown indicates that the formation of AgNPs. The AgNPs precipitates were collected by centrifugation. The pellets were collected and dried using hot air oven at 50°C for 1hr. The dried powders were stored at eppendorf tubes and can be used for further analysis. The green synthesized nanoparticles were examined under UV-Vis spectroscopy at range 350nm to 500nm for confirmation of AgNp synthesis.

Preparation of wound healing gels

Wound dressing gels was developed by the method described by Natarajan *et al.*, 2018. Poly vinyl alcohol (2%) is mixed with the distilled water and stirred under room temperature. Under stirring conditions 2% of leaf extracts were added drop wise. 2% citric acid is added to the solution and stirred for 30mins. The films were poured in a plastic petri dish and kept undisturbed overnight. Later the gels were used for further analysis.

Antibacterial activity against wound causing pathogens

The antibacterial efficacy of the Ag Np and wound healing gel was evaluated against the two major wound pathogen *Escherichia coli* and *Klebsiella pneumoniae* by well diffusion method [9]. Nutrient Agar was prepared and sterilized, and poured into plates. (Nutrient agar Composition (for 100ml): Peptone: 0.5g; Yeast extract: 0.5g, Beef extract: 0.3g, Sodium chloride: 0.5g, Agar 1.5 g; Total pH: 7.0 ± 0.2). Overnight cultures of test pathogens were cultured and 0.1% of culture solution of each test organisms was streaked throughout the petri plate with the sterile cotton swab by rotating the plate at 60° angle for each streaking. 6mm well borer was used to bore wells on the agar surface of each NA plates. About 100µl of the samples were loaded into the well and the plates were incubated in an incubator at 37°C for 48h. The antibacterial activity was determined in terms of inhibitory zones around the wells loaded with samples in all the Nutrient Agar plates containing test pathogens. The obtained clear zones were observed and measured in millimetre (mm).

Determining Minimum inhibitory concentration (MIC) of the green synthesized Ag Np

Minimum inhibitory concentration (MIC) of the green synthesized Ag Np was determined by well diffusion method. Briefly, four concentrations of Ag Np (25 μ g, 50 μ g, 75 μ g & 100 μ g) were added to the wells against two test pathogens. The minimum concentration showing inhibitory zones was recorded as MIC

RESULTS AND DISCUSSION

Collection and Processing of plants

Hemigraphis colorata plant was collected from the areas of Palakkad, Kerala (Figure-1). It was traditionally used for treatment of wounds by the native people. The common name of the plant was Murikooti (wound healer). The plant can be easily identified by its colour. The upper region of the leaves was dark green in colour and the lower region is purple in colour. The leaves were collected and dried in room shade. The dried leaves were grounded and turned to fine powders for extraction and synthesis of silver nanoparticles.



Figure-1: Collection and Drying of *Hemigraphis colorata* leaves

Green synthesis of silver nanoparticles

In green synthesis method, plant extracts used as the reducing agent. Plant extracts was added to the silver nitrate solution. On continuous stirring the colour of the solution turned from colourless to black colour. Formation of black colour indicates the synthesis of silver oxide

nanoparticles. Further the nanoparticles were collected by centrifugation at 10,000rpm for 5 minutes (Figure-2). The particles were dried in oven and stored in a sterile vials.

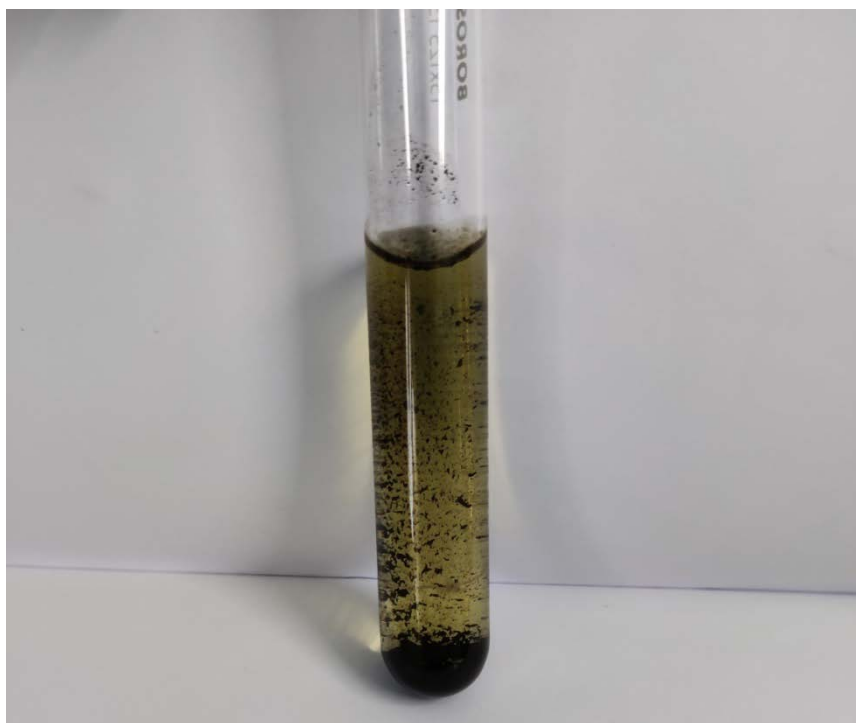


Figure-2: Formation of green synthesized Silver nanoparticles

Characterization of nanoparticles using UV-Vis spectroscopy and FE-SEM

The green synthesized nanoparticles were characterized using UV-Vis spectroscopy and Field emission scanning electron microscope (FE-SEM). From UV-Vis analysis, the absorbance peak was observed at 415nm which is in between standard the range (390nm to 450nm) (Figure-3). FE-SEM images showed the fine aggregates at the size of nanometres. The nanoparticles were found to be scattered and evenly distributed. The AgNps were found to be irregular and uneven in shape (Figure-4).

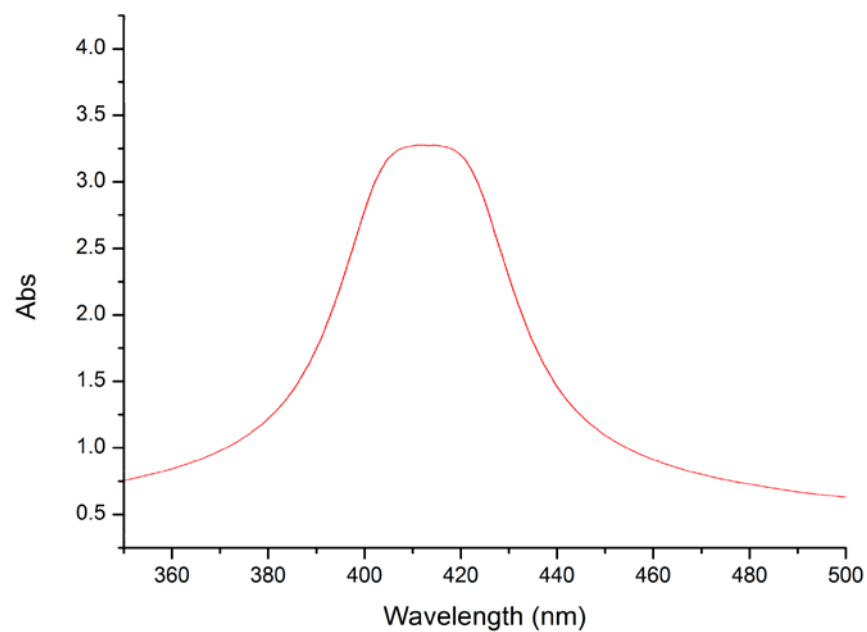


Figure-3: UV-Vis analysis of green synthesized Ag Nps

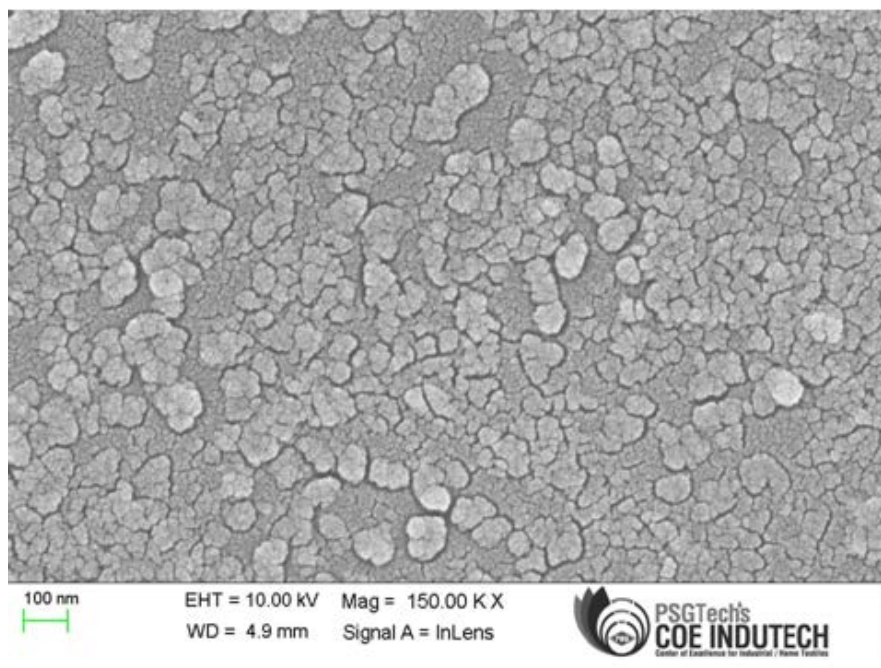


Figure-4: FE-SEM image of green synthesized nanoparticles

Antibacterial activity against wound pathogens

Figure-5: Antibacterial activity against *Escherichia coli*

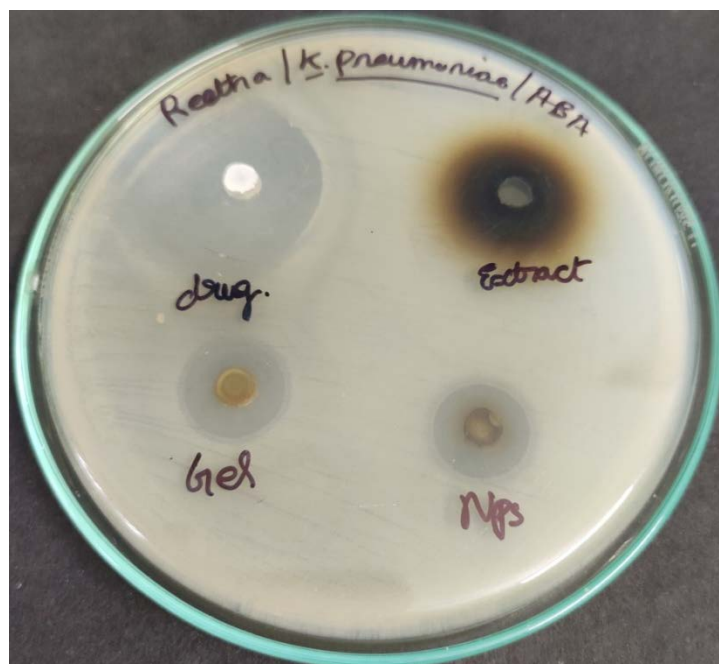


Figure-6: Antibacterial activity against *Klebsiella pneumoniae*

Table-1: Antibacterial activity against wound pathogens

S. No	Samples	Inhibitory zones (mm)	
		<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
1	Extract	0	0
2	Ag Nps	14	10
3	Wound healing gel	12	11
4	Drug (ciprofloxacin)	26	24

Plant extract showed no inhibitory zones against *Escherichia coli* and *Klebsiella pneumonia* (Table-1). Silver nanoparticles showed 14mm against *Escherichia coli* and 10mm against *Klebsiella pneumonia*. Similarly, wound healing gel showed 12mm against *Escherichia coli* and 11mm against *Klebsiella pneumonia*. Whereas, standard drug Ciprofloxacin showed 26mm against *Escherichia coli* and 24mm against *Klebsiella pneumonia* (Figure-5 and 6).

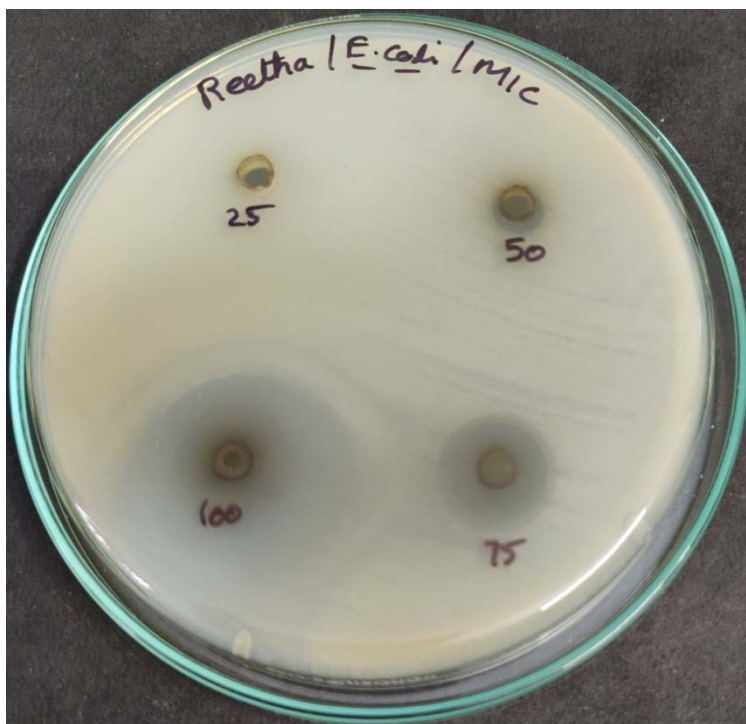
MIC of the Ag Nps

Figure-7: Determining MIC against *Escherichia coli*



Figure-8: Determining MIC against *Klebsiella pneumoniae*

Table-2: MIC of the green synthesized silver nanoparticles

S. No	Concentration (µg)	Inhibitory zones (mm)	
		<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
1	25	0	0
2	50	7	0
3	75	13	8
4	100	19	12

Minimum inhibitory concentration (MIC) is the lowest concentration required to inhibit the bacteria. 25µg of green synthesized AgNps showed no inhibitory zones (Table-2). 50µg of AgNps showed slight zone (7mm) against *Escherichia coli* and no zone against *Klebsiella pneumoniae*. 75µg showed 13mm against *Escherichia coli* and 8mm against *Klebsiella pneumoniae*. 100µg of AgNps showed 19mm against *Escherichia coli* and 12mm against *Klebsiella pneumoniae*. Therefore, the MIC of the green synthesized nanoparticles against *Escherichia coli* and *Klebsiella pneumoniae* was found to be 50µg and 75µg (Figure–7 and 8).

DISCUSSION:

Microorganisms are responsible for wound infection, significant controversy nevertheless exists concerning the precise mechanisms through which they reason infection and additionally their importance in nonhealing wounds that don't showcase medical symptoms and symptoms of contamination. Untreated wound is liable to infections resulting from micro organism which include *S. aureus*. Broad-spectrum antimicrobial hobby of AgNPs has inspired the improvement of AgNPs-primarily based totally dressing for wound recuperation.[10]pronounced that AgNPs had been capable of deal with irritation via cytokine modulation and result in wound restoration

with reduced scar formation. [11] additionally substantiated that AgNPs dealt with institution presentations reduced launch of increase elements and inflammatory cytokines (which might be secreted from immune cells), in human dermal keratinocytes. Our research proved the ability for Ag-AgNPs in bacterial mobileular disruption which collectively with its free-radical scavenging capacity may be applied for improvement of wound dressings.

Healing of the wound is a complicated procedure that includes synchronous association amongst numerous chemical parts to permit reconstruction of the impaired tissues and to restore the ordinary pores and skin function[12]AgNPs hydrogel is capable of limitation the infectivity of each Gram superb and Gram bad bacterial kinds and offer a sterile surroundings feasible to resource energetic wound recovery. The adverse impact of AgNPs hydrogel on bacterial increase and survival, couple with well-timed launch of AgNPs upon software make it viable as a wound dressing. [13]proven that the topical utility of AgNPs inspired wound-recovery system protected remodeling, re-epithelialization, and wound contraction processes.

Silver is likewise one of the major additives withinside the diverse ointments for restoration wounds [14]. However, silver nanoparticles are actually being brought as an opportunity antibacterial agent changing silver ions. Both silver ions and silver nanoparticles have inhibitory and deadly outcomes on bacterial species which include *Escherichia coli*, *Staphylococcus aureus*, or even yeast. But, the formation of complexes for silver ions is confined and the impact of the silver ions in some way stays most effective for a quick period [15].

Silver nanoparticles have garnered outstanding role in ailment control because of their precise homes due to small size and big floor area, mechanical and thermal stability, chemical inertness, electric conductivity, biosensor, and antimicrobial hobby [16]. The inexperienced synthesis of AI-AgNPs turned into obtrusive from alternate in coloration of answer that is attributed to floor plasma resonance phenomenon. A clean dominant height at four hundred nm in UV-Vis spectroscopy and microscopic evaluation (SEM, TEM) installed the particle size (~33 nm) and round form of synthesized AI-AgNPs, which turned into according with the literature [17]. Traditional medicinal flowers are being explored as supply of latest tablets in opposition to growing quantity of antibiotic resistant micro organism[18]. In this study, silver nanoparticles synthesized from *Hemigraphis colorata* plant indicates antibacterial sports than the ordinary

plant extract and It indicates the minimal inhibitory awareness approximately 50g for *Klebsiella pneumoniae* and 75g for *Escherichia coli*.

Various antibacterial mechanism of motion of AgNPs may be summarized as mobile membrane adhesion and harm, era of ROS and cellular stress, lack of balance of mobile proteins and RNA, leakage of DNA from nucleus, and alternation of cellular signaling pathway [19]. Conventional dry dressings fabric including gauze, plasters, bandages are making manner for plant- primarily based totally wound dressings included films, foams, and gels [20]. Quality and amount of biosynthesized AgNPs rely on response parameters and their residences are regularly ruled via way of means of the presence of secondary metabolites [21].

According to [22] a wound takes extra than 14 days to heal absolutely with software of chemically synthesized AgNPs, loaded on gelatin hydrogel. Interestingly our end result confirmed that AgNPs hydrogel brought about higher recuperation impact in only 10 days. This turned into because of non-stop launch of AgNPs from hydrogel, which well timed entered the physiological device and interacted with inflammatory cells gift withinside the wound sites. This sluggish launch ensured no harm to the ordinary cells at the same time as prolonging the wound recuperation impact. These AgNPs did now no longer have any tremendous poisonous impact.

Conclusion:

Wound healing hydrogel from *Hemigraphis colorata* plant extract shows antibacterial activity against major wound pathogens *Escherichia coli* and *Klebsiella pneumoniae*. Study evaluated the wound healing activity of methanolic extract ointment of dried leaves of *H. colorata*. *Hemigraphis colorata* leaves will helps to prevent infections and promote wound healing process. Thus the developed biodegradable hydrogels can be used for wound healing treatments.

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