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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *Zingiber officinale* RHIZOME EXTRACT AND ITS APPLICATION ON DIABETIC FOOT ULCER

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Abstract: The biosynthesis of metal nanoparticles is a vast research area due to the application of novel technologies. Plant extract are cost effective and eco-friendly for green synthesis of silver nanoparticles. To evaluate the diabetics foot ulcer activity from aqueous silver nitrate through the extract of *Zingiber officinale* rhizome . Ginger extract act as reducing and capping agent. The formation of silver nano particles are confirmed by UV- Vis spectrophotometer and characterization using Scanning Electron Microscopy, EDAX and Fourier Transform Infra red spectroscopy . SEM images indicated the presence of silver nanoparticles as spherical shape and range of between 62 nm to 92 nm. EDAX shows strong signal from silver atom . FTIR spectra suggest that the biological molecules possibly perform dual function of formation and stabilization of silver nanoparticles in aqueous medium. Minimal inhibitory concentration showed effective activity in 3mm concentration.

Index Terms **– Diabetic foot ulcer, Ginger extract, UV- Vis spectrophotometer, SEM-EDAX, FTIR and Minimal inhibitory concentration .**

I. INTRODUCTION

Diabetes mellitus is a chronic disorder and a major public health problem. Infections are a common complication in diabetic patients, and they are usually more severe than infections in non-diabetic patients. Foot infections are among the most common complications in diabetic patients and a common case of morbidity and mortality. Twenty- five percent of all diabetics develop severe foot or leg problems and foot infections account for about 20% of all hospital admissions in diabetics (Cunha BA. -2000).

There are a number of predisposing factors, which determine the severity of this complications: 1.According to impaired immunologic response, 2. peripheral neuropathy, 3. peripheral arterial disease. Depending on the control of diabetes, these factors can be present in varying degrees (Lipsky BA, et.al.,-2004). In case of poor control, an infection can develop, spread rapidly and produce significant and irreversible tissue damage, resulting in a high rate of amputations (Caputo GM-1994).

Foot infection in diabetic patients usually begins in a skin ulceration. Although most infections remain superficial, ~25% will spread contiguously from the skin to deeper subcutaneous tissues and / or bone. Up to half of those who have a foot infection will have another within a few years. About 10-30% of diabetic patients with a foot ulcer will eventually progress to an amputation, which may be minor (i.e., foot sparing) or major. Conversely, an infected foot ulcer precedes ~60% of amputations, making infection perhaps the most important proximate cause of this tragic outcome.(Amsterdam,1999).

S. aureus is the most important pathogen in diabetic foot infections, even when it is not the only isolate, it is usually a component of a mixed infection (Breen JD et.al.,-1995). Serious infection in hospitalized patients are often caused by 3-5 bacterial species, including both aerobes and anaerobes (Wheat LJ et al., -1986).

Zingiber officinale, commonly known as ginger, belongs to the Zingiberaceae family that originates in Southeast Asia. *Z.officinale* is an edible rhizomatic herb that is often used as seasoning throughout the world. The ginger rhizome is elongated, reasonably flattened, and its coloration ranges from golden yellow to bight brown, containing longitudinal grooves (Butt MS and Sultan M., 2011).

Ginger (Zingiber officinale) is a world known spice and has been used for over 2500 years in traditional medicine for the treatment of respiratory diseases(rhinitis, asthma), inflammation (rheumatism), heart disease (hypertension, palpitations, cardiopathies), gastrointestinal tract (diarrhea, constipation, vomiting, poor digestion) and metabolic disease(Diabetes)(Mozaffari KH et al., 2014).

Ginger can also be presented in powdered, crystallized form or in solution, which promotes further changes in its composition (Ali BH et al., 2008). The chemical components of ginger can be volatile or non-volatile, the later being responsible for the characteristic

smell and taste of ginger. Among the volatile compounds are sesquiterpene hydrocarbons, such as zingiberene (35%), curcumene (18%) and farnesene (10%) (Butt MS et.al., 2011). The non-volatile compounds give the ginger the spicy taste, these being zingerone derivatives, although other other non- volatile substances are also recognized for their pharmacological properties, such as gingerols, shogaols and paradols (Arablou T et al., 2014 and Chen CY et al., 2012). Both gingerols and shogaols are very important phenolic components, since they have pharmacological properties that are beneficial to health (Mozaffari KH et al., 2014).

Nanotechnology can be termed as the synthesis, characterization, exploration and application of nano-sized (1-100nm) materials for the development of science. It deals with the materials whose structure exhibit significantly novel and improved physical, chemical and biological properties. Nanotechnology holds a trusting future for the design and development of many types of novel products that are used in early detection , treatment, prevention of various diseases in food technology. It also plays a role in pharmaceutical by using green synthesis from plant extracts and microorganism favou.6rs numerous benefits to ecofriendliness application (Thanaa.I.Shalaby et al.,2015)

The upcoming applications in various fields like optical sensors, in spectrally coating for absorption of solar energy (G.Haripriyaa et al., 2014), bioremediation of radioactive wastes, functional electrical coating, synthesis of enzyme electrodes, in medicine such as delivery of antigen for vaccination, gene delivery for treatment or prevention of genetic disorders, in medical field as a bactericidal and a therapeutic agent. Silver Nanoparticles have wide reactivity due to large surface to volume ratio and plays a crucial role in inhibiting bacterial growth, It is a well known that silver is very toxic to the microorganism (Chandan singh et al., 2011).

Hence nanosized organic and inorganic particles are finding increased attention in medical application due to their amenability to biological functionalization. Byased on enhanced effectiveness, the new age drugs nanoparticles of polymers, metals or ceramics, which can combact conditions like cancer and fight human pathogens like bacteria (Govindaraju et al.,2010).

Silver is a non- toxic, safe, inorganic antibacterial agent being used for centuries and capable for killing microorganism that cause disease. It has a significant potential for a wide range of biological applications such as antibacterial agent for antibiotic resistant bacteria, preventing factors, healimg wounds and anti- inflammatory. Silver ions (Ag+) and its compounds are highly toxic to microorganism exhibiting strong biocidal effects on many bacterial species but have a low toxicity towards animal cells.

Biosynthetic methods have been investigated as an alternative to chemical and physical one. These methods can be divided into two categories depending on the place where the nanoparticles or nanostructures are created as many microorganism can provide inorganic materials either intra or extracellularly. For example, the organism isolated from silver mine materials is able to reduce Ag+ ions and accumulates silver nanoparticles. The metabolic activity of microorganisms can lead to precipitation of nanaoparticles in external environment of a cell (Sadowski et al., 2008). Among noble metal nanomaterials , silver nanoparticles have received considerable attention due to their attractive physicochemical properties. The surface plasmon resonance and large effective scattering cross section of individual nanoparticles make them ideal candidates for molecular labelling (Schultz et al., 2000, Elchiguerra et al., 2005).

Nanoparticle characterization is necessary to establish understanding and control of nanoparticle synthesis and application .Characterization is done by using a variety of techniques, mainly drawn from material science .Common techniques are Electron microscopy (TEM ,SEM) , Atomic force microscopy (AFM), Dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS) and UV spectroscopy (Das et al ., 2009).SEM shows macroscopic aggregates composed of nanosized silver particles. Obviously these particles have only a limited use as biocidal materials in liquid systems because of their low colloidal stability (Sondi and Sondi ,2004).

Silver nanoparticles are attractive as these are nontoxic to human body at low concentration and having broad spectrum antimicrobial nature. Silver nanoparticles inhibit the bacterial growth at very low concentrations than antibiotics as of no side effects are reported (Dharnendra and Behari., 2009).

Major benefits of the project was effective AgNPs against Diabetic Foot Ulcer by biosynthesis process. The biosynthesis process helps to avoid many chemicals. These process doesn't contain any chemical and which are not harmful. At 30mM concentration gives effective activity against DFU. Overall its an cost effective and eco-friendly work.

Further studies of the works such as toxicity checking by MTT Assay, animal studies and formulation of product as ointment or spray will be performed later.

II. MATERIALS AND METHODS

2.1 Collection of Sample:

The Diabetic Foot Ulcer sample was collected from a local private hospital by providing a nutrient broth and swab for taking a swab from the wound and then inoculate it into the nutrient broth. The broth was incubated for 24hrs at 37°C. Then serial dilution were made, from that some colonies are inoculated in a nutrient agar for isolation of pure culture. Those cultured are subcultured , identified and used for this study.

2.2 Identification of Microorganism:

The culture was processed for identification such as : Gram staining, motility, Biochemical Test (IMVIC, oxidase, TSI, urease) and enrichment media are used (EMB, Mac conkey, Mannitol Salt Agar, Blood Agar).

2.3Preparation of Ginger Extract:

Ginger (Zingiber officinale) rhizome extract was used in the study. The materials were collected from farmers market in Coimbatore. Then rinsed with sterile distilled water to remove any associated debris. The extract was prepared by taking 10gm thoroughly washed ginger rhizome, taking out the upper part by the knife, chopped into the fine pieces and converting it into paste using mortar and pestle followed by addition of 40ml of MilliQ water, boiling for 10 mins then filtered by Whatman filter paper , centrifuged at 5000rpm for 15mins and collection of suspension , which was further used as ginger rhizome broth for the experiment(Chandan singh et al.,2011).

2.4 Bio-Synthesis of Silver Nanoparticles:

For the reduction of Ag+ ions 5ml of ginger rhizome broth was added to 50ml of 1mM Silver Nitrate solution, 10ml for 3mM and 15ml for 5mM silver nitrate solution. The ginger broth was slowly and drop by drop added to the silver nitrate solution, which kept in a magnetic stirrer in 40°C at 500 rpm for 1.30 hours in dark.

2.5 UV- Visible Spectrophotometer Analysis:

Bioreduction of Ag+ in aqueous solution was monitored by UV-Vis spectroscopy. UV- Visible spectrophotometer analyses of silver nanoparticles synthesized were recorded as a function of time at room temperature using SHIMADZU UV-2600 spectrophotometer, operated between 800 to 400nm wavelength ranges at a resolution of 1nm.

2.6 FTIR Analysis: (Fourier Transform Infrared Spectroscopy)

After the completion of Ag+ ions by the ginger rhizome extract, 10ml solution of silver nanoparticle was centrifuged at 4000 rpm for 5 mins and these process to make nanoparticle free from proteins or other bioorganic compounds present in the solution. There after 5ml of purified suspension was given for analysis in SHIMADZU -FTIR for % Transmittance at 4cm-¹ resolution.

2.7 Scanning Electron Microscopy - EDAX:

The powdered silver nanoparticle were characterized using SEM-EDAX. The biosynthesized nanoparticles were lyophilized to make powder form then given for analysis.

SEM analysis was done to study the size and the elements present in the sample. The energy dispersive analysis of X-ray (EDAX) was also done along with the SEM analysis.

2.8 Anti-Bacterial Activity:

The Diabetic Foot Ulcer sample were serial diluted and plated on nutrient agar medium and various bacterial colonies were isolated and those organisms are: *Staphylococcus aureus, Proteus* sp, *Klebsiella* sp, *Pseudomonas* sp, *Escherichia coli and Bacillus* sp.

Well Diffusion method was used for checking anti-bacterial activity. Sterile Muller Hinton Agar plates were prepared. These plates were swabbed with the respective clinical isolates. Using a sterile cork borer, wells were cut on the Muller Hinton Agar plates. The biosynthesized liquid silver nanoparticles of ginger was then added, and in the remaining wells silver nitrate solution and ginger rhizome broth also added in the respective wells. The plates were the incubated at 37°C for 24 hours. Control plates with silver nitrate solution and culture broth were kept for incubation along with the test plates. After incubation the zones were examined and the zone diameter was measured. For identifying Minimal Inhibitory Concentration (MIC) various value such as 1mM, 3mM and 5mM of silver nanoparticles are added.

III. RESULT AND DISCUSSION

3.1 Isolation and Identification of bacterial pathogens from Diabetic Foot Ulcer:

The samples were collected and the isolates predominantly showed 7 bacterial colonies which are subcultured and identified based on morphological and biochemical analysis. The isolated bacterial species were examined by gram staining, some isolates showed gram negative rods and few were gram positive cocci in shape. In the study , the most common isolates were *Escherechia coli, Klebsiella* sp, *Pseudomonas* sp, *Staphyloccus aureuc, Streptococcus pyogenes, Proteus* sp *and Bacillus* sp.

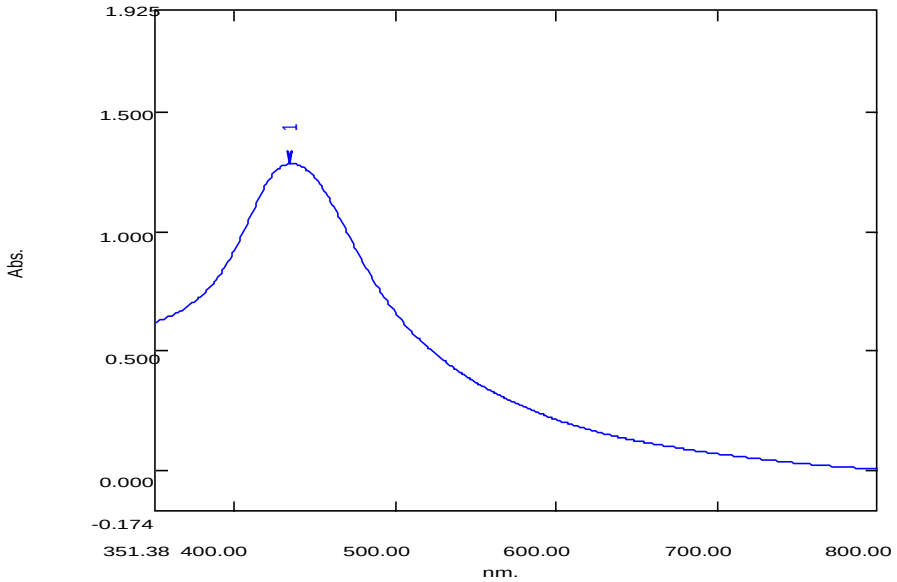
3.2 Ginger Rhizome Silver Nanoparticle:

Ginger broth and 1mM, 3mM and 5mM of Silver nitrate solution were mixed in order to reduce the silver nitrate to Ag+ ions. The process of silver reduction was taken place in magnetic stirrer at 40°C at 500 rpm. The reduction was confirmed by Colour change and UVVisible spectrophotometer , then which are characterized by SEM-EDAX and FTIR.

• **Colour Change:** The ginger broth was pale yellow in colour before the addition of AgNO3. Then colour changed into golden brown on completion of the reaction with Ag+ ions for 1.30 hours. This change in colour is primarily due to the surface plasma resonance of silver nanoparticles. In the case of silver nitrate solution alone, no change in colour was observed. The intensity of the colour increased with time. At the end of the reaction golden or brown colour was observed in the reaction mixture. Reduction of silver ions was reflected in colour change of the ginger broth from pale yellow to golden brown colour. The appearance of the brown colour indicates the silver nanoparticles formation (Nithya and Raghunathan, 2009). The periodical analysis in colour of the ginger with silver nitrate as control was observed and results were recorded.

• **UV-Spectrophotometer Absorbance:** Ginger Rhizome capped AgNPs showed absorbance peak with maximum at 435nm wavelength indicated the reduction of Silver nitrate into silver nanoparticles.(fig.1)

Figure 1: UV-Vis absorption spectra of biologically synthesized AgNPs using ginger rhizome .

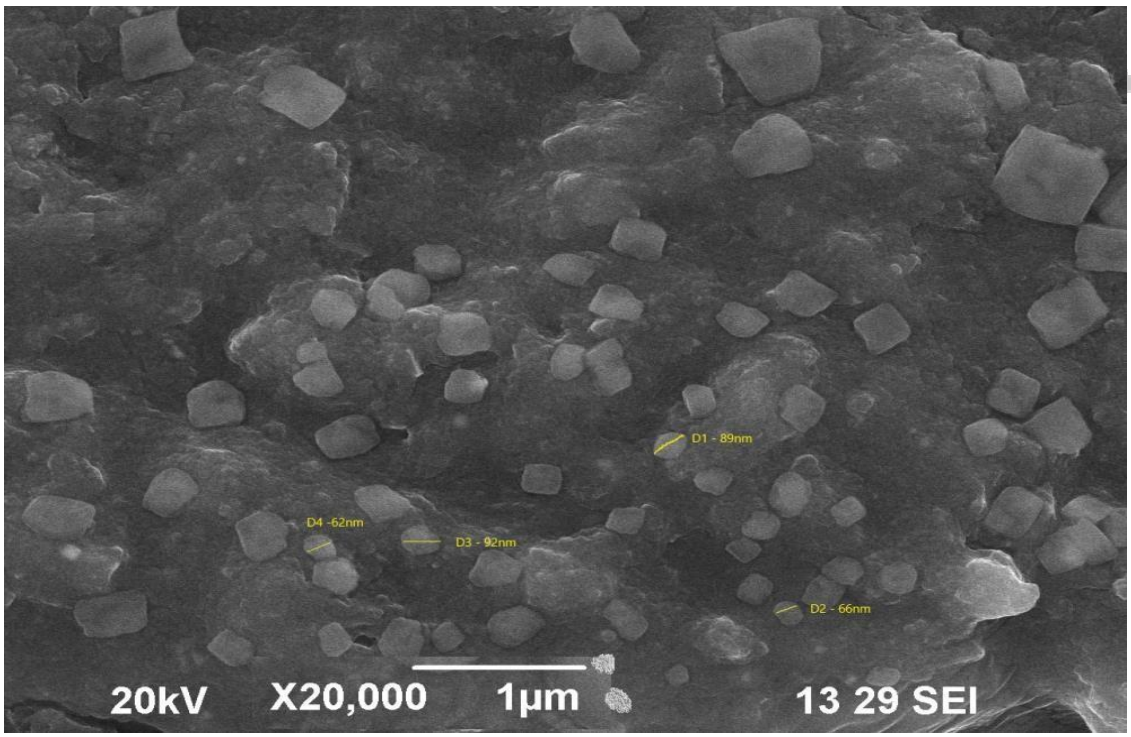


The (figure 1)reveals the UV Vis absorption peak at 435nm wavelength.

• **SEM -EDAX Analysis:**The SEM Analysis enables the high- resolution imaging of single nanoparticles (NPs) with sizes well below 10nm (V-D Hodoroaba et al.,2016). The SEM Analysis of Ginger rhizome silver nano compounds were observed. The micrograph of nanoparticles obtained, which showed that silver nanoparticles are spherical shape well distributed without aggregations in solution with an average range between 62nm-92nm.EDAX spectrum recorded in the spot- profile mode from one of the densely populated silver nanoparticles regions on the surface of film. Strong signals from the silver atoms in the nanoparticles are observed (Varshney et al., 2009).EDAX study showed strong signals from silver and from oxide. These are likely to be due to X-Ray emission from some plant compounds (fig 2 and 3) .

SEM ANALYSIS

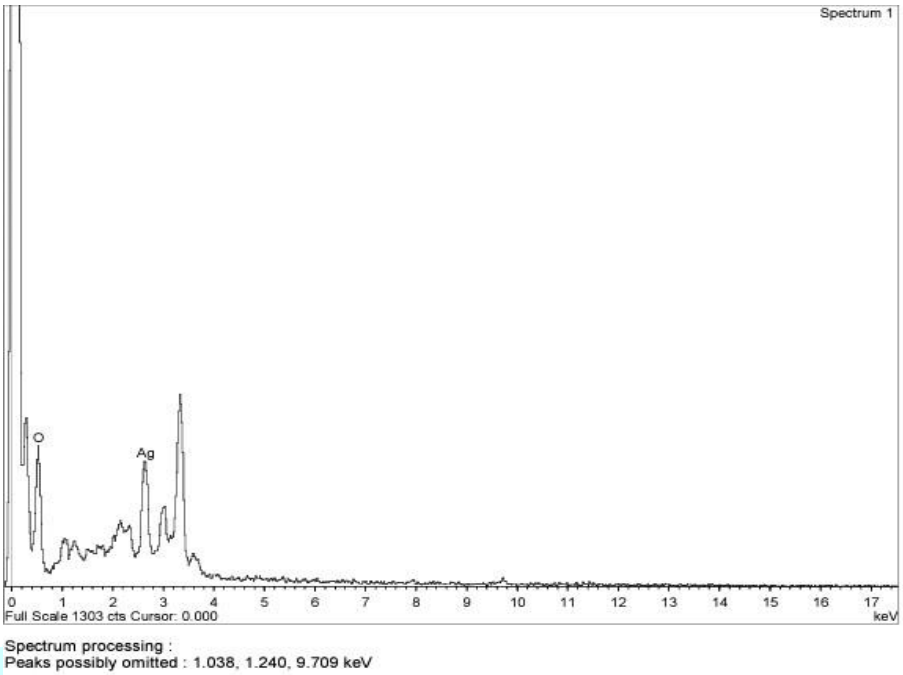
Figure 2: SEM Image of AgNPs biosynthesized using Ginger rhizome extract



The SEM image reveals the size if AgNPs ranges between 62nm-92nm.(fig.2)

EDAX Analysis

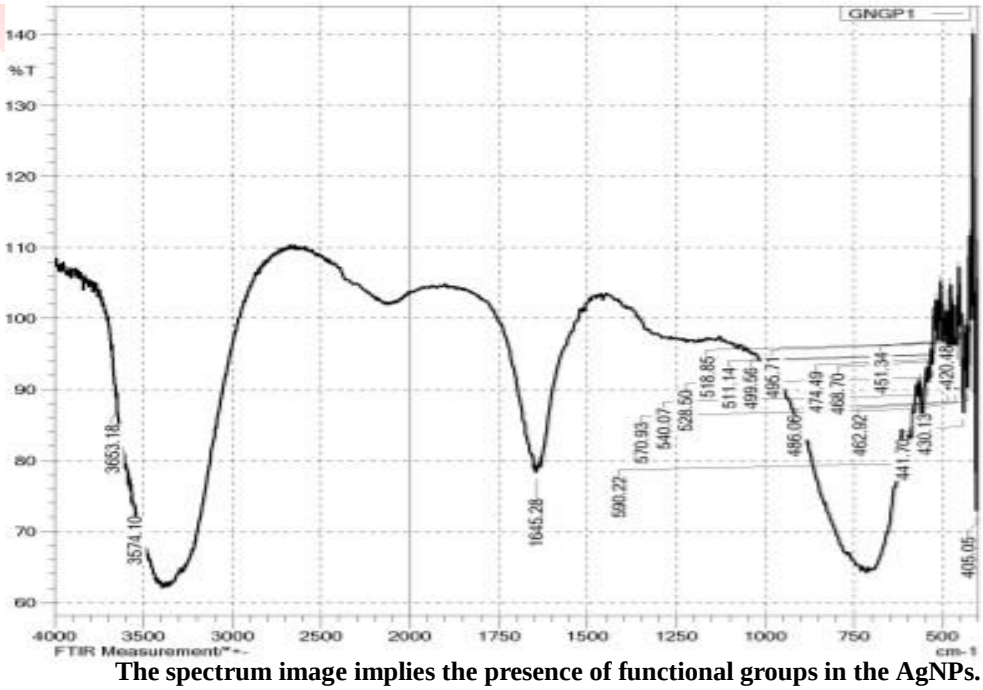
Figure 3: EDAX signals of biosynthesized AgNPs



The EDAX study showed the strong signal towards Ag=ions(fig.3).

• **FTIR:**FTIR Analysis was used for the characterization of the extract and the resulting nanoparticles. FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of metal nanoparticles synthesized by plant root extract (M. Kurian et al., 2016).FTIR measurement were performed to identify the potential biomolecules in the ginger rhizome responsible for the reduction and then for stability to the bio-reduced AgNPs. The peak (fig.4) was centered at 1645.28cm-¹ which indicates the presence of alkanes (C=C) in the residual solution. The band at 3547cm-¹ and 3653cm-¹ (fig.4) indicates OH stretching in alcohols. From the analysis of FTIR spectra, we conclude that the presence of these functional groups proves the plant extract act as capping and stabilizing agent for silver nanoparticles.

Figure 4: FTIR Spectrum of bio-synthesized silver nano particle using ginger rhizome



The spectrum image implies the presence of functional groups in the AgNPs.

3.3 Anti-Bacterial Activity:

The silver nanoparticles obtained from ginger rhizome were tested for antibacterial activity against clinical isolates of DFU (*Staphylococcus aureus*, *E. coli*, *Proteus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Bacillus* sp., *Streptococcus pyogenes*). 20mm zone of inhibition was observed in 3mM concentration (tab.1) of this formulation reveals most effective against *S. aureus.*

3.4 Minimal Inhibitory Concentration (MIC):

The MIC of silver nanoparticles from ginger was showed inhibition zone(tab.1) for 1mM (*Staphylococcus aureus*- 10mm, *E.coli*- 12mm and *Klebsiella* sp- 8mm).

For 3mM AgNPs (*Staphylococcus aureus*-20mm, *E.coli*-18mm,*Pseudomonas* sp17mm, *Klebsiella* sp-13mm and *Bacillus* sp- 16mm), zone of inhibition was observed.

For 5mM of AgNPs (*Strephylococcus aureus*-14mm, *E.coli*-13mm,*Proteus* sp12mm, *Pseudomonas* sp-10mm,*Klebsiella* sp-13mm,*Bacillus* sp-14mm and *Streptococcus pyogenes*-15mm).AgNPs most effective against all the clinical isolates at 3mM concentration when compared to other different concentration, so the MIC of ginger rhizome silver nanoparticles is 3mM concentration.

Table 1. Antibacterial Activity of biosynthesized AgNPs towards DFU pathogens

S.No	Test Organism	Zone of Inhibition (Diameter in mm)		
		1mM of AgNPs	**3mM of AgNPs**	**5mMof AgNPs**
1.	*Staphylococcus aureus*	10	20	14
2.	*Escherechia coli*	12	18	13
3.	*Klebsiella sp*	8	13	13
4.	*Bacillus sp*	-	16	14
5.	*Pseudomonas sp*	-	17	10
6.	*Streptococcus pyogenes*	-	-	15
7.	*Proteus sp*	-	-	12

The table implies the Triplicates mean values of each organism in all concentration. Average value for *Staphylococcus aureus*- 15 -, *E.coli* 14, *Klebsiella sp*- 11, *Bacillus sp*- 10, *Pseudomonas sp*- 9, *Strephylococcus pyogenes*- 5 and *Proteus sp*- 4.

SUMMARY AND CONCLUSION:

The present study was made to isolates the bacterial populations from Diabetic Foot Ulcer. And the clinical isolates are identified as *Staphylococcus aureus, E. coli, Proteus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Bacillus* sp., *Streptococcus pyogenes.*

Silver had huge antimicrobial activity, and it is a non-toxic to humans. So Silver Nanoparticles are formed in this study. Ginger Rhizome had many benefits and it also had more physiochemical properties, even it shows antibacterial activity and cost effective.

Here, Ginger Rhizome broth was prepared by adding silver nitrate solution of various concentration to form Silver Nanoparticle. Where ginger rhizome act as a capping and stabilizing agent. Magnetic Stirrer method was used in 40°C at 500 rpm for 1.30 hours.

Silver Nanoparticles were formed , which are confirmed by different characterization such as colour change, shows pale yellow to golden yellow colour. Then UV- spectrophotometer was used to prove that silver Nanoparticles were formed by absorbance peak at 435nm.

SEM performed to show the size of the silver nanoparticles which ranges from 62nm- 92nm at 20X magnification. EDAX study, showed a strong signal from silver that indicates the presence of Ag+ ions.

FTIR measurement indicates, the presence of functional group such as alkanes (C=C) and the presence of alcohol whereas some plant residues. These shows the ginger rhizome act as a capping and stabilizing agent for Silver Nanoparticles.

Antibacterial assay was performed by Well diffusion method. To find MIC at three different concentration such as 1mM, 3mM,and 5mM are plated for the isolated 7 clinical isolates. It shows a highly effective zone of inhibition for *Staphylococcus* aureus of 20mm. Finally the effective MIC is 3mM concentration. The minimal concentration of 3mM able to inhibit the causative agent of Diabetic Foot Ulcer. Further, study of toxicity checking and animal studies for the product formation will be performed later.

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V. Conflicts of Interest

The authors have no conflicts of interest to publish this research article in this journal

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