GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING GUM ODINA AND ITS ANTIBACTERIAL EVALUATION

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CERTIFICATE OF APPROVAL

This is to certify that the thesis entitled **"Green synthesis of silver nanoparticles by using gum odina and its antibacterial evaluation"** submitted to Jadavpur University, Kolkata for the partial fulfilment of the Master degree in Pharmacy, is a faithful record of bonafied and original research work carried out by **Sudipto Mandal** under my supervision and guidance.

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I, **Sudipto Mandal.**, a student of Master of Pharmacy, 4th semester, bearing Roll No. **M4PHA1607** , Department of Pharmaceutical Technology, Jadavpur University, Kokata declare that my thesis work titled - **"Green synthesis of silver nanoparticles by using gum odina and its antibacterial evaluation."**, is original and presented in accordance with academic rules and ethical conduct and no part of this project work has been submitted for any other degree of mine. All the given information and works are true to the best of my sense and knowledge.

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I deem it a privilege to work under the guidance of **. Prof. (Dr.) Amalesh Samanta,** Division of Microbiology, Department of Pharmaceutical Technology, Jadavpur University on the very ongoing current topic **"Green synthesis of silver nanoparticles by using gum odina and its antibacterial evaluation."**. I am blessed to work under his inspiring guidance, constructive and valuable suggestion throughout my work. His expertise supervision with undue patience fathered my work at every stage, without his engagement I would not be able to complete my task further.

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Sudipto Mandal

M4PHA1607

DEDICATED TO MY

BELOVED PARENTS

AND

MY RESPECTED

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CHAPTER 1

1. INTRODUCTION

The nanometerials have a great health impact in the environment so it become an important topic in recent year. Every year, different type of nano-materials are synthesized. Their type of application is several and more importance to our daily life. "Nanotechnology is the application of science to control matter at the molecular level". Developments in nanotechnology has opened up novel fundamental and applied frontier in engineering and materials science, such as bionanotechnology,[1] and applied microbiology[2]. Due to size and shape-dependent properties nanoparticles are of great interest in advanced reserch .The physical and chemical differences in their properties depends on to their extremely small size and large surface to volume ratio compared to bulk of the same chemical composition. [3] Therefore, production of materials and design with there novel applications can be achieved by controlling shape and size in nanometre scale.. Nanoparticles are of interest or application ranging from biosensing and catalysts to antimicrobial activity depend on the property of size and shape. These particles also have many application in different fields such as nano composites filters drug delivery, medical imaging.[4] Metal nanoparticles have a important applications for in medicine and pharmacy. Silver and gold nanoparticles are the most common ones used for biomedical applications and in emerging inter disciplinary field of nano biotechnology. The synthesis and research of metal and semiconductor nanoparticles have a potential applications which was implemented in the development of novel technologies. The nanotechnology field is a one of the advance areas of research in the modern field of material science. The completely new or improved properties of nanoparticle, are size, distribution and morphology of the particles etc. The novel applications of nanomaterials and nanoparticles are emerging rapidly on different fields. The tremendous applications of Nano-crystalline silver particles have been found in the fields of diagnostics, high sensitivity biomolecular detection, therapeutics, antimicrobials, micro-electronics and catalysis. However, economic commercially viable as well as environmentally clean synthesis route is still required to synthesize the silver nanoparticles. Silver have well known an inhibitory effect toward many bacterial strains and microorganisms commonly present in industrial processes and medical. [5]The chemical route is adopted for the synthesis of various NPS In most of the cases; however, the chemical route has potential hazards to health and environment. Hence the green synthesis of nanoparticles has emerged on one of the active areas of resarch in recent years. It has no

potential hazards as well as chemical synthesis and several advantages. Such as cost effectiveness, simplicity as well as compatibility for pharmaceutical and bio medical application.[6]

1.1 Historical perspective

Nanoparticles have been use in medicine and pottery from the ancient times. There are several historical evidences suggest that during the priod of 2500 BC the Gold nanoparticles were used as drug by Chinese. Red colloidal gold is still in use under the name of *Makaradhwaj* and *a Swarna Bhasma*" in traditional medicine system of India called Ayurveda, which dates back to 1st millennium BC. [7] In 16th Century Europe an aqueous form of colloidal gold called "Aurum Potabile (drinkable gold)" was thought to have curative properties for many diseases. [8] In 1857 Michael Faraday described the methods for the synthesis of stable aqueous dispersions and optical properties of gold nanoparticles (Faraday 1857). However the credit of realising the enormous potential of nanoparticles and their possible implications in different fields was first highlighted to Richard P. Feynman. In his classical lecture in 1959 at California Institute of Technology (Caltech) during Annual meeting of the American Physical Society. Because of such brilliant foresight and visionary thinking about the properties of nanoparticle and their future implications, Feynman is often referred as Father of the field of nanoparticle research.[9] Later the book"Engines of Creation: The Coming Era of Nanotechnology" Published by Eric Drexler which brought Feynman's vision to a broader audience (Wilsdon 2004). It give a detail concepts on nanoparticle, its potentials and even dangers if it is misused.

1.2 Nanoparticles

The term "nanoparticles" describe, that the particle size range from 1nm to 100nm, at least in one of the three possible dimensions. The size range is great interest because of the physical, chemical and biological behavior of the nanoparticles changes in fundamental ways from the properties of both individual molecules and of the bulk materials. Nanoparticles can be made of

materials of defferent chemical nature, the most common being metals, metal oxides, non-oxide ceramics, silicates, organics, polymers, carbon and biomolecules. Nanoparticles haves different morphologies such as cylinders, spheres, platelets, tubes etc. The nanoparticles has a enormous diversity. **(**Figure. 1) It has wide chemical nature, shape and morphologies, so that the medium in which the particles are present in the form of dispersion of the particles . The surface modifications of the nanoparticles is an important active field of science now-a-days.

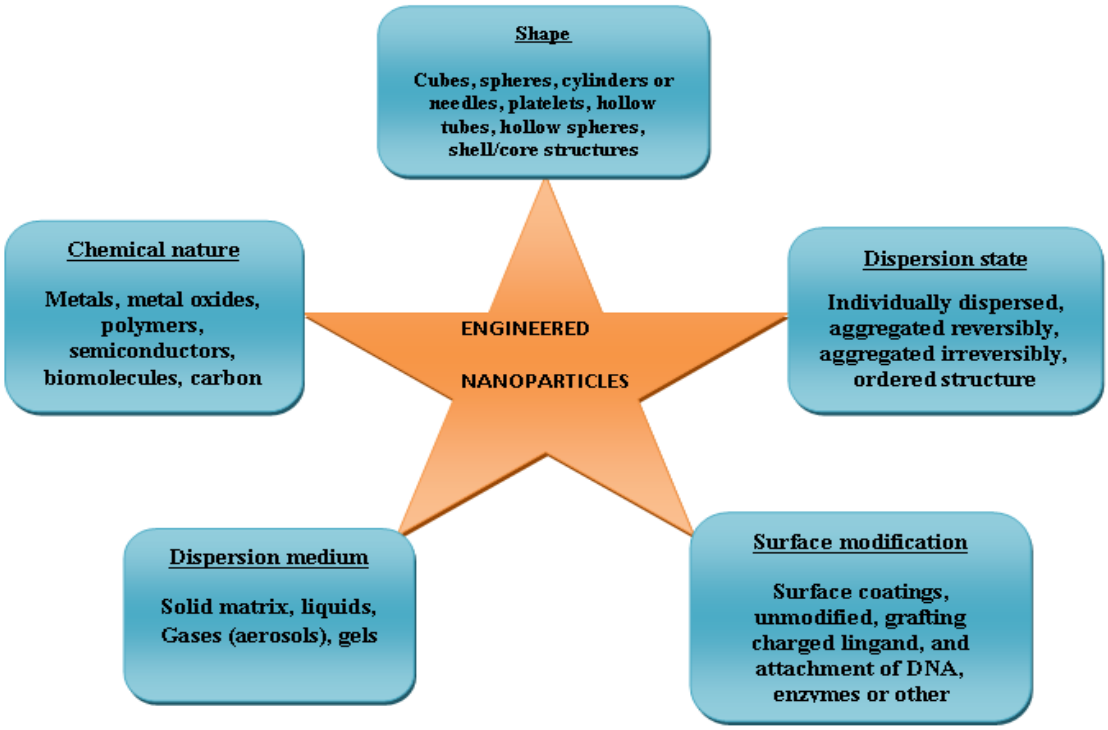


Fig. 1: Various features contributing to the diversity of engineered nanoparticles. The same chemical can generate a wide variety of nanoparticles.

1.3 Application of nanoparticles

When the particles are more divided its surface area will more increase compare to bulk molecule which enhances the activity of the particle in nano dimension therefore properties of the particle like mass transfer, catalytic activity etc are all increases. Metal nanoparticles have more industrial application then non –metal nanopartical. Nanoparticles enhance the research and development in the field of biomedicine and bio-nanotechnology and other specific area like

- As medical diagnostic tools,
- Drug delivery,
- As a cancer treatment agent.

1.4 Types of nanoparticles

Nanoparticles can be broadly divided into two grouped

- Organic nanoparticles : it is include
    - carbon nanoparticles (fullerenes)
- inorganic nanoparticles : it include
    - magnetic nanoparticles
    - noble metal nanoparticles : e.g. Gold, silver
    - semi-conductor nanoparticles :e.g titanium oxide , zinc oxide.

Inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases. so, Inorganic nanoparticles like of noble metal nanoparticles (Gold and silver) are growing interest in research. It have advantages over available chemical imaging their size features. [10]

1.5 Silver nanoparticles

Silver nanoparticles are of more interest because of the unique properties (*e.g.*, size and shape depending electrical, optical, and magnetic properties) which can be incorporated into antimicrobial applications, cosmetic products, biosensor materials, composite fibers, cryogenic superconducting materials, and electronic components. [11] Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles. Recently, nanoparticle can synthesis by green synthesis method. Green synthesis approaches include polysaccharides, mixed-valence polyoxometalates, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity.

1.6 Synthesis of silver nanoparticles

▪ Physical approaches

- Evaporation-condensation

One of the important physical approaches include evaporation-condensation.Various metal nanoparticles such as silver, lead sulfide, gold, cadmium sulfide, and fullerene have previously been synthesized using the evaporation-condensation method.The absence of solvent contamination in the prepared thin films and the uniformity of nanoparticles distribution are the advantages of physical approaches in comparison with chemical processes.[12] It was demonstrated that silver nanoparticles could be synthesized via a small ceramic heater with a local heating source. [13] The temperature gradient in the vicinity of the the evaporated vapor can cool at a suitable rapid rate heater surface is very steep in comparison with that of a tube furnace so that the. So this prosses makes possible the formation of small nanoparticles in high concentration. This type of physical method can be useful as a nanoparticle generator for long-term experiments for inhalation toxicity studies, and also used as a calibration device for nanoparticle measurement equipment. [13]

- Laser ablation

The other physical approach include lesar ablation technic. Laser ablation of metallic bulk materials in solution could be synthesized Silver nanoparticles . The ablation efficiency and the characteristics of produced nanosilver particles depend upon many factors such as the duration of the laser pulses (in the femto-, pico- and nanosecond regime), the wavelength of the laser impinging the metallic target, the ablation time duration, the laser fluence and the effective liquid medium, with or without the presence of surfactants.[14] The important advantage of laser ablation technique is that production of metal colloids is the absence of chemical reagents in solutions. so the, pure and uncontaminated metal colloids for further applications can be prepared by this lesar ablation technique.[15]

▪ Chemical approaches

The chemical approach for synthesis of silver nanoparticles is reduction by organic and inorganic reducing agents. Different reducing agents such as ascorbate, sodium citrate, sodium borohydride (NaBH4), elemental hydrogen, Tollens reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers are used for reduction of silver ions (Ag+) in non-aqueous oraqueous solutions. The aforementioned reducing agents reduce silver ions (Ag+) and it formed the metallic silver (Ag0). The metallic silver (Ag0) is followed by agglomeration into oligomeric clusters. These clusters formation of metallic colloidal silver particles. [16] The protective agents used to stabilize dispersive nanoparticles during the course of metal nanoparticle preparation, and its protect the nanoparticles that can be absorbed or bind onto nanoparticle surfaces,and also avoiding their agglomeration. The presence of surfactants comprising functionalities like thiols, amines, alcohols and acids for interactions with particle surfaces.which can be stabilize particle growth, and protect particles from agglomeration, sedimentation, or losing their surface properties. Recently, Tollens method, has been used for the synthesis of silver nanoparticles with a controlled size. In the modified Tollens procedure in the presence of ammonia,the silver ions are reduced by saccharides,and yielding silver hydrosols (20-50 nm) , silver nanoparticle films (50-200 nm), and silver nanoparticles of different shapes.[17]

▪ Biological approaches

In recent years, the development of green chemistry methods employing natural reducing, stabilizing, and capping agents to prepare silver nanoparticles. Biological methods can be used to synthesize silver nanoparticles without the use of any toxic and chemical substances. [18]

➢ SYNTHESIS OF SILVER NANOPARTICLES BY BACTERIA

There are some microorganisms that can survive metal ion concentrations and can also grow under those conditions, and this phenomenon is due to their resistance to that metal.The mechanisms involved in the resistance are alteration of solubility and toxicity via reduction or oxidation, efflux systems, bioaccumulation, biosorption, extracellular complex formation or precipitation of metals, and lack of specific metal transport systems. The first evidence of bacteria synthesizing silver nanoparticles was established using the *Pseudomonas stutzeri* AG259 strain which was isolated from silver mine. The most widely accepted mechanism of silver biosynthesis is the presence of the nitrate reductase enzyme. The enzyme converts nitrite from nitrate. There is a using bacteria, in in vitro synthesis of silver the presence of alpha-nicotinamide adenine dinucleotide phosphate reduced form the enzyme (NADPH) - dependent nitrate reductase would remove the downstream processing step that is required in other cases. [19]

➢ SYNTHESIS OF SILVER NANOPARTICLES BY FUNGI

Fungi can produce larger amounts of nanoparticles in comparison with bacteria, because they can secrete larger amounts of proteins which directly translate to higher productivity of nanoparticles.[20] The mechanism of silver nanoparticle production by fungi is trapping of Ag+ ions at the surface of the fungal cells and the subsequent reduction of the silver ions by the enzymes present in the fungal system . Though the exact mechanism involved in silver nanoparticle production by fungi is not fully understood, it is believed that the above mentioned phenomenon is responsible for the process.

- SYNTHESIS OF SILVER NANOPARTICLES BY PLANTS

The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, nontoxic and safe. They have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. The main mechanism considered for the process is plant-assisted reduction due to presence of different phytochemicals e.g. flavones, ketones, aldehydes, terpenoids, amides, and carboxylic acid. It was suggested that the phytochemicals are directly involved in the reduction of the ions and formation of silver nanoparticles. [21]

1.7 **Need for green synthesis**

Biosynthesis of nanoparticles is a kind of bottom up approach where it is a simple reduction/oxidation process. The need for biosynthesis of nanoparticles by physical and chemical processes was costly. Often, chemical synthesis method also has adverse effect in the medical applications . But biosynthesis of nanoparticles via green synthesis route there is no adverse effect. [22] Green synthesis provides advancement over physical and chemical method as it is environment friendly, cost effective, easily scaled up for large scale synthesis and in this method there is no need to use of energy, high pressure, temperature and toxic chemicals .

1.8 **Nanosilver**

The silver (nanosilver) used in nanoformulation due to its antimicrobial properties. The metallic silver converted to nanosilver by several methods; include , solution irradiation, electrochemical reduction, spark discharging, and cryo- chemical synthesis .The size of Nano-silver particles are smaller than 100 nm and consist of about 20-15,000 silver atoms . Nanosilver is nowadays used in an increasing number of consumer and medical products due to the properties of silver at the nanoscale. The strong antimicrobial activity is the major direction for development of nano-silver products. Examples are food packaging materials, medical advices, cosmetics, electronics, room sprays and water disinfectants.

Why silver?

Silver is one of the basic elements in our planet. It is a naturally occurring element, slightly harder than gold and very ductile. Pure silver has the highest electrical and has the lowest contact resistance. Silver have different oxidation states: Ag0, Ag2+, Ag3+. The first two are the most abundant ones, the latter are unstable in the aquatic environment. Metallic silver is insoluble in water, but metallic salts such as AgNO3 and Silver chloride are soluble in water (WHO, 2002). Metallic silver is used for the splints, surgical prosthesis and fungicides. Soluble silver compounds such as silver slats, have been used in treating epilepsy, mental illness, gastroenteritis, nicotine addiction, and infectious diseases including gonorrhea and syphilis. Although acute toxicity of silver in the environment is dependent on the availability of free silver ions. The wide variety of silver allows exposure through various routes of entry into the body. Ingestion is the primary route for entry for silver compounds. Dietary intake of silver is estimated at 70-90μg/day. Since silver is not considered to be carcinogenic, therefore silver is relatively non-toxic. Silver demand will likely to rise as silver find new uses, particularly in medical industries, textiles, plastics and changing the pattern of silver emission as these technologies and products diffuse through the global economy.

1.9 Action of silver nanoparticles on microbes

The exact mechanism which to cause antimicrobial effect of silver nanoparticles is not clearly known. However there are various theories on the action of silver nanoparticles on microbes to cause the microbicidal effect. Silver nanoparticles have the ability to anchor to the bacterial cell wall and penetrate it, thereby it causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. Formation of pits on the cell surface, and there is accumulation of the nanoparticles on the cell surface.[23] The formation of free radicals by the silver nanoparticles , and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death. [24]The bacterial cells in contact with silver take in silver ions, which inhibit several functions in the cell of bacteria and damage the cells. The interaction of the silver nanoparticles with the sulfur and phosphorus of

the DNA can lead to problems in the DNA replication of the bacteria cell and thus terminate the microbes. [25] (Figure 2)

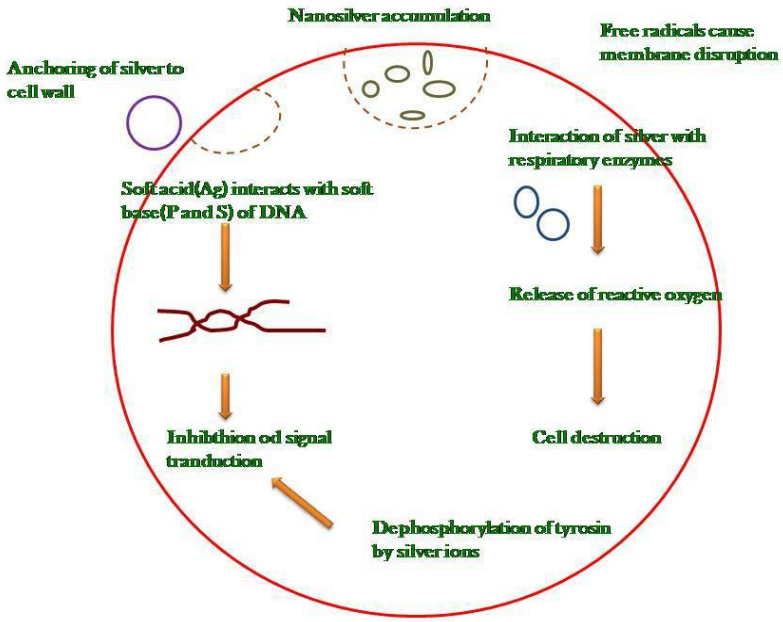


Fig. 2: Various modes of action of silver nanoparticles on bacteria.

1.10 Applications of silver nanoparticles and their incorporation into other materials

Nanoparticles are of great interest due to their extremely small size and large surface area and both chemical and physical differences in their properties compared to bulk of the same chemical composition, such as biological, mechanical, and catalytic activity, sterical properties, thermal and electrical conductivity, optical absorption and melting point . Nanoparticles exhibit size and shape-dependent properties which are of interest for applications ranging from antimicrobial activity, biosensing and catalysts to optics, electrometers, chemical sensors, and wireless electronic logic. These particles also have many applications in different fields such as medical imaging, drug delivery, hyperthermia of tumors nano-composites, and filters. Silver nanoparticles have been used extensively as antimicrobial agents in health industry, textile

coatings, food storage, and a number of environmental applications. Antimicrobial properties of silver nanoparticles caused the use of these nano-metals in different fields of medicine, cosmetics, health and military various industries, animal husbandry, packaging, accessories. For instance, it was shown that silver nanoparticles mainly in the range of 1-10 nm attached to the surface of *E. coli* cell membrane, and disturbed its proper function such as permeability and respiration (Figure. 3).

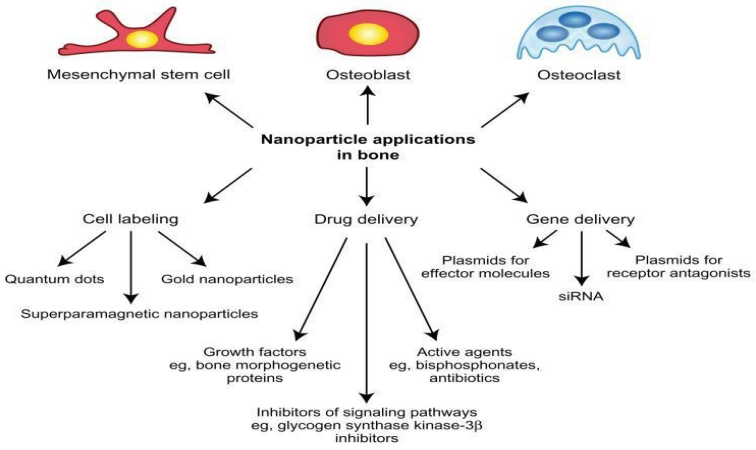


Fig 3: Overview of nanoparticle applications.

In general, therapeutic effects of silver particles (in suspension form) depend on important aspects, including), particle shape (catalytic activity), particle size (surface area and energy particle concentration (therapeutic index) and particle charge (oligodynamic quality). [26] Mechanisms of antimicrobial effects of silver nanoparticles are still not fully understood, but several studies suggest that silver nanoparticles may attach to the negatively charged bacterial cell wall and rupture it, which leads to denaturation of protein and finally cell death (Table 1). Catalytic mechanism of silver nanoparticle composites and their damage to the cell by interaction with sulfur and phosphorous containing compounds such as DNA have been also investigated. [27] Another mechanism involves the silver association with oxygen and its reaction with sulfhydryl groups on the cell wall to form R-S-S-R bonds, for that it cause blocking respiration and causing cell death (Table 2). [28]

Table 1: Applications of silver nanoparticles in pharmaceutics, medicine, and dentistry

PHARMACEUTICS & MEDICINES	➢ **Treatment of dermatitis; inhibition of HIV-1 replication** ➢ **Treatment of ulcerative colitis & acne** ➢ **Remote laser light-induced opening of microcapsules** ➢ **Detection of viral structures (SERS & Silver nanorods)** ➢ **Antimicrobial effects against infectious organisms** ➢ **Silver/dendrimer nanocomposite for cell labeling** ➢ **Molecular imaging of cancer cells** ➢ **Coating of hospital textile (surgical gowns, face mask)** ➢ **Additive in bone cement** ➢ **Implantable material using clay-layers with starch-stabilized Ag NPs** ➢ **Orthopedic stocking** ➢ **Hydrogel for wound dressing**
DENTISTRY	✓ **Additive in polymerizable dental materials Patent** ✓ **Polyethylene tubes filled with fibrin sponge embedded with Ag NPs dispersion** ✓ **Silver-loaded SiO2 nanocomposite resin filler (Dental resin composite)**

Table 2: Mechanisms of antibacterial effects of silver nanoparticles

Mechanisms of Antibacterial Effects of Ag NPs
Cell death due to induction of free radical formation
Cell death due to uncoupling of oxidative phosphorylation
Interference with respiratory chain at Cyt C level
Interference with components of microbial ETS
Interactions with protein thiol groups & membrane bound enzymes
Interaction with phosphorous- and sulfur-containing compounds such as DNA

1.11 Toxicity of silver nanoparticles

The silver nanoparticles are excellent candidates for many purposes in the medical field depends on its unique physical and chemical properties. But there are many reports suggest that nanosilver cause adverse effects on humans as well as the environment. It is estimated that tonnes of silver are released into the environment from industrial wastes, and also the toxicity of silver in the environment is due to free silver ions in the aqueous phase. The adverse effects of these free silver ions on humans and living body include permanent bluish-gray discoloration of the eyes (argyrosis),or the skin (argyria) and exposure to soluble silver compounds may produce toxic effects like kidney and liver damage; skin,eye, respiratory, and intestinal tract irritations; and changes in blood cells. [29] Nanosilver aggregates are said to be more cytotoxic than asbestos. Nanosilver have also toxic effects on the male reproductive system. Research shows that nanosilver can cross the blood-testes barrier and it deposited in the testes where they adversely affect the sperm cells.[30] Nanosilver with its antimicrobial activity can hinder the growth of many "friendly" bacteria in the soil. Due to Loss of environmental denitrification through reduction of plant productivity can lead to eutrophication of lakes ,rivers, and marine ecosystems and destroy the ecosystem. Nanosilver also has toxic effects on aquatic animals because silver ions can interact with the gills of fish and inhibit basolateral Na+-K+-ATPase

activity, which can lead to inhibit osmoregulation in the fish. [31] Though many studies suggest that nanosilver can induce toxicity to living beings, and also it has to be understood that the studies on nanosilver toxicity were done in vitro conditions which are different from in vivo conditions.

1.12 Green synthesis of silver nanoparticles by using gum

The term "gum" is used to describe a group of naturally occurring polysaccharides and/or proteins that's originated from different sources (i.e. plant, animal, and microbial). Natural plant gums are usually safe for oral consumption and are analogous synthetic gums also preferred due to their safety (non-toxic), low cost and availability.[32] Plant gums are usually heteropolysaccharide gums composed of simple hexoses and deoxy sugar units such as glucose, arabinose, mannose, xylose, galactose, uronic acids and etc. depends on their biological properties, chemical composition and molecular structure of polysaccharide plant gums play a significant role . In fact, the functional properties of polysaccharide plant gums are governed by the molecular weight, sequence of monosaccharide, chemical composition, configuration of glycoside linkages, and the position of glycoside linkages in the side chains and backbone. [33] The main goal of the current study was to investigate the isolation , purification characterization of gum odina(Figure 4(a)) and also study the synthesis of silver-nano by using this gum.Green synthesis of silver nanoparticles(AgNps)by using plant leaf extracts, plant latex, microorgaisms and some biopolymer have been reported earlier[34] polymer such as PVP,[35] PAN[36] have been widely used as reducing and stablising agent for synthsis of well dispersed AgNps. Biopolymer like natural rubber, [37] polysaechonides, [38] gum acacia polymer[39] have been used as matrics or stabilizer for synthesis of NPS because of their biocompatibility and nontoxic nature. However, Gum odina has not been explored yet as a reducing & stabilizing agent for AgNps. In recent past described the use of gum odina as an excellent substitute of starch paste as a tablet binder. [40] *Odina wodier,* Roxb. family *Ana-cardiaceae* is a large tall tree (Figure 4(b)) found in de-ciduous forest in India, Myanmar, Srilanka, China, Malaysia, Cambodia and Philippine Islands. [41] It is popularly known as Kashmala, Odimaram, Jiol in local language and in English it is called Rhus olina. [42] Various parts of this plant have been found to be used as medicines in Ayurveda. The leaves have been reported to use in Elephantiasis of the legs. [42] Juice of green branches is used

as an emetic in case of coma or insensibility produced by narcotic. The dried and powdered bark is found to use as tooth

(a) **(b)**

Fig 4. (a) Transparent reddish brown needle shape gum liberating from the bark of the plant; (b) Tree of *Odina wodier*, Roxb., family *Anacardiaceae.*

powder by poor villagers. [41] The bark extract has been reported to be useful in vaginal trouble, curing ulcer, heart diseases etc. [43] we report the characterization of Gum odina & application of AgNps for the development of Gum based Nanoparticles. The natural availability, non-toxic nature, low cost and medicinal values of Gum odina intrigued us to use this biopolymer for the silver nanoparticle synthesis. In this context, we have designed a facile, biosynthetic route for the production of silver nanoparticles employing a renewable, biodegradable natural plant polymer, Gum odina as both the reducing and stabilizing agent. The intend of the present study was on the synthesis, characterization of gum odina, and synthesis of silver nanoparticles. We have also shown the antibacterial activity of the prepared nanoparticles on Gram-positive and Gram-negative bacteria for prospective biological applications.

CHAPTER 2

2. LITERATURE REVIEWS

Vigneshwaran et al.[44] explained a novel one-pot 'green' synthesis of stable silver nanoparticles using soluble starch.They synthesized the Stable silver nanoparticles by using soluble starch as both the reducing and stabilizing agents. In this report,the aldehyde terminal of soluble starch is used to reduce silver nitrate while the starch itself stabilized the silver nanoparticles..This reaction was carried out in an autoclave at 15 psi, 121 °C for 5 min. Nanoparticles thus prepared are found to be stable in aqueous solution over a period of three months at room temperature (25° -C). The size of these nanoparticles was found to be in the range of 10–34 nm as analyzed using transmission electron micrographs. The X-ray diffraction analysis revealed the face-centred cubic (fcc) geometry of silver nanoparticles.

Murali Mohan et al.[45] reported a facile approach for the spontaneous formation of silver nanoparticles in the presence of gum acacia polymer as a natural polymer without the addition of any typical reducing agent under mild conditions. Silver nanoparticles (~ 5 nm) have been obtained by the mixing of equal amounts of 0.5 wt % aqueous solutions of acacia and silver nitrate. The formation of silver nanoparticles has been confirmed with ultraviolet–visible, Fourier transform infrared, X-ray diffraction, and X-ray photoelectron spectroscopy analyses.

Iravani et al.[2] showed that most of the plant used in metal nanoparticle synthesis, are more stable and the rate of synthesis is faster than in the case of microorganisms. He has investigated in order to find an eco-friendly technique for production of well-characterized nanoparticles.

Samanta et al.[43] investigated the efficacy of gum odina as new pharmaceutical excipients, in particular, as an emulsifying agent.

Sinha et al.[46] studied & establish the potential of Odina gum as a novel pharmaceutical aid for development of controlled release drug delivery systems. They influence of varying the proportion of the gum, the nature of diluents and their ratio in the preparation was also evaluated.

Kostadinovic et al.[47] studied the chemical composition and molecular structure of polysaccharide-protein biopolymer from *Durio zibethinus* seed and its extraction and purification process. The main objective of the present study was to characterize the chemical and molecular structure of a natural biopolymer from *Durio zibethinus* seed. In addition, the extraction and further processing conditions significantly influence the chemical and molecular structure of the plant biopolymer.

Bhattacharyya et al.[48] investigated the gum and they also reported arabinose, galactose, and galacturonic acid as constituent units. The gum examined had been purified by repeated precipitation by ethanol from an aqueous solution and alkaline solution was avoided to protect from deesterification.

Jindal et al.[49] investigated the physical properties of bael fruit (*Aegle marmelos*). They exhibited fair flow (angle of repose 37.2°) and moderate compressibility (Carr's Index 17.65%) desired of an excipient. High swelling index (4.2) indicated its promising functionality in pre-disperse systems.

Malsawmtluangi et al.[50] studied and investigated The physicochemical properties of *Prunus cerasoides* D. Don gum exudates. Determination of the angle of repose, Carr's index, Hausner ratio and Swelling index indicate the gum possess fairly good powder flow property.

Kamboj et al.[51] investigate the physicochemical, rheological and antioxidant potential of corn fiber gum.various attempt was made to understand various properties of CFG to promote its uses for food and pharmaceuticals. A good flow property was indicated from the results of % compressibility (12.9%), Hausner ratio (1.14) and angle of repose (35.45_) in comparison to beal fruit gum [% compressibility (17.65%), Hausner ratio (1.21) and angle of repose (37.2_)]. Further, Reff.p (1.97 mm), Swelling index (SI)(1.51 +_ 0.12) suggested highly porous nature of CFG which makes CFG acceptable for the use of diluent in various pharmaceutical preparations.

Tagad et al.[52] reported a green approach for the synthesis of silver nanoparticles (Ag NPs) by using locust bean gum (LBG). Ag NPs were synthesized by mixing optimized weight percent of LBG with a known quantity of silver nitrate ($AgNO_3$) at 55–60°C. Synthesized Ag NPs were characterized by UV–vis spectroscopy. The size of synthesized Ag NPs was in the range of 18–51 nm depending upon the concentration of LBG and $AgNO_3$.

Kora et al.[53] has been developed a facile and ecofriendly green method for the synthesis of silver nanoparticles from silver nitrate using gum ghatti (*Anogeissus latifolia*) as a reducing and stabilizing agent.The influence of gum concentration on the morphology of the nanoparticles was investigated with 0.1% gum and 1 mM AgNO3, autoclaved for 30 min. UV–visible spectroscopy, were used to characterize the synthesized nanoparticles.

Kora et al.[54] developed an ecofriendly method for the synthesis of silver nanoparticles from silver nitrate using gum kondagogu (*Cochlospermum gossypium*) a natural biopolymer, as a reducing and stabilizing agent. The influence of different parameters such as gum particle size,

concentration of gum, concentration of silver nitrate and reaction time on the synthesis of nanoparticles was studied. The synthesized nanoparticles were characterized using UV–visible spectroscopy. The synthesized silver nanoparticles had significant antibacterial action on both the Gram classes of bacteria.

Kaviya et al.[55] reported the Synthesis of silver nanoparticles (AgNPs) using *Polyalthia longifolia* leaf extract as reducing and capping agent along with Dsorbitol used to increase the stability of the nanoparticles . The reaction is carried out at two different concentrations (10−3M and 10−4 M) of silver nitrate, and the effect of temperature on the synthesis of AgNPs is investigated by stirring at room temperature 25°C and at 60°C. The UV-visible spectra of NPs showed a blue shift with increasing temperature at both concentrations.

CHAPTER 3

3 MATERIALS AND METHOD

3.1 Materials

3.1.1 Isolation of the gum

The absolute ethanol(99.9%) were purchased from Merk Specialities, Mumbai, India. Dry ether, glacial acetic acid and hydrochloric acid were procured from Thermo Fischer scientific, India. Acidified ethanol(1.0M HCL) prepared by acidified 50% v/v ethanol in Milli-Q water then adding 4.4 ml concentrated HCl (10N) to 1 liter of 50% v/v ethanol. De-ionized (Milli-Q) water was used for all experiments. All other chemicals used were of analytical reagent grade. Used fine Muslin cloth for filtering.

3.1.2 Characterization of gum

Source: After isolation & purification of the gum, it dried on B.O.D. shaker incubator. Then collected the dry gum sample.

Materials – Dried gum odina sample, Milli –Q water, alcohol, n-hexane.

Apparatus – Beaker, Funnel, Measuring cylinder, Pipette, Micropipette tips (white 2 ml), conical flask, petri plates, whatman filter paper,ruller scale.

Equipments – B.O.D. shaker incubator, Mechanical grinder, standard sieve according to B.S.S. 4.10/69 Grade (sector india), Bulk & tapped density machine (Model-Vtap/Matic-I), Heating mantel, thermometer.

3.1.3 Silver nanopariticle synthesis & characterization

Source: After isolation & purification of the gum, it dried on B.O.D. shaker incubator. Then collected the dry gum sample. The dry gum sample of Gum Odina was powdered in a

high-speed mechanical blender and sieved to obtain a mean particle size of 300-38µm.

Materials – Dried gum odina powdered sample , Milli –Q water , alcohol , n-hexane. Silver nitrate ($AgNO_3$,analytical-reagent-grade) was purchased from E. Merck, Mumbai,India . Whatman filter paper.

Apparatus – Beaker, Funnel, Measuring cylinder, Pipette, Micropipette tips (white 2 ml), conical flask, petri plates, ruller scale. Centrifugal tube,Test tube.Drug plate ,Tray.

Equipments – B.O.D. shaker incubator, Mechanical grinder, standard sieve according to B.S.S. 4.10/69 Grade (sector india), Bulk & tapped density machine (Model-Vtap/Matic-I), Heating mantel, thermometer,Autoclave,Hot air oven, Incubator, Mechanical stirrer, Multiskan GO (Thermo scientific) , Heraeus Biofuge Stratos centrifuge (Thermo scientific).

Composition of media:

Nutrient Broth

Ingredients	**Gms / Litres**
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Final pH (at 25 ^{o}C) 7.4 ± 0.2	

3.2 Experimental Method

3.2.1 Collection of gum odina

The sample of the gum odina were obtained as an exudate from the tree Odina wodier , family Anacardiaceae. The gum was colleceted from Mathurapure of South 24 Pargana District, West Bengal during the month of August-October. It was collected in the dry condition. After collection the gum was dried in B.O.D. shaker incubator at 40 ºC for 3 to 5 days in Microbiology laboratory Department of Pharmaceutical Technology Jadavpur University.

3.2.2 Isolation and purification of the gum

The gum was purified as per the method reported earlier by Bhattacharyya et al.[48] Brifly, 160 gm of raw gum exhudates dissolved in 100 ml of Milli-Q water for overnight swelling . Then the viscous solution formed which vigorously stirred at room temperature for 6 hour by mechanical stirrer at 750 rpm. We get the brown colour homogenized viscous solution then it was filtered through fine muslin cloth. A clear solution was added slowly into acidified ethanol (p^H 4-5). Then a white amorphous precipitate was formed. Then collected the precipitate and further treatment with absolute ethanol & dry ether. After 24 hour a brown colour precipited was formed which is dried in B.O.D. incubator shaker at 35°C. Yield 53.92 gm of raw gum powder.

3.3.3 Sieving

The powder was pulverized in a mechanical grinder. Then 20 gm. of the powder was sieved through Sieve No.44 & 85. Then the powder obtained after sieving was again passed through a fine sieve to obtain powder of 38 µm particle size which was used for nanoparticle synthesis.[53]

3.2.4 Determination of angle of repose

Angle of repose was measured by a funnel which is clamped to a stand at a fixed height of 3cm with over a plane Whatman filter paper surface (the funnel is 60 , 7.7cm in base diameter,5cm internal stem diameter with 7.5cm stem length). About 10gm of the dried powder sample

accurately weighed and carefully introduced into a funnel and allowed to flow freely to the Whatman filter paper surface.[50]The height of the cone(h),formed after complete flow and the radius of the cone (r) were measured and used to calculate the angle of repose using the equation:

$$\text{Angle of repose} = \tan^{-1}(h/r) \qquad [56]$$

3.2.5 Compressibility index

The compressibility index of the GO powder sample was determined after determining bulk and tapped densities. About 10 gm. of the dried powder sample was taken into 50 ml graduated measuring cylinder then the filled powder gave the initial volume (v_i) or bulk volume was recorded. The measuring cylinder was then clamped to the USP I tapper of a USP tap density tester. The cylinder was then tapped 100 times to achieve a final volume (v_f). The bulk or tapped density was calculated as the ratio of the weight of powder to the bulk or tapped volume, respectively.

$$\text{Bulk density} = \text{Mass/ initial volume } (M/V_i)$$

$$\text{Tapped density} = \text{Mass/ final volume } (M/V_f) \qquad [50]$$

Compressibility index were then determined by the following equation:

$$\text{Carr's index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100 \qquad [57]$$

3.2.6 Hausner ratio

Hausner ratio was then determined by the ratio of tapped density and bulk density based on following equations:

$$\text{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}} \quad [49]$$

3.2.7 Swelling index (SI)

The gum odina powder sample filled into micro pipette tips (white ,2ml) for estimating swelling index. Then first blocked the tip outlet with a tiny swab of nylon fiber to avoid any leakage of the powder during experiment. After placing the sample GO powder into the tip, it was tapped 10 times by dropping on a hard surface from a 10cm. height to obtain possibly the same packing of the bed. Take the weighed (W_a) of plastic tip and then it dipped into a 2-3 mm. layer of deionised water,when the bed became wetted with liquid then the tip was again weighed ($W_{b)}$ to find the amount of the liquid taken in by the powder. The whole experiment were repeated six time and average values were taken for calculation.[51]

The swelling index was estimated by this equation:

$$\text{Swelling index (SI)} = \frac{\text{Wb}-\text{Wa}}{\text{Wa}} \times 100 \quad [51]$$

3.2.8 Effective pore radius

The sample GO powder was filled into micropipette plastic tip (white, 2ml) and weighed (w_A). Then n-hexane (surface tension, (γ **)** 18.4 N/m, $\theta = 0°$) was added dropwise to the top of packed bed till the solvent filtered out at the bottom of the tip. Then the tip was weighed again (w_B) . [51] The effective pore radius ($R_{eff.p}$) was calculated using formula:

$$R_{eff.p} = \frac{WB-WA}{2\pi\gamma} \quad [51]$$

3.2.9 Moisture contents (percent loss on drying)

Moisture content of the gum was also expressed as percentage weight loss on drying (% LOD). About 2gm. of the GO gum sample was weighed and oven dried at 105℃ for 5 h to a constant weight. The experiment was done in three replications and an average of the three replicates was taken. [50] The percent loss on drying was then calculated by this equations:

$$\%\ \text{loss on drying (\%LOD)} = \frac{\text{weight of water in sample}}{\text{total weight of wet sample}} \times 100 \quad [58]$$

3.2.10 Synthesis of silver nanoparticles

For the synthesis of AgNPs,0.625 and 0.9375gm of Gum Odina powder was dissolved in separate 250 ml of conical flask each contain 125 ml of Milli-q water under constant stirring overnight at room temperature in mechanical stirrer for dissolution of GO to achieve 0.5%and 0.75% (w/v) solution. After dissolution ,2 different concentration of GO solution transfer in each separate test tube, then sonication each test tube for 10 minutes.After sonication each solution transfer into 2 separate centrifugal tube .Centrifuge the gum solution at 12000 rpm for 10 minutes .Take 100 ml of each supernatant in 2 test tube.Prepared of 100 ml of 1mM $AgNO_3$ stock solution to dissolved 0.016987gm of AgNO3 in 100 ml of Milli q water in a volumetric flask.Take 50ml of 1mM AgNO3 for each 2 individual GO Solution .Then silver nanoparticles were synthesized by autoclaving the silver nitrate solution containing various concentratin of Gum Odina at 121°c and 15 psi for 30 minute. The effect of concentration of gum on nanoparticle synthesis was studied.

3.2.11 Characterization of synthesized silver nanoparticles

I. UV–visible spectra

In order to study the formation of silver nanoparticles, the UV–visible absorption spectra of the prepared colloidal solutions were recorded using an Elico SL 196 spectrophotometer (Hyderabad, India), from 250 to 800 nm, against autoclaved gum blank. The absorption spectra of gum before and after autoclaving were also recorded against ultra pure water blank.

II. Antibacterial activity

Microorganisms

2 Gram positive and 2 Gram negative bacterial strains were used in this experiment. These were obtained from Division of Microbiology, Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata – 32. These strains are

1. *Staphylococcus aureus* 29737,
2. *Bacillus cereus* 11778,
3. *Escherichia coli* K88,
4. *Escherichia coli* 871,

Preparation of silver nanoparticle solution

This nanoparticles ware prepared with 0.5% gum solution containing 1mM $AgNo_3$, autoclaved for 30 minute.

Preparation of inoculums:

These strains were grown in Nutrient broth (Hi-Media) at 37° C for 24 hrs and the suspension was prepared by matching a 0.5 McFarland standard. [59]

Evaluation of Zone of inhibition:

Zone of inhibition of test drugs were evaluated by well diffusion methods as per NCCLS protocol (NCCL 2004). 0.1 ml of bacterial suspension was spread on agar plates with sterile bent glass rod to achieve uniform growth. Wells were dug by sterile borer(8 mm) and appropriate concentration(50 µl) of nanoparticle solution was added to the wells aseptically. The plates were incubated at 37°C for 24 hrs and clear zone of inhibition around wells were recorded**.**

Procedure

Nutrient agar medium was prepared and sterilized. Then the sterilized nutrient agar media was poured in sterilized Petri plates and allowed to solidify. 0.1ml of inoculums was taken aseptically and spread on nutrient agar plates. The wells (8 mm) were dug by using sterile borer and 50 µL of nanoparticle solution poured into each well on all plates. The plates were incubated at 37º C for 24 hrs and after sufficient incubation clear zone around the wells were observed and measured. [55]

CHAPTER 4

4. RESULT AND OBSERVATION

4.1 Characterization of gum

4.1.1 Size separation

The degree of fineness of a powder is known with the help of sieve through which the powdered material is passed. All particle(20gm) pass through sieve no 44,but less than 40% (7.48gm) pass through sieve no.85 .so the powder is moderately fine powder. The relevant grades of powder and sieve numbers according to I.P. along with a nominal mesh aperture are shown in Table-3

Table 3.Grade of powder

Sl no	Grade of powder	Sieve through which all particles must pass	Nominal mesh aperture size	Sieve through which 40% of particles pass	Nominal mesh aperture size
1	Coarse powder	10	1.7mm	44	355µm
2	Moderately coarse powder	22	710µm	60	250µm
3	Moderately fine powder	44	355µm	85	180µm
4	Fine powder	85	180µm	-	-
5	Very fine powder	120	125µm		

4.1.2 Angle of repose

From the below Table(4&5) the angle of repose mean value 41.89° indicate passable flow character with no need of adding flow promoter.

Table 4. angle of repose

No. of experiment	Weight of powder gum	Diameter of Pile (cm)	Average Diameter of pile (cm)	Average radius of pile (cm) (r)	Height of pile (cm) (h)	Angle of repose / $\theta = \tan^{-1} h/r$
1	10	6.8 6.6 6.6	6.66	3.33	3	42.01
2	10	6.9 6.2 6.8	6.63	3.31	3	42.18
3	10	6.5 6.4 6.4	6.43	3.21	3	43.06
4	10	6.9 6.7 6.9	6.83	3.41	3	41.34
5	10	7.1 6.9 6.7	6.9	3.45	3	41
6	10	6.6 6.8 6.8	6.73	3.36	3	41.76

Table 5. flow properties of powder.

Flow properties	Angle of repose (°)
Excellent	25 - 30
Good	31 - 35
Fair	36 - 40
Passable	41 - 45
Poor	46 - 55
Very poor	56 - 65
Very very poor	> 66

4.1.3 Compressibility index & Hausner ratio

The bulk and tapped densities give an insight into the packing arrangement of the particle and the compaction profile of a material. From the Table no 6 . the weight of powder is 10 gm. Mean

initial volume 16ml and after 100 tapped mean volume 12.41ml. so the bulk density 0.625 and tapped densities 0.805.

Table 6. Tapped volume

Serial no	Initial volume	weight	After 100 tapped	
			volume	USP-II
1	15	10	13	
2	16	10	12.5	
3	16	10	12.5	
4	16	10	12.5	
5	16	10	12	
6	17	10	12	

The compressibility index and Hausner ratio of gum odina powder were found to be 22.36 and 1.22 respectively, indicating the gum has a fair flow property and compressibility .

4.1.4 Swelling index

Table 7.Swelling index

Micropipette tips (2ml)	Powder weight (mg)	Powder weight +tip(wa)	After dipped into 2-3 layer of demonized water(wb)	Swelling index
Tip 1	102	388	395	1.80
Tip 2	102	371	378	1.88
Tip 3	102	378	385	1.85
Tip 4	104	383	390	1.82
Tip 5	102	384	391	1.82
Tip 6	103	386	393	1.81

The swelling behavior of gum odina can be expected to be useful for modulating the drug release from dosage form. So from the above experiment table the average value of swelling index of gum odina in water is 1.83.

4.1.5 Effective pore radius

The effective pore radius is the descriptor that was used to indicate the porosity of powder . Higher the effective pore radius higher is the porosity. From the table the average effective pore radius of gum odina (0.94 mm) suggest high porosity. These properties indicates a potential role of Gum odina as diluent/ disintegrant in tablet formulations and food items.

Table 8. Effective pore radius

Micropipette tips (2 ml)	Powder weight + tip (mg) (w_A)	After passed with solvent (mg)(w_B)	Difference (mg) (w_B - w_A)	Effective pore radius ($R_{eff,p}$)
Tip 1	578	691	113	0.98
Tip 2	572	682	110	0.95
Tip 3	595	703	108	0.93
Tip 4	591	700	109	0.94
Tip 5	592	701	109	0.94
Tip 6	595	702	107	0.92

4.1.6 Moisture content

Take 1000 mg of gum sample oven dried at 105 °c for 4 hour to give a constant weight 863mg.so the percent loss on drying of gum odina sample is 13.7%.

4.2 **Synthesis of silver nanoparticles**

The present experimental investigation reports the green synthesis of silver nanoparticles using gum odina by autoclaving. This method utilizes a non-toxic, renewable gum odina which functions as both reducing and stabilizing agent during synthesis. By virtue of being a natural polymer, this gum is also amenable for biodegradation. The process of autoclaving makes the silver nanoparticles intrinsically safe and sterile, in environmentally benign solvent water. After autoclaving the silver nitrate containing gum solution ,the appearance of yellow color in the reaction mixture (fig- 5) was observed. This is a clear indication for the formation of silver nanoparticles by the gum. Moreover, generation of gum-silver nanoparticles by autoclaving is a prerequisite for microbiological applications. Thus, the adopted method is meeting the requirements of green chemistry principles.

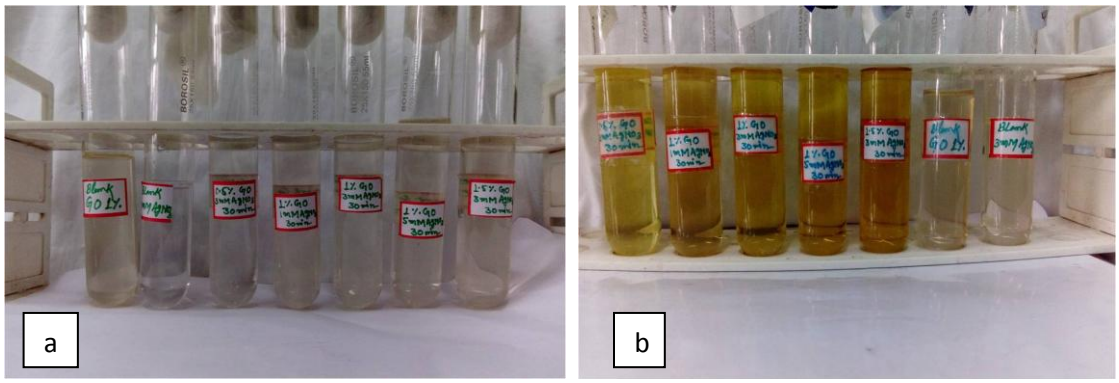


Fig -5. (a) before autoclave **(b) after autoclave**

Proposed mechanism of reduction

During the time of autoclaving at 121°C under the influence of temperature and pressure, this biopolymer expands and becomes more accessible for the silver ions to interact with the available functional groups on the gum, as observed earlier for starch.[44]The large number of hydroxyl and carboxylic groups on this biopolymer facilitates the complexation of silver ions. Subsequently, these silver ions oxidize the hydroxyl groups to carbonyl groups, during which the silver ions are reduced to elemental silver. It was noticed that the autoclaving at 121°C and 15 psi

of pressure, increased the extent of synthesis and stabilization of the nanoparticles. It is known that elevated temperature and pressure accelerate the synthesis of nanoparticles.

4.3. Characterization of synthesized silver nanoparticles

I. UV–visible spectroscopy

The UV–visible absorption spectroscopy is one of the most widely used simple and sensitive techniques for the observation of nanoparticle synthesis. In order to monitor the formation of silver nanoparticles, the absorption spectra of synthesized silver nanoparticles were recorded against respective autoclaved gum blanks. Fig. 6 is indicating (a) gum tears of good quality, (b) gum powder sieved to 38_m particle size and (c) gum solution of 0.5% (w/v). The experiments were carried out using the gum solution obtained with 38_msized gum. Fig. 7 shows the UV–vis spectra of the produced silver nanoparticles with different concentrations of gum (0.5%,0.75%) at 1mM AgNO3 and 30 minute of autoclaving. It reveals that the efficiency of nanoparticle synthesis increases with increasing concentration of gum. It also indicates the increased colour intensity of the nanoparticle solution with increase in gum concentration.

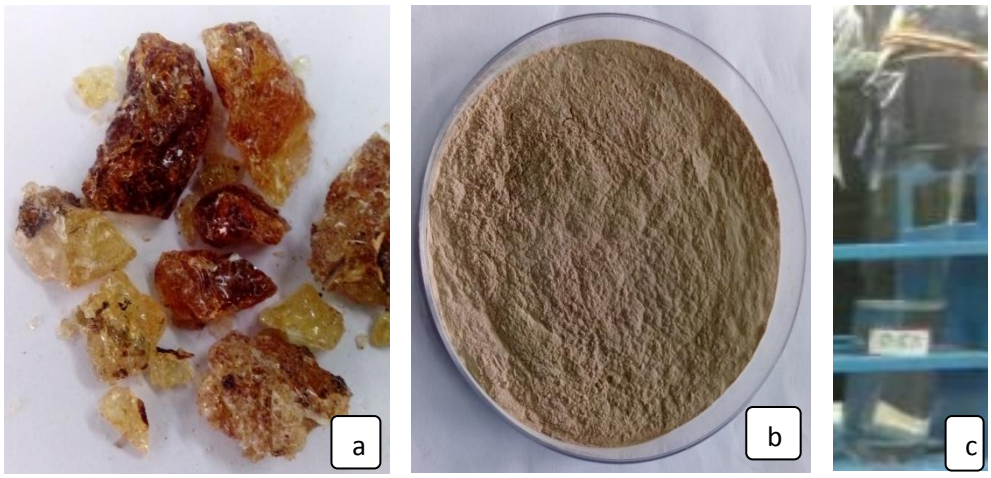


Fig 6. (a) Gum tears of good quality, (b) gum powder sieved to 38µm particle size and (c) gum solution of 0.5% (w/v).

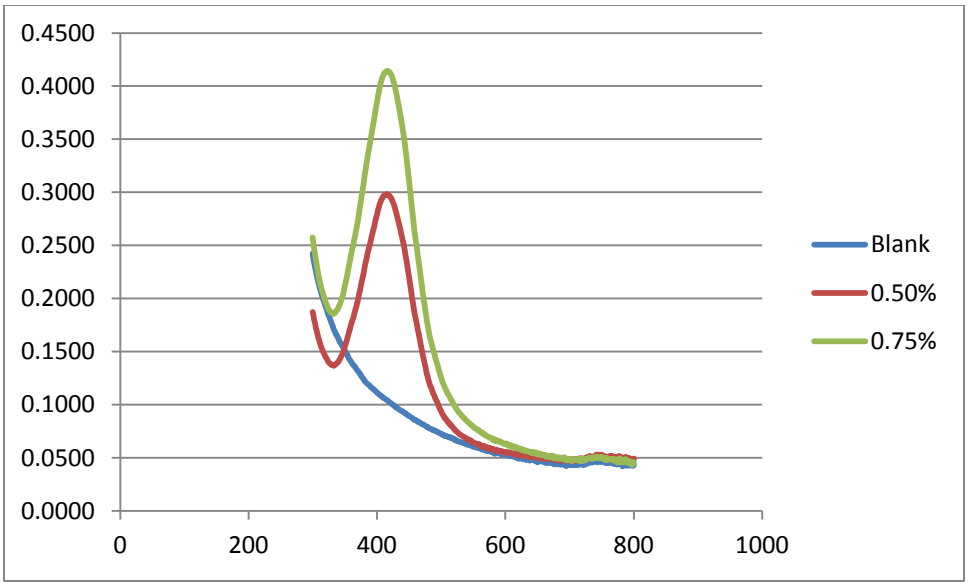


Fig 7: UV-vis spectra of the produced silver nanoparticles with different concentrations of gum

II. Antibacterial activity

Representation of the zone diameter of biologically synthesized silver nanoparticles against the tested organisms.

Table 9. zone diameter of organism.

Organism	Zone diameter (mm)
Staphylococcus aureus 29737	11.5
Bacillus cereus 11778	10.25
Escherichia coli K88	7.6
Escherichia coli 871	9.0

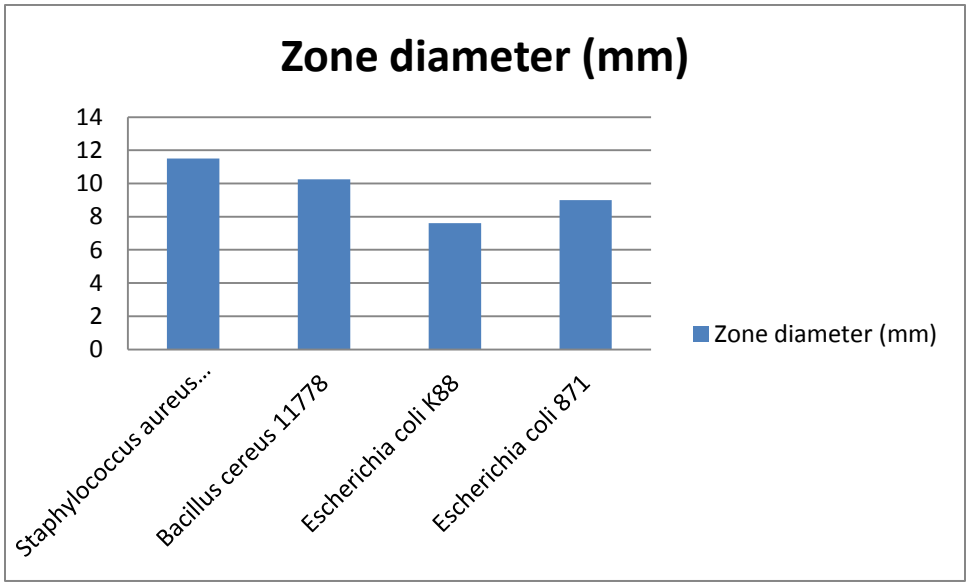
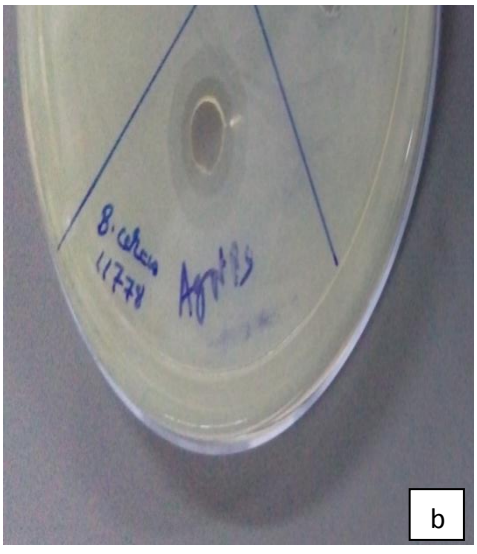


Fig. 8:Zone diameter in different organism



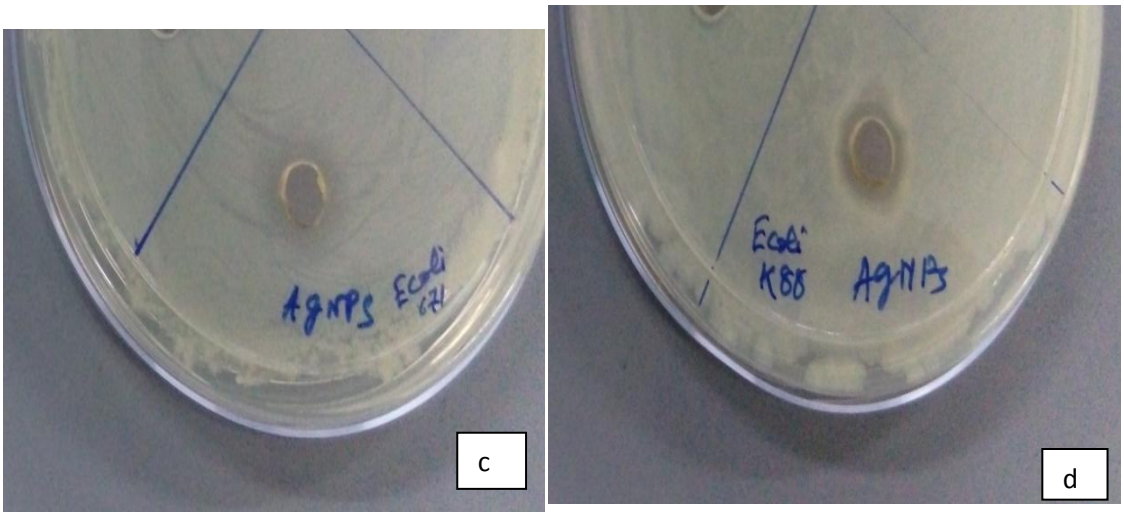


Fig. 9:- Zone of inhibition (in mm) of compound is shown here against *Staphylococcus aureus* 29737 *(a) Bacillus cereus* 11778 *(b) Escherichia coli* 871(c) *Escherichia coli* K88(d)

The compound screened against the bacteria strain by well diffusion method showed that the compound had moderate antibacterial activity.Bacterial growth inhibition around the well is due to the release of diffusible inhibitory compound from silver nanoparticles.The ZOI of around 11.25mm & 10.25 was observed for the gram-positive bacterial strain *Staphylococcus aureus* 29737 & *Bacillus cereus* 11778. In the case of gram-negative bacterial strain *Escherichia coli* 871 & *Escherichia coli* K88 ; the detected ZOI were 9.0 and 7.6 respectively. Based on these results, it can be concluded that synthesized silver nanoparticles had significant antibacterial action on both the Gram classes of bacteria.

CHAPTER 5

5.CONCLUSION

The main aim of this study is purification of gum odina and few characteristics also study the synthesis of silver nanoparticles and its antibacterial activity.we observed from the present study that the gum odina powder have a fair flow property .The gum powder can be used as a synthesis of silver nano which have antibacterial action of Gram –organism.The adopted method is compatible with green chemistry principles that the gum served as a reducing and stabilizing agent.however, further studies are needed for moleculer structure and function of gum nanoparticle in biological.

Chapter 6

6. REFERENCES

1. Goodsell, D., Bionanotechnology: Lessons from Nature, Willey-Less, New Jersey, USA, 2004, 1–8.
2. Iravani, S., Green synthesis of metal nanoparticles using plants. Green Chem, 2011, 13, 2638.
3. Bogunia, K.K and Sugisaka, M. BioSystems, 2002, 65, 123–138.
4. Pissuwan, D., Valenzuela, S.M., Cortie, M.B. Trends Biotechnol., 2006, 24, 62–67.
5. Jiang, H.M.S., Wong, A.C.L., Denes, F.S. ″Plasma enhanced deposition of silver nanoparticles onto polymer and metal surfaces for the generation of antimicrobial characteristics″, J Appl Polym Sci, 2004, 93, 1411-1422.
6. Abdel-Halim, E.S., El-Rafie, M.H., Al-Deyab, S.S. Polyacrylamide guar gum graft copolymer for preparation of silver nanoparticles. Carbohydrate Polymers, 2011, 85, 692–697.
7. Bhattacharya, R., Mukherjee, P. Biological properties of "naked" metal nanoparticles. Advanced Drug Delivery Reviews, 2008, 60(11), 1289-306.
8. Caseri, W. Nanocomposites of polymers and metals or semiconductors: Historical background and optical properties. Macromolecular Rapid Communications, 2000, 21(11), 705-22.
9. Erren, T.C. Prizes to solve problems in and beyond medicine, big and small: It can work. Medical Hypotheses, 2007, 68(4), 732-4.
10. Xu, Z. P., Z.Q.P., Lu, G.Q., Yu, A.B. *"*Inorganic Nanoparticles As Carriers For Efficient Cellular Delivery*"*. Chemical Engineering Science, 2006, 61, 1027-1040.
11. Klaus, T.J., R.; Olsson, E. & Granqvist, C.Gr., ″Silver-based crystalline nanoparticles, microbially fabricated.″. Proc Natl Acad Sci USA,, 1999, 96, 13611-13614.

12. Magnusson, M.D., K.; Malm, J., Bovin, J. & Samuelson, L., ″Gold nanoparticles: production, reshaping, and thermal charging.″. J Nanoparticle Res, 1999, 1, 243-251.

13. Jung, J.O., H.; Noh, H.; Ji, J. & Kim, S., ″Metal nanoparticle generation using a small ceramic heater with a local heating area.″. J Aerosol Sci, 2006, 37, 1662-1670.
14. Mafune, F.K., J.; Takeda, Y.; Kondow, T. & Sawabe, H., ″Formation of gold nanoparticles by laser ablation in aqueous solution of surfactant.″. J Phys Chem B, 2001,105, 5114-5120.

15. Tsuji, T.I., K.; Watanabe, N. & Tsuji, M., ″Preparation of silver nanoparticles by laser ablation in solution: influence of laser wavelength on particle size″. Appl Surf Sci, 2002. 202, 80-85.

16. Wiley, B.S., Y.; Mayers, B. & Xi, Y., "Shape-controlled synthesis of metal nanostructures: the case of silver.″. Chem Eur, 2005, 11, 454-463.

17. Yin, Y.L., Z-Y.; Zhong, Z.; Gates, B. & Venkateswaran, S., ″Synthesis and characterization of stable aqueous dispersions of silver nanoparticles through the Tollens process″. J Mater Chem, 2002, 12, 522-527.

18. Ahmad, A.M., P.; Senapati, S.; Mandal, D.; Khan, M.I.; Kumar, R. & Sastry, M., ″Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum″. Colloids and Surfaces B: Biointerfaces, 2003, 28, 313-318.

19. Vaidyanathan, R., Gopalram, S, Kalishwaralal, K, Deepak, V, Pandian, SR, Gurunathan, S, ″Enhanced silver nanoparticle synthesis by optimization of nitrate reductase activity″. Colloids Surf. B Biointerfaces, 2010, 75, 335-341.

20. Mohanpuria, P., Rana, KN, Yadav, SK, ″Biosynthesis of nanoparticles: technological concepts and future applications″. J. Nanopart. Res., 2008, 10, 507-517.

21. Jha, A., Prasad, K, Prasad, K, Kulkarni, AR, ″Plant system: nature's nanofactory″. Colloids Surf. B Biointerfaces, 2009, 73, 219-223.

22. Begum N A, M.S.a.B.L.R.A., *‘‘*Mondal Colloids and Surface B*’’.* Biointerfaces, 2009, 71, 113-118.

23. Sondi, I., Salopek-Sondi, B, ″Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria″. J. Colloid Interface Sci., 2004, 275,177-182.

24. Danilcauk, M., Lund, A, Saldo, J, Yamada, H, Michalik, J, ″Conduction electron spin resonance of small silver particles″. Spectrochimaca. Acta. Part A., 2006, 63,189-191.

25. Morones, J., Elechiguerra, JL, Camacho, A, Holt, K, Kouri, JB, Ramirez, JT, Yacaman, MJ, ″The bactericidal effect of silver nanoparticles″. Nanotechnology, 2005, 16, 2346-2353.

26. Yoon, K.Y.B., J.H.; Park, J.H. & Hwang, J., ″Susceptibility constrants of Escherichia coli and Bacillus subtilis to silver and copper nanoparticles″. Science of the Total Environment, 2007, 373, 572-575.
27. Sharma, V.K.Y., R.A. & Lin, Y., ″Silver nanoparticles: green synthesis and their antimicrobial activities″. Advances in Colloid and Interface Science, 2009, 145, 83-96.

28. Kumar, V.S.N., B.M.; Shashikala, V.; Padmasri, A.H.; Madhavendra, S.S. & Raju, B.D., "Highly efficient Ag/C catalyst prepared by electro-chemical deposition method in controlling microorganisms in water″. J Mol Catal A, 2004, 223, 313-319.

29. Panyala, N., Pena-Mendez, EM, Havel, J:, ″Silver or silver nanoparticles: a hazardous threat to the environment and human health″. j. Appl. Biomed., 2008, 6, 117-129.

30. McAuliffe, M., Perry, MJ, ″Are nanoparticles potential male reproductive toxicants?″. A literature review. Nanotoxicol, 2007, 1, 204-210.

31. Wood, C., Playle, RC, Hogstrand, C, ″Physiology and modeling of mechanisms of silver uptake and toxicity in fish″. Environ. Toxicol. Chem., 1993, 18, 71-83.

32. Rana V, Rai P, Tiwary AK, Singh RS, Kennedy JF, Knill CJ: Modified gums: approaches and applications in drug delivery. Carbohydr Polym 2011, 83, 1031–1047.

33. Chaubey M, Kapoor P.V.,Structure of galactomannan from the seeds of Cassia angustifolia Vahl. Carbohydr Res 2001, 332 ,439–444.

34. Sharma V.K., Yngard R.A., Lin Y., Silver nanoparticles: green synthesis and their antimicrobial activities, Advances in Colloid and Interface Science, 2009, 145,83–96.

35. Zheng M.P., Gu M.Y., Jin Y.P., Jin G.L., Optical properties of silver-dispersed PVP thin film, Materials Research Bulletin, 2001, 36, 853–859.

36. Zhang Z.P., Zhang L.D., Wang S.X., Chen W., Lei Y.. A convenient route to polyacrylonitrile/silver nanoparticle composite by simultaneous polymerization-reduction approach, Polymer, 2001, 42, 8315–8318.

37. Abu Bakar N.H.H., J Ismail., Abu Bakar M., Synthesis and characterization of silver nanoparticles in natural rubber, Materials Chemistry and Physics , 2007 ,104 ,276–283.

38. Huang H.Z., Yang X.R., Synthesis of polysaccharide-stabilized gold and silver nanoparticles: a green method, Carbohydrate Research ,2004,339, 2627–2631.

39. Mohan Y.M., Joseph D.K., Geckeler K.E., Poly(N-isopropylacrylamide-cosodium acrylate) hydrogels: interactions with surfactants, Journal of Applied Polymer Science ,2007, 103, 3423–3430.

40. Mukherjee, B., Samanta, A. and Dinda, S.C., Gum odina-a new tablet binder. Trends in Applied Sciences Research. 2006, 1(4**)**, 309-316.

41. Chidanbarathanu, S., Index of herbs in languages. Siddha Medical Literature Research centre, Madras. 1995.

42. Kiritikar, K.R. and Basu, B.D. , *Indian Medicinal Plants*, 2nd Edition, International Book Distributors, Book Sellers and Publishers, Dehradun., 1935.

43. Samanta A., Ojha D., Mukherjee B., Stability analysis of primary emulsion using a new emulsifying agent gum odina, natural science, 2010,.2(5), 494-505 .

44. Vigneshwaran, N., Nachane, R. P., Balasubramanya, R. H., & Varadarajan, P. V. A novel one-pot 'green' synthesis of stable silver nanoparticles using soluble starch. Carbohydrate Research, 2006,341, 2012–2018.

45. Mohan Y. M, Raju K. Mohana, K. Sambasivudu, Satyendra Singh, B. Sreedhar, Preparation of Acacia-Stabilized Silver Nanoparticles:A Green Approach. Journal of Applied Polymer Science, 2007, 106, 3375–3381 ,

46. Sinha V. R., Azaki,A.A. Kumar AL- R. V., carbohydrate polymers, 2011, 83, 1492-1498, Novel Lannea Woodier gum matrices for controlled release of drugs.

47. Bahareh Tabatabaee Amid, Hamed Mirhosseini, Sanja Kostadinovic., Chemical composition and molecular structure of polysaccharide-protein biopolymer from Durio zibethinus seed: extraction and purification process, Chemistry Central Journal 2012, 6,117.

48. Bhattacharyya a. k. and rao c. v**.** n., gum jeol: the structure of the degraded gum derived from it**,** Canadian Journal of Chemistry., 1964, 42 .

49. Jindal M. , Kumar V., Rana V., Tiwary A.K., Exploring potential new gum source Aegle marmelos for food and pharmaceuticals: Physical, chemical and functional performance, Industrial Crops and Products ,2013, 45,312– 318.

50. Malsawmtluangi C., Thanzamia K., Lalhlenmawiaa H., Selvanb V., Palanisamyb S., Kandasamyb R., Pachuaua L., Physicochemical characteristics and antioxidant activity of Prunus cerasoides D. Don gum exudates, International Journal of Biological Macromolecules ,2014, 69 ,192–199.

51. Kamboj S., Rana V., Physicochemical, rheological and antioxidant potential of corn fiber Gum, Food Hydrocolloids, 2014, 39, 1-9.

52. Tagad C. K., Dugasani S. R., Aiyer, Park R. S., Kulkarni A., Sabharwal S. Green synthesis of silver nanoparticles and their application for the development of optical fiber based hydrogen peroxide sensor, Sensors and Actuators B , 2013, 183,144– 149.

53. Kora, A. J. Beedu S. R. and A. Jayaraman, Size-controlled green synthesis of silver nanoparticles mediated by gum ghatti (Anogeissus latifolia) and its biological activity, Organic and Medicinal Chemistry Letters 2012, 2,17.

54. Kora A. J., Sashidhar R.B., Arunachalam J., Gum kondagogu (Cochlospermum gossypium): A template for the green synthesis and stabilization of silver nanoparticles with antibacterial application, Carbohydrate Polymers 2010 ,82 ,670–679.

55. Kaviya S., Santhanalakshmi J., Viswanathan B., Green Synthesis of Silver Nanoparticles Using Polyalthia longifolia Leaf Extract along with D-Sorbitol: Study of AntibacterialActivity Journal of Nanotechnology, 2011, 152970.

56. Avachat A.M., Dash R.R., Shrotriya S.N., Ind. J. Pharm. Educ. Res. ,2011, 45 86–99.

57. Janaki B., Sashidhar R.B., Food Chem. 1998, 61, 231–236 .

58. Singh K., Kumar A., Langyan N., Ahuja M., AAPS PharmSciTech, 2009, 10, 1121–1127.

59. McFarland J., Am J.. Med. Assoc , 1907, 14, 1176-1178.