NANOPARTICLE

Research Article

Formulation And Evaluation Of Soapfilm Incorporated with Green Synthesized *Azadirachta indica* Silver Nanoparticles

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ABSTRACT

The present research aimed at developing the formulation and evaluation of soap film incorporated with green synthesised *Azadirachta indica* silver nanoparticles for its anti-bacterial activity. SEM analysis OF *Azadirachta indica* silver nanoparticles was performed for the study. SEM images of soap impregnated with silver nanoparticles exhibited spherically agglomerated particles due to the presence of soap. Antibacterial studies of *Azadirachta indica* silver nanoparticles were done in this study. The bactericidal properties of soap AgNPs were confirmed in studies against gram-positive (Staphylococcus Aureus) and gram-negative (E. coli) bacterial strains. For silver nanoparticles, the highest concentration of 100 µg/ml showed activity ranging between 20 for E. coli and 19 mm zone of inhibition for Staphylococcus Aureus. Silver nanoparticle-impregnated biomedical fibre showed efficient antimicrobial activity. It is reported that silver nanoparticles have the advantage of high anti-microbial activity even at low concentrations. The antibacterial study for *Azadirachta indica* silver nanoparticles was conducted in both gram-positive and gram-negative bacteria. The results were found that both gram-positive and gram-negative bacteria showed good antibacterial activity. But by comparison, the *Azadirachta indica* silver nanoparticles showed more effect in gram-positive bacteria. Formulation and optimisation of different types of soap films were carried out in this study. Different types of soap formulations were formulated by using sodium hydroxide and potassium hydroxide. Among this formulation, the soap containing sodium hydroxide was used because the soap containing potassium hydroxide was irritating to the skin. Another reason was the potassium hydroxide-containing soap didn't provide the texture of a film. The study revealed the efficacy of the synthesis of silver nanoparticles (AgNPs) using green principles and its potential application in health and the environment.

Keywords: Azadirachta indica, Silver Nanoparticles, Green Synthesis, Antibacterial Activity.

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INTRODUCTION

Nanobiotechnology is a rapidly growing scientific field of producing and constructing devices. An important area of research in nanobiotechnology is the synthesis of NPs with different chemical compositions, sizes and morphologies, and controlled disparities. Nanobiotechnology has turned up as an elementary division of contemporary nanotechnology and untied novel epochs in the fields of material science receiving global attention due to its ample applications. It is a multidisciplinary approach resulting from the investigational use of NPs in biological systems, including biology, biochemistry, chemistry, engineering, physics and medicine. Moreover, the Nano bio-technology also serves as an imperative technique in the development of clean, nontoxic, and eco-friendly procedures for the synthesis and congregation of metal NPs having the intrinsic ability to reduce metals by specific metabolic pathways. There has been a rapid increase in interest in nanotechnology and the use of nanoparticles in commercial applications. However, little is known about the fate and behaviour of engineered nanoparticles in the environment. The properties of nanoparticles differ remarkably from small molecules and their chemistry and synthesis necessitates that they be considered more like complex mixtures than small molecules. The ability of the molecules to attach to the surface of nanoparticles and exchange with other molecules already placed there indicates that careful consideration of the chemistry of nanoparticles and how they relate to their fate in surface waters and sediments is key to predicting their final fate. We have set out to briefly introduce the properties and synthesis of nanoparticles at a basic level and then review the state of current understanding relating to the fate and behaviour of nanoparticles in the environments with a particular focus on engineered nanoparticles.

Silver NPs are of interest because of their unique properties which can be incorporated into antimicrobial applications, biosensor materials, composite fibres, cryogenic super-conducting materials, cosmetic products, and electronic components. Some important applications of silver NPs are pharmaceutics, medicine, and dentistry. Several physical and chemical methods have been used for synthesizing and stabilizing silver NPs [1].

In recent years, increasing antibiotic resistance by microbes has posed a serious threat to the health sector. Nanoparticles have proved to be a likely candidate for antimicrobial agents since their large surface-to volume ratio ensures a broad range of attacks on bacterial surfaces. One of the most promising nanoparticles which act as a highly effective antimicrobial agent is silver. Various investigations on silver nanoparticles have been done to study their antimicrobial activity. AgNPs exhibited significant antibacterial activity in E. coli, staphylococcus aureus and antifungalactivity [2].

GREEN SYNTHESIS

The present work aims to use the leaf extract of *Azadirachta indica* known as neem) a member of the *Meliaceae* family used for the green synthesis of silver nanoparticles. This is is a medicinal plant and has been used for the treatment of bacterial, fungal, viral and many types of skin ailments since ancient times. The aqueous neem extract is used in the synthesis of various nanoparticles such as gold, zinc oxide, silver, etc. Terpenoids and flavanones are the two important phytochemicals present in neem which play a vital role in stabilizing the nanoparticle and also act as a capping and reducing agent. Aqueous neem leaf extract reduces silver salt to silver nitrate, this capped nanoparticle with neem extract exhibits antibacterial activity.

Earlier methods used for the synthesis of silver nanoparticles were toxic as hazardous chemicals were used for their synthesis. Thus, the use of eco-friendly processes, for the synthesis of silver nanoparticles are known as "Green synthesis".Green synthesis is preferred over conventional synthesis because it is an eco-friendly, cost-effective, single-step method that can be easily scaled up for large-scale synthesis and does not require high pressure, temperature, energy and toxic chemicals. Many researchers have reported the use of materials such as plant leaf extract, root, stem, bark, leaf, fruit, bud and latex, fungi, bacteria and enzymes for the synthesis of silver nanoparticles. A lot of work has been done on the green synthesis of silver nanoparticles using microorganisms including bacteria, fungi and plants because of their antioxidant properties capable of reducing metal compounds in their respective nanoparticle. Plant extracts produce the best capping material for the stabilization of silver nanoparticles.

In the present study, the antibacterial effect of green synthesized silver nanoparticles and its role in water purification was studied. Even the concentration of silver nanoparticles was determined to be the most effective in controlling the growth of Gram-positive and Gram-negative bacteria isolated from the water sample. The effect of silver nanoparticles on the bacterial count was also studied.

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Azadirachta indica (Neem) tree contains many natural substances in its different parts, leaves, seeds, bark, and has many biological activities against disease-causing organisms, and it contains about 140 chemical compounds. The leaves and seeds of the Neem tree contain active material known as Azadiractrin (AZ) (C35H44O16) and have the ability to kill some disease-causing fungi, viruses and parasites. Neem extract is very active against skin fungi which cause ringworm disease. The AZ content in neem oil was highly correlated with its bioactivity against test insects. A marked difference has been reported in the yield of AZ from neem seeds from different geographical origins, even in different seasons in the same geographical area. Recently, two new tetranortriterpenoids, 11epiazadirachtin H and AZ-K, have been isolated from neem seeds. Although the bark, heartwood, leaves, fruit, and seeds of neem have been investigated chemically for their main biocidal components, the renewable parts (seeds and leaves) received major research attention. The neem oil is Eco-friendly and an important essential oil due to its insecticidal and fungicidal value in Entomological practices [3].

ADVANTAGES OF GREEN SYNTHESIS

Environmentally friendly.

Easily scale up for large synthesis of Nanoparticles.

No need for temperature, pressure, energy, and toxic chemicals.

More advantageous to the microorganism by a less elaborate processof maintaining cultures.

Reduce the cost of micro-organism isolation and their culture media.

DISADVANTAGE OF GREEN SYNTHESIS

Plant cannot be manipulated as the choice of nanoparticle through optimised synthesis through genetic engineering.

Plant produces a low yield of secreted protein which decreases the synthesisrate.

SYNTHESIS OF SILVER NPs

Silver nanoparticles are particles of silver, with particle sizes between 1 and100 nm in size. While frequently described as being "silver" some are composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms. Like gold nanoparticles, ionic silver has a long history and was initially used to stain the glass for yellow. Currently, there is also an effort to incorporate silver nanoparticles into a wide range of medical devices, including bone cement, surgical instruments, surgical masks, etc. Moreover, it has also been shown that ionic silver, in the right quantities, is suitable for treating wounds. Silver nanoparticles are now replacing silver sulfadiazine as an effective agent in the treatment of wounds. Additionally, Samsung has created and marketed a material called Silver Nano, which includes silver nanoparticles on the surfaces of household appliances. Moreover, due to their attractive physiochemical properties, these nanomaterials have received considerable attention in biomedical imaging using SERS. The surface plasmon resonance and large effective scattering cross-section of individual silvernanoparticles make them ideal candidates for molecular labelling. Thus many targeted silver oxide nanoprobes are currently being developed.

Silver nanoparticles are widely incorporated into wound dressings and are used as an antiseptic and disinfectant in medical applications and consumer goods. Silver nanoparticles have a high surface area per unit mass and release a continuous level of silver ions into their environment. The silver ions are bioactive and have broad-spectrum antimicrobial properties against a wide range of bacteria. By controlling the size, shape, surface and agglomeration state of the nanoparticles, specific silver ion release profiles can be developed for a given application.

The antibacterial effects of silver nanoparticles have been used to control bacterial growth in a variety of applications, including dental work, surgery applications, wounds and burns treatment, and biomedical devices. It is well known that silver ions and silver-based compounds are highly toxic to microorganisms. The introduction of silver nanoparticles into bacterial cells can induce a high degree of structural and morphological changes, which can lead to cell death. Scientists have demonstrated that the antibacterial effect of silver nanoparticles is mostly due to the sustained release of free silver ions from the nanoparticles, which serve as a vehicle for silver ions [4].

Physical methods

Evaporation-condensation and laser ablation are the most important physical approaches. The absence of solvent contamination in the prepared thin films and the uniformity of NP distribution are the advantages of physical synthesis methods in comparison with chemical processes.

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Silver NPs could be synthesized by laser ablation of metallic bulk materials in solution. The ablation efficiency and the characteristics of produced nano-silver particles depend upon many parameters, including the wavelength of the laser impinging the metallic target, the duration of the laser pulses (in the femto-, pico- and nanosecond regime), the laser fluence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants.

Chemical methods

The most common approach for the synthesis of silver NPs is chemical reduction by organic and inorganic reducing agents. In general, different reducing agents such as sodium citrate, ascorbate, sodium borohydride (NaBH4), elemental hydrogen, polyol process, Tollen's reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers are used for reduction of silver ions (Ag+) in aqueous or non-aqueous solutions. These reducing agents reduce Ag+and lead to the formation of metallic silver (Ag0), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of metallic colloidal silver particles.

Bio-based methods

Several reports in the literature indicate that the synthesis of nanoparticles by chemical approaches is eco-friendly and expensive. Thus, there is a growing need to develop environmentally and economically friendly processes, which do not use toxic chemicals in the synthesis protocols. This has conducted researchers to look at the organisms. The potential of organisms in nanoparticle synthesis ranges from simple prokaryotic bacterial cells to eukaryotic fungi and plants. Some examples of nanoparticle production include using bacteria for gold, silver, cadmium, zinc, magnetite, and iron NPs; yeasts for silver, lead and cadmium NPs; fungi for gold, silver and cadmium NPs; algae for silver and gold NPs; plants for silver.

Plant produces a low yield of secreted protein which decreases the synthesis rate. Abacterium is a single, but complex, cell. It can survive on its own, insideor outside the body.

Most bacteria aren't harmful. We have many bacteria on and inside our body, especially in the gut to help digest food. However, some bacteria can cause infections. Bacterial infections can affect the throat, lungs, skin, bowel and many other parts of the body. Many are mild; some are severe.

Most bacterial infections can be effectively treated with antibiotics. They either kill bacteria or stop them from multiplying. This helps the body's immune system fight the bacteria. Antibiotic resistance is a growing problem so antibiotics may beprescribed only for serious bacterial infections. AgNPs have been extensively used in household utensils, food storage, the health care industry, environmental applications, and biomedical applications such as wound dressings, surgical instruments, and disinfectants. Furthermore, due to their optical activities, these NPs have been used in catalysis, electronics and biosensors.

SOAP

Soap is a mixture of sodium salts of various naturally occurring fatty acids. Air bubbles added to a molten soap will decrease the density of the soap and thus it will float on water. If the fatty acid salt has potassium rather than sodium, a softer lather is the result. Soap is produced by a saponification or basic hydrolysis reaction of a fat or oil. Currently, sodium carbonate or sodium hydroxide is used to neutralize the fatty acid and convert it to salt [5].

$$\text{Fat} + \text{NaOH} \rightarrow \text{glycerol} + \text{sodium salt of fatty acid}$$

Cleansing Action of Soap

The cleansing action of soap is determined by its polar and non-polar structures in conjunction with an application of solubility principles. The long hydrocarbon chain is of course non-polar and hydrophobic (repelled by water). The "salt" end of the soap molecule is ionic and hydrophilic (water soluble). Monolayer: When soap is added to water, the ionic-salt end of the molecule is attracted to water and dissolved in it. The non-polar hydrocarbon end of the soap molecule is repelled bywater. A drop or two of soap in water forms a monolayer on the water surface as shown in the graphics on the left. The soap molecules "stand up" on the surface as the polar carboxyl salt end is attracted to the polar water. The non-polar hydrocarbon tails are repelled by the water, which makes them appear to stand up.

The soap should have good ingredients that can kill bacteria but not damage body tissues. Several bacteria including Gram-positive and Gram-negative are deposited from the environment on the surface of the skin and cause skin infection. Examples of these bacteria include *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*. The spread of infection by such bacteria can be prevented by the use of antiseptic soaps, as they contain antimicrobial

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chemicals, but overuse of soaps might result in antimicrobial resistance and evenrender a person more sensitive to allergies, and skin rashes [6].

A soap is a salt of a compound, known as a fatty acid. A soap molecule has a long hydrocarbon chain with a carboxylic acid group on one end, which has an ionic bond with a metal ion, usually sodium or potassium. The hydrocarbon end is non-polar which is highly soluble in non-polar substances and the ionic end is soluble in water. The cleaning action of soaps is because of their ability to emulsify or dispersewater insoluble materials and holds them in the suspension of water. This ability is seen in the molecular structure of soaps. When soap is added to water that contains oil or other water-insoluble materials, the soap or detergent molecules surround the oil droplets. The oil is, dissolved in the alkyl groups of the soap molecules while the ionic end allows it to be dissolved in water. As a result, the oil droplets are to be dispersed throughout the water and can be washed away.

A good soap is biodegradable when it does not contain chemicals that cannot be made to their natural elements. Neither does it contain chemicals that can be harmful to the environment or cause undue destruction to the environment.

A good soap gets dissolved easily and removes stains from the clothes, human skin or any material being cleaned. The cleansing action of soap is determined by its polar and non-polar structures in conjunction with an application of solubility principles. The long hydrocarbon chain is of course non-polar and hydrophobic (repelled by water). The "salt" endof the soap molecule is ionic and hydrophilic (water soluble). Monolayer: When soap is added to water, the ionic-salt end of the molecule is attracted to water and dissolved in it. The non-polar hydrocarbon end of the soap molecule is repelled bywater. A drop or two of soap in water forms a monolayer on the water surface as shown in the graphics on the left. The soap molecules "stand up" on the surface as the polar carboxyl salt end is attracted to the polar water. The non-polar hydrocarbon tails are repelled by the water, which makes them appear to stand up.

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Silver: an effective antibacterial agent

Ag-based particles have exhibited helpful and compelling antibacterial applications, yet because of the remarkable activities of nanotechnology-based materials have given immense support to improve the activities of Ag. Metallic particles with a size in the nanometer range have exhibited physical characteristics with a unique relation to both the particle and the mass material. This makes them show astounding properties, for example, expanded synergist action because of morphologies with profoundly dynamic aspects. A few electron microscopy strategies could be applied to concentrate the system by which AgNPs interface with these microbes. Also, a high point annular dull field (HAADF) filtering transmission electron microscopy (STEM) could be used, followed by the formation of a novel specimen planning that maintains a strategic distance from the utilization of substantial metal-based mixtures [8].

The advancement of new benign microbial strains to the present anti-infective agents has been a major issue in

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common health systems; subsequently, there is a prerequisite to develop novel bactericidal agents]. The microbial strains have been reported to exist with varied film assemblies which allow for recognition of them as Gram-negative (G–ve) or Gram-positive (G+ve). The main mechanism lies behind the conjugation with the main sites of the layers of peptidoglycan (PG). G–ve microorganisms have a thin PG layer of 1–5 nm that exists between the external layer and the cytoplasmic film, however, the G+ve microscopic organisms do not have the external film yet have a thick PG layer of ~30 nm.

The complexes of Ag have likewise been utilized as a part of the therapeutics to treat blazes and a variety of diseases. Also, Ag and its salt forms have been significantly utilized as a potential tool as bactericidal agents for the treatment of various microbial infections. Excellent endeavours have been performed to investigate the bactericidal features of Ag-based materials through electron microscopy and observe the possible interfaces of AgNPs with microorganisms. AgNPs have also been used as a material medium for the transportation of antibiotics, as a blending agent to form composites used in purification processes and also as casing material. However, in any phase, the antibacterial properties of these AgNPs depend upon their steadiness in the system, as this exhibits greater reaction time for the interaction between the AgNPs and bacterial membranes. Thus, the stability of the AgNPs in the reaction medium plays a crucial role in hindering the growth and progression of the microbes [9].

MATERIALS AND EQUIPMENTS

Table 1. List of materials

MATERIALS	SOURCE
Coconut Oil	Local market
Sodium Hydroxide	Lobachemie Pvt, Ltd, Mumbai
Methylcellulose	Himedia, Mumbai
Ethanol	Zhuhai Chemico Industries
PEG 400	Lancaster synthesis
Sodium lauryl sulphate	SD Fine Chemical Limited

Table 2. List of equipment

EQUIPMENT	MODEL / COMPANY
Plastic Circular moulds	Quantum Biomedicals
Centrifuge	Remi Motors
Digital balance	Sri Mahalakshmi Enterprises
FT-IR Spectrophotometer	Jasco FTIR 4100
Heating Mandle	Guna Enterprises, Chennai
Magnetic stirrer	Remi motors
UV- Visible Spectrophotometer	Jasco v 530
Scanning electron microscope	Hitachi X650, Tokyo Japan

EXPERIMENTAL METHODOLOGY

i. Collection, Processing, and Extraction of Azadirachta indica

Fresh leaves of neem leaves were collected and washed off to remove impurities and dirt. The leaves were cut into small pieces and extraction was done for 10 minutes [9].

ii. Preparation of 1mm Silver Nitrate Solution

A silver nitrate solution of 1MM was prepared by adding 0.1699 silver nitrate to 1L of distilled water [9].

iii. Preparation of *Azadirachta indica* Silver Nanoparticles

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The leaves were finely cut 20 G of boiled in 100ml water for 10 minutes and filtered to obtain Neem leaf extract. The extract of Neem leaves 5ml was mixed with 45 ml of 1MM silver nitrate and the colour changes were observed indicating the formation of AgNPs [9].

iv. Characterisation of *Azadirachta Indica* Silver Nanoparticles

a. UV Visible Spectroscopy Of *Azadirachta indica* Silver Nanoparticles

The formation of nanoparticles was monitored based on the effect of surface plasmon resonance effect of UV light on nanoparticles. The development of color in the solution was due to the formation of silver nanoparticles which was attributed to the characteristic surface plasmon vibrations of the respective nanoparticles. The absorbance maxima were recorded at 430 nm for silver nanoparticles. As the concentration increases the absorbance also slightly increases [10].

b. FTIR Analysis of *Azadirachta indica* Silver Nanoparticles

FOURIER-TRANSFORM INFRARED (FTIR) analysis of silver nanoparticles and their physical mixture are obtained using an FTIR spectrophotometer. The weighed amount of the drug was mixed with the PEG 400 (polymer) and their FTIR was observed [10].

c. Sem Analysis of *Azadirachta indica* Silver Nanoparticles

The scanning electron microscopy analysis of the *Azadirachta indica* silver nanoparticles in powdered form was observed 10000X, 5500x, and 950x [10].

d. Antibacterial Studies of *Azadirachta indica* Silver Nanoparticles

The bactericidal properties of AgNPs were studied against gram-positive (Staphylococcus Aureus) and gram-negative (Staphylococcus epidermidis) bacterial strains [10].

PREPARATION OF METHYL CELLULOSE FILM

Methylcellulose was weighed to 5.66g and 75ml of water was added and 25 ml of ethanol ,heated to 85°C with continuous stirring. 300mg of sodium lauryl sulfate and 2ml of PEG 400. The mixture was maintained at the temperature for 15 minutes. The mixture was removed and kept in a magnetic stirrer at 600rpm for 10 minutes [11].

PREPARATION OF SOAP

Coconut oil of 9.03ml was measured and poured into a China dish then heated to 60℃. The lye solution was prepared by mixing 4.32g ofsodium hydroxide in 8.4 ml of water. The lye was cooled to 60℃ and added to the coconut oil with continuous stirring till the soap was formed [11].

PREPARATION OF SOAP INCORPORATED WITH METHYLCELLULOSE FILM

The prepared soap of about 5 g was mixed with 5g of methyl cellulose polymer and the film was poured into the Petri dish and allowed to set it down [11].

EVALUATION OF SOAP INCORPORATED METHYLCELLULOSE FILM

a. PH of soap incorporated methylcellulose film

A soap solution of 10% was prepared by dissolving 10 gm of soap in distilled water in a volumetric flask of 100 ml. For the determination of pH, a pH meter was used. The electrode was introduced into the solution and the pH was noted down.

b. Colour and clarity characterization of soap incorporated methylcellulose film

The soap was visualized against a white background the determine its colour and to see the clarity of the formulated soap film.

c. Foam forming ability of soap incorporated methylcellulose film

For the determination of the soap film for its ability to form foam about 1.0 gm of soap wastaken and dissolved in distilled water (about 50ml) in a 100 ml graduated measuring cylinder. The measuring cylinder was then shaken for about 2-3 minutes and it was allowed to stand for about 10 min. Foam height was measured after 10 minutes. Record the observation for three consecutive experiments and the mean was taken.

d. The retention time of foam in soap incorporated methylcellulose film

Foam retention time refers to the time for which the foam produced by the soap is retained. The above procedure was repeated and the foam interval was measured for about 5-10

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minutes.

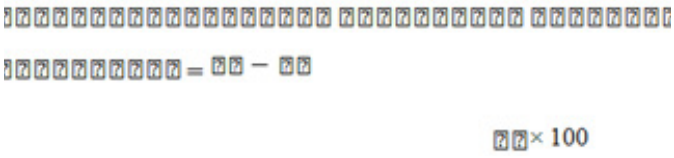
e. Saponification value determination of soap incorporated methylcellulose film

The saponification value was determined by adding 0.5M KOH solution to a conical flask containing 2 grams of soap sample.. This mixture was heated to about 55 degrees Celsius along with stirring continuously on a hot water bath. Then the temperature was further increased to 100 degrees Celsius and boiling was continued for about 1 hour Titration was performed with pheophytin as an indicator and 0.5M HCl. The endpoint observed is pink colour disappearance.

The saponification value is calculated as:

f. Determination of TFM (total fatty matter) of soap incorporated methylcellulose film

In this procedure, approximately 10g of the soap sample was taken and dissolved in 150 of water (distilled). It was dissolved by heating. Then this soap solution was treated with 20% sulphuric acid and heated till the solution was cleared. Fatty acids would be observed at the surface of the film which were then solidified by the addition of 7 mg of beeswax and again heated. Cake formation takes place and it is removed and weighed.



Where, X= weight of wax A= weight of wax+ oil W= weight of soap

g. Dissolution Time of soap incorporated methylcellulose film

In this procedure, a small square-shaped soap film is cut down and allowed to wash the film with water in hand. The time required to dissolve the soap film was noted down.

PREPARATION OF SILVER NANOPARTICLES IMPREGNATED SOAP FILM

From the above formulations optimized soap film formulations were carried out. 4.80g of soap base was weighed accurately and taken in a beaker. 0.20g of silver nanoparticles were added, mixed well and warmed when slurry was formed. It was stirred well poured into a mould and allowed to cool.

IX. EVALUATION OF SILVER NANOPARTICLES IMPREGNATED SOAP FILM.

a. Antibacterial Studies of *Azadirachta indica* Silver Nanoparticles in Corporated Soap Film

The bactericidal properties of AgNPs incorporated in soap film were studied against gram-positive (Staphylococcus Aureus) and gram-negative (Staphylococcus epidermidis) bacterial strains. The soap alone does not provide any antibacterial study [10].

b. UV Visible Spectroscopy of *Azadirachta indica* Silver Nanoparticles in Corporated Soap Film

The formation of nanoparticles was monitored based on the effect of surface plasmon resonance effect of UV light on nanoparticles. The development of color in the solution was due to the formation of silver nanoparticles which was attributed to the characteristic surface plasmon vibrations of the respective nanoparticles. The absorbance maxima were recorded at 430 nm for silver nanoparticles [10,12-35].

RESULTS AND DISCUSSION

Collection, Processing, and Extraction of *Azadirachta indica*

The neem extract obtained was the pale yellow colour solution.

Preparation of 1mm Silver Nitrate Solution

The prepared solution of silver nitrate solution was colour and when it was mixed with the neem extract it changed to pale brown.

Preparation of *Azadirachta indica* Silver Nanoparticles

The prepared solution of silver nitrate solution was colour and when it was mixed with the neem extract it changed to pale brown. Keep on placing the silver nanoparticle solution it will change to dark brown.

Standard Graph of *Azadirachta indica* Silver Nanoparticle

Silver nanoparticles were estimated using the UV spectrophotometric method by measuring the absorbance at 430nm using deionized water. It obeyed Beer's Lambert's

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law in the range of .5- 4µg/ml. The correlation coefficient was found to be 0.993.

Table 3. Standard graph of *Azadirachta indica* silver nanoparticles usingDeionized Water at absorbance430 nm

S.No	Concentration(µg/ml)	Absorbance at 430nm
1	0.5	0.232
2	1	0.426
3	2	0.643
4	3	0.932
5	4	1.132

Figure 1. Standard graph of *Azadirachta indica* silver nanoparticles using Deionized Water.

UV visible absorption spectra for *Azadirachta indica* lambda max were observed at 440nm and the calibration curve of *Azadirachta indica* was found tobe a linear graph following Beer's Lambert's law.

CHARACTERISATION OF *AZADIRACHTA INDICA* SILVER NANOPARTICLES

a. UV Visible Spectroscopy of *Azadirachta indica* Silvernanoparticles

The formation of nanoparticles was monitored based on the plasmon resonance effect of UV light on nanoparticles. The development of colour in the solution was due to the formation of silver nanoparticles which was attributed to the characteristic surface plasmon vibration of the respective nanoparticles. The absorption maxima were recorded at 440 nm.

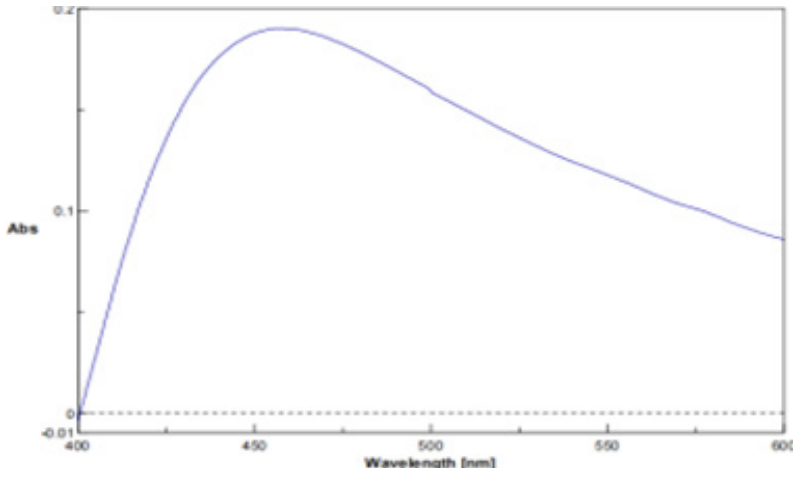

Figure 2. Spectrum of *Azadirachta indica* silver nanoparticles.

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b. FTIR Analysis of *Azadirachta indica* Silver Nanoparticles

The compatibility between the silver nanoparticles and PEG 400 was evaluated using the FTIR method. There was no appearance or disappearance of peaks in the silver nanoparticles - polymer PEG 400, which confirmed the absence of any chemical interaction between the silver nanoparticles and PEG 400.

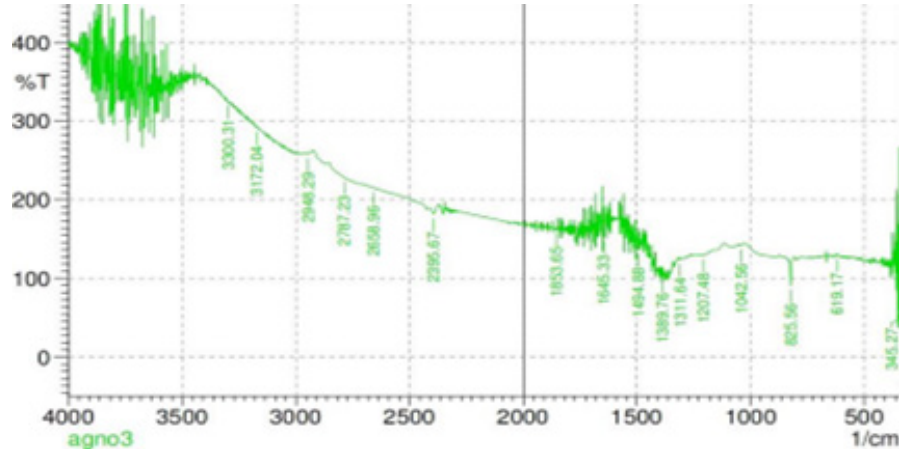

Figure 3. FT IR of *Azadirachta indica* Silver Nanoparticles.

Table 4. FT IR of *Azadirachta indica* Silver Nanoparticles

Materials	Functional group	Type of vibration	Characteristic vibration (Cm^{-1})	Test absorption (Cm^{-1})
Silver nanoparticles	C-H	Stretching	2850 – 3000	2998.29
	-NH2	Stretching	3200-3500	3300.31
	Ag ions	Shoulder peak	-	1645.33, 1389.76

Table 5. For FTIR of *Azadirachta indica* silver nanoparticles + PEG 400

Materials	Functional group	Type of vibration	Characteristic vibration (Cm^{-1})	Test absorption (Cm^{-1})
Silver nanoparticles + PEG 400	C-H	stretching	2850 – 3000	2883
	-NH2	stretching	3200-3500	3569.39
	Ag ions	stretching		1633.76, 1383.97

FORMULATION AND OPTIMIZATION OF DIFFERENT TYPE OF SOAP FILMS

The various soap films were prepared using sodium hydroxide and potassium hydroxide.

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Table 6. Formulation of different types of soap films

Name of ingredient	Quantity				
	F1	F2	F3	F4	F5
Coconut oil	9ml	9ml	9ml	9ml	9ml
Sodium hydroxide	4.3 g	4.3 g	4.3g	4.3 g	4.3
Methylcellulose	5.6 g	5.6 g	5.6g	5.6g	5.6g
Ethanol	25 ml	25 ml	25 ml	25ml	25ml
Water	75 ml	75 ml	75 ml	75 ml	75ml
PEG 400	2ml	-	-	2ml	2ml
PEG 1000	-	2ml	-	-	-
PEG 6000	-	-	2g	-	-
Sodium lauryl sulphate	-	-	-	300mg	-
Potassium hydroxide	-	-	-	-	4.32g
Silver nanoparticles	-	-	-	.20g	-

F1 formulation containing polymer poly ethylene glycol 400 and the soapfilm obtained after drying show good texture but the soap seems to be brittle.

F2 formulation containing polymer poly ethylene glycol 1000 and the soapfilm obtained after drying shows a hard clumpy mass.

F3 formulation containing polymer poly ethylene glycol 6000 and the soapfilm obtained after drying shows sticking to the Petri dish and is difficult to remove.

F4 formulation containing polymer poly ethylene glycol 400 along with sodium lauryl sulphate showing good texture good foaming ability and easy to handle.

F5 formulation contains potassium hydroxide instead of sodiumhydroxide and it shows a watery texture and is difficult to dry.

Different type of soap formulations was formulated by using sodium hydroxide and potassium hydroxide. The formulations prepared were tabulated above. Among this formulation, the soap containing sodium hydroxide was used because the soap containing potassium hydroxide was irritating to the skin. Another reason was the potassium hydroxide-containing soap doesn't provide the texture of a film. The formulation F4 was used for the study because it shows all the characteristics of a soap.

c. SEM Analysis of *Azadirachta indica* Silver Nanoparticles

SEM images of soap impregnated with silver nanoparticles exhibited spherically agglomerated particles due to the presence of soap.

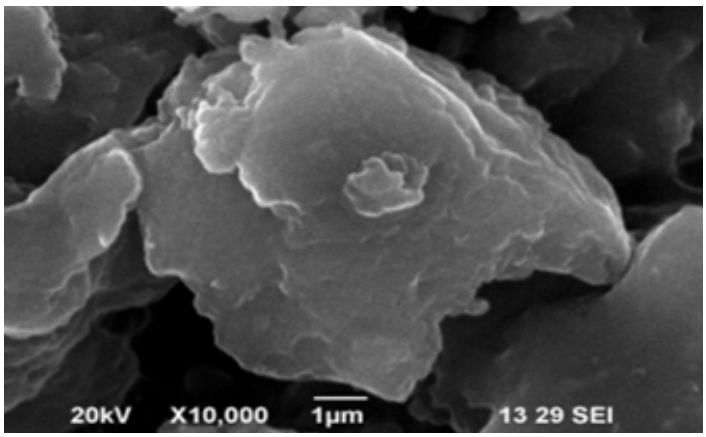

Figure 5. SEM Images for Silver Nanoparticles with 10000X magnification.

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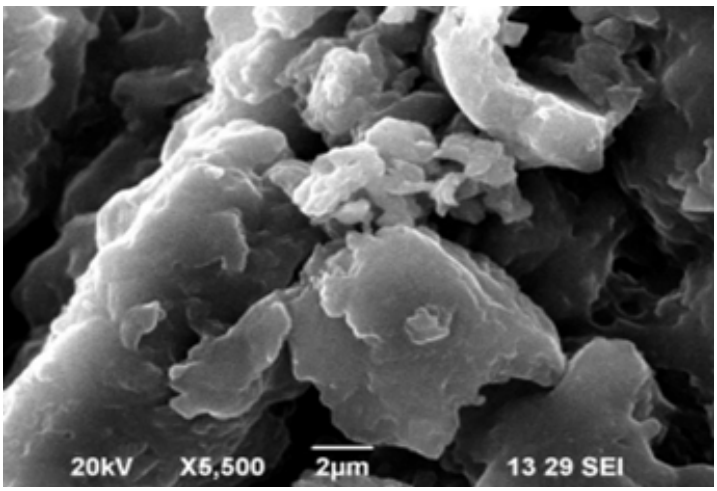

Figure 6. SEM Images for Silver Nanoparticles with 5500X magnification.

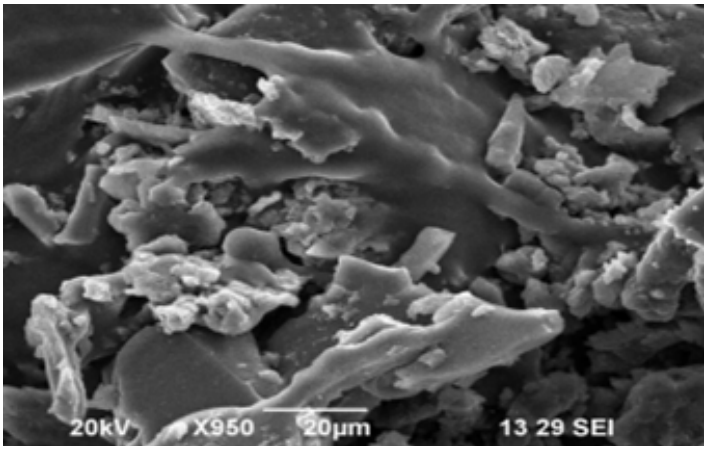

Figure 7. SEM Images for Silver Nanoparticles with 950X magnification.

d. Antibacterial Studies of *Azadirachta indica* Silver Nanoparticles

The bactericidal properties of AgNPs were confirmed in studies against gram-positive (Staphylococcus Aureus) and gram-negative (E. coli) bacterial strains. For silver nanoparticles, the highest concentration of 100 µg/ml showed activity ranging between 20 for E. coli and 19 mm zone of inhibition for Staphylococcus Aureus. silver nanoparticle-impregnated biomedical fibre showed efficient antimicrobial activity. It is reported that silver nanoparticles have the advantage of high anti-microbial activity even at low concentrations.

Table 7. Zone of inhibition for gram-positive (staphylococcus aureus) for *Azadirachta indica* silver nanoparticles

CONCENTRATION (µg/mL)	ZONE OF INHIBITION (mm)
25	12
50	15
75	16
100	20
Standard (Amikacin)	23

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At a concentration of 25µg/mL, the zone of inhibition was found to be 12 mm and showed a positive result. In comparison with the 25µg/mL, the zone of inhibition for the 50 µg/mL was increased by 3nm. For 75µg/mL, the zone of inhibition was found to be increased by 1nm. For the 100µg/mL, the zone of inhibition was found to be19 and increased by 4nm.

The different concentrations of 25 -100 µg/mL of *Azadirachta indica* silver nanoparticles were prepared and the antibacterial study was done against staphylococcus aureus. The Azadirachta indica silver nanoparticle shows activity even in small concentrations. It is showing good compatibility against standard Amikacin.

Figure 8. Antibacterial study for Gram-positive Azadirachta indica silvernanoparticles.

Table 8. Zone of inhibition of gram-negative (Escherichia coli) for Azadirachta indica silver nanoparticles

CONCENTRATION (µg/mL)	ZONE OF INHIBITION (mm)
25	10
50	13
75	15
100	19
Standard (Amikacin)	21

At a concentration of 25µg/mL, the zone of inhibition was found to be10mm and showing positive results. In comparison with the 25µg/mL, the zone of inhibition for the 50 µg/mL was increased by 3nm. For 75µg/mL, the zone of inhibition was found to be increased by 2 nm. For the 100µg/mL, the zone of inhibition was found to be19 and increased by 5nm.

The different concentrations of 25 -100 µg/mL of Azadirachta indica silver nanoparticles were prepared and the antibacterial study was done against Escherichia coli. The Azadirachta indica silver nanoparticle shows activity evenin small concentrations. It is showing good compatibility against standard Amikacin.

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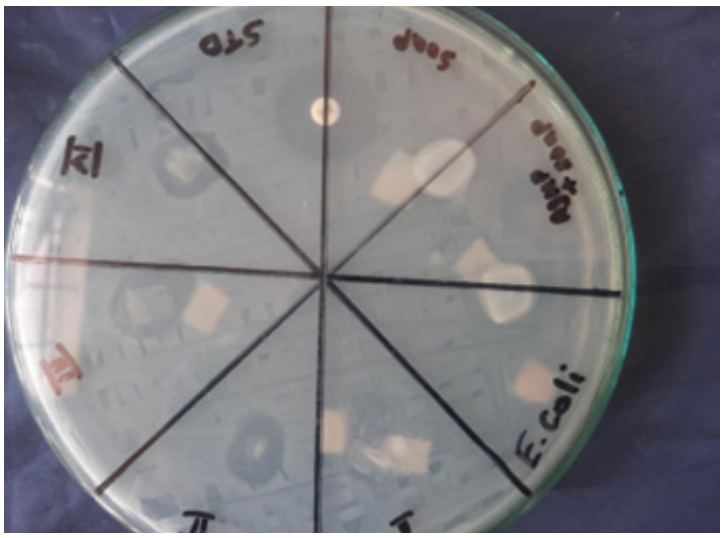

Figure 9. Antibacterial study for Gram Negative for Azadirachta indica

Silver nanoparticles

The antibacterial study for Azadirachta indica silver nanoparticles was conducted in both gram-positive and gram-negative bacteria. The result was foundthat both gram-positive and gram negative bacteria showed good antibacterial activity. But by comparison, the Azadirachta indica silver nanoparticles showed more effect in gram-positive bacteria.

EVALUATION OF SOAP INCORPORATED METHYL CELLULOSE

Preparation and evaluation of physicochemical parameters

The physicochemical parameters of the prepared soap were determined. Parameters such as colour, odour, appearance, and pH were tested. The formulations exhibited good appearance characteristics as well as the pH was found in the range 7.0 which is the desired pH. Other parameters such as percentage Foam height, Foam retention, total fatty matter (TFM), time taken to dissolve the soap and saponification value were determined; The results are tabulated.

Table 9. Evaluation parameters for soap-incorporatedmethylcellulose film

CHEMICAL PARAMETER	SOAP FILM	STANDARDS
Colour	White	-
pH	7.3	6.5-8
Foam height	3cm	2.5-3
Foam retention	2.5cm	0.5-2.5
Total fatty matter	77.6	70-90
Saponification value	0.184	-
Time taken to dissolve the soap	47 Seconds	-

The prepared soap incorporated methyl cellulose film showed a PH of 7.3 approximately equal to the skin pH. So, the soap film does not show any allergic reaction. The foam height and the foam retention were excellent. Bothlie in between the standards. The time taken to dissolve the soap film was found to be 47 seconds. The total fatty matter was found to be 77.6 indicating that it helps to remove the dirt easily.

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EVALUATION OF SILVER NANOPARTICLES IMPREGNATEDSOAP FILM.

a. Antibacterial Studies of Azadirachta indica Silver Nanoparticles in Corporated Soap Film

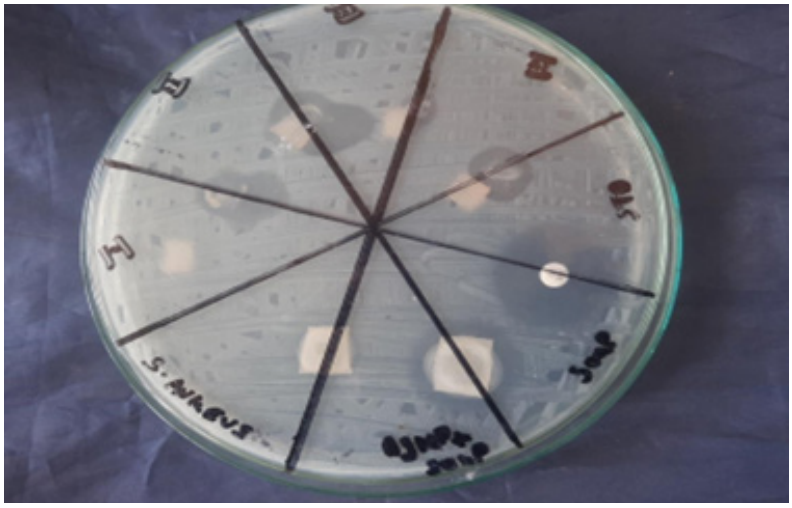

Figure 10. Antibacterial study for Gram-positive Azadirachta indicasilver nanoparticles.

The Azadirachta indica silver nanoparticles were prepared and incorporated in soap film and the antibacterial study was done against staphylococcus aureus. The Azadirachta indica silver nanoparticle shows activity even in small concentrations. It is showing good compatibility against standard Amikacin.

Table 8. Antibacterial study for Gram-positive for Azadirachta indica Silver nanoparticles

CONCENTRATION (µg/mL)	ZONE OF INHIBITION (mm)
Soap alone	7
Soap and AgNP	20mm
Standard (Amikacin)	23

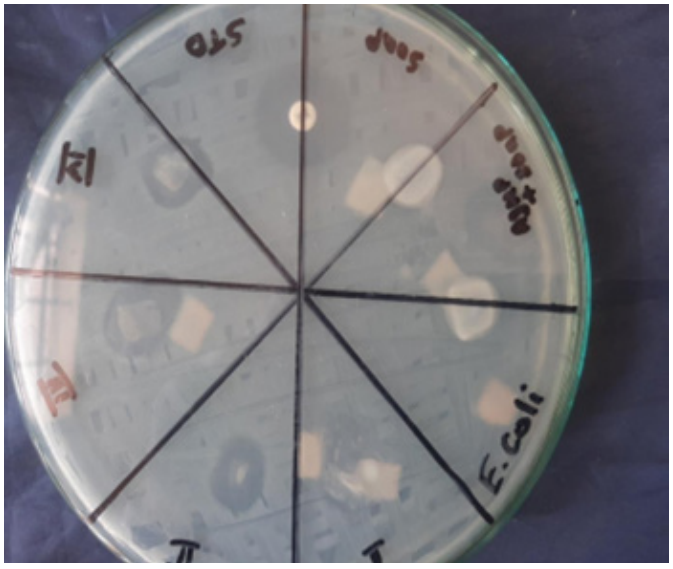

Figure 11. Antibacterial study for Gram Negative for Azadirachta indicasilver nanoparticles.

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The Azadirachta indica silver nanoparticles were prepared and incorporated in soap film and the antibacterial study was done against Escherichia coli. The Azadirachta indica silver nanoparticle shows activity even in small concentrations. It is showing good compatibility against standard Amikacin.

Table 9. Antibacterial study for Gram Negative for Azadirachta indica silvernanoparticles

CONCENTRATION (µg/ML)	ZONE OF INHIBITION (mm)
Soap alone	0
Soap and AgNP	20
Standard (Amikacin)	23

The anti-bacterial study for Azadirachta indica silver nanoparticles incorporated soap film was conducted in both gram-positive and gram-negative bacteria. The result was found that both gram-positive and gram-negativebacteria showed good antibacterial activity. But by comparison, the Azadirachta indica silver nanoparticles showed more effect in gram-positive bacteria.

b. UV Visible Spectroscopy of Azadirachta indica Silver Nanoparticles in Corporated Soap Film

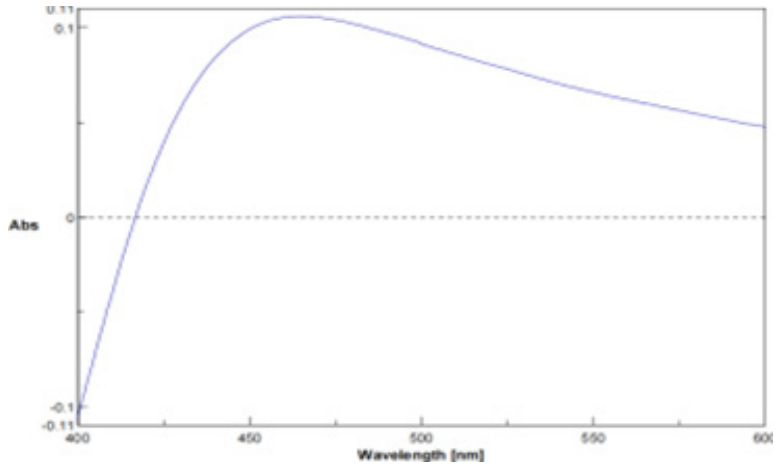

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Figure 12. Spectrum of Azadirachta indica silver nanoparticles incorporatedsoap film.

The UV visible spectrum of Azadirachta indica silver nanoparticles incorporated soap film showing the absorbance at 440 nm and indicating the formation of nanoparticles.

Soap is a mixture of sodium salts of various naturally occurring fatty acids. Soap is produced by a saponification or basic hydrolysis reaction of a fat or oils. The soap should have good ingredients which can kill bacteria but not damage body tissues. The bacteria including Gram-positive and Gram-negative are deposited from the environment on the surface of the skin and cause skin infection. Examples of these bacteria include *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*. The spread of infection by such bacteria can be prevented by the use of antiseptic soaps, as they contain antimicrobial chemicals, but overuse of soaps might result in antimicrobial resistance and even render a person more sensitive to allergies, skin rashes The objective of the studies is to Formulation and evaluation of soap film incorporated with green synthesized Azadirachta indica silver nanoparticles.

UV visible absorption spectra for Azadirachta indica lambda max were observed at 440nm and the calibration curve of Azadirachta indica was found to be a linear graph

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following Beer's Lambert's law. The green synthesised silver nanoparticles were done using *Azadirachta indica* leaves along with silver nitrate solution. The formation of nanoparticles was monitored based on the plasmon resonance effect of UV light on nanoparticles. The development of color in the solution was due to the formation of silver nanoparticles which was attributed to the characteristic surface plasmon vibration of the respective nanoparticles. The absorption maxima were recorded at 440 nm.

FTIR analysis of *Azadirachta indica* silver nanoparticles was performed. The compatibility between the silver nanoparticles and PEG 400 was evaluated using the FTIR method. There was no appearance or disappearance of peaks in the silver nanoparticles -polymer PEG 400, which confirmed the absence of any chemical interaction between the silver nanoparticles and PEG 400.

SEM analysis OF *Azadirachta indica* SILVERNANOPARTICLES was performed for the study. SEM images of soap impregnated with silver nanoparticles exhibited spherically agglomerated particles due to the presence of soap. Antibacterial studies of *azadirachta indica* silver nanoparticles were done in this study. The bactericidal properties of soap AgNPs were confirmed in studies against gram-positive (Staphylococcus Aureus) and gram-negative (E. coli) bacterial strains. For silver nanoparticles, the highest concentration of 100 µg/ml showed activity ranging between 20 for E. coli and 19 mm zone of inhibition for Staphylococcus Aureus. Silver nanoparticle-impregnated biomedical fibre showed efficient antimicrobial activity. It is reported that silver nanoparticles have the advantage of high anti-microbial activity even at low concentrations. The antibacterial study for *Azadirachta indica* silver nanoparticles was conducted in both gram-positive and gram-negative bacteria. The results were found that both gram-positive and gram negative bacteria showed good antibacterial activity. But by comparison, the *Azadirachta indica* silver nanoparticles showed more effect in gram-positive bacteria. Formulation and optimization of different types of soap films were carried out in this study. Different types of soap formulations were formulated by using sodium hydroxide and potassium hydroxide. The formulations prepared were tabulated above. Among this formulation, the soap containing sodium hydroxide was used because the soap containing potassium hydroxide was irritating to the skin. Another reason was the potassium hydroxide-containing soap didn't provide the texture of a film.

The formulation F4 was used for the study because it shows all the characteristics of soap. Evaluation of soap-incorporated methylcellulose film was done using various methods. The physicochemical parameters of the prepared soap were determined. Parameters such as colour, odour, appearance, and pH were tested. The prepared soap incorporated methyl cellulose film showed a pH of 7.3 approximately equal to the skin PH.

So, the soap film does not show any allergic reaction. The foam height and the foam retention were excellent. Both lie in between the standards. The time taken to dissolve the soap film was found to be 47 seconds. The total fatty matter was found to be 77.6 indicating that it helps to remove the dirt easily.

Evaluation of silver nanoparticles impregnated soap film was done using in vitro drug release. The anti-bacterial study for *Azadirachta indica* silver nanoparticles incorporated soap film was conducted in both gram-positive and gram-negative bacteria. The result was found that both gram-positive and gram-negative bacteria showed good antibacterial activity. But by comparison, the *Azadirachta indica* silver nanoparticles show more effect in gram-positive bacteria. The green synthesis of stable silver nanoparticles as small as 10nm using endemic plant species *Azadirachta indica* aqueous leaf extract was reported. Synthesis was found to be efficient in terms of reaction time as well as the stability of synthesized nanoparticles without any external stabilizers and reducing agents. Ag-NPs using *Azadirachta indica* can be advantages over other biological entities which can overcome the slow route of using microorganisms and sustain their culture which can lose their potential towards the production of NPs. Other advantages of synthesis from plant extracts are the provision of a hygienic working environment, health and environment shielding, lesser wastage and the most stable products. Ag-NPs synthesized by green route have important aspects of nanotechnology through numerous applications. Ag-NPs have emerged in the present and future era. The study revealed the efficacy of synthesizing silver nanoparticles (AgNPs) using green principles and its potential application in health and the environment.

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