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Green Synthesis of Silver Nanoparticles using *Hybanthusenneaspermus* Plant Extract against Nosocomial Pathogens with Nanofinished Antimicrobial Cotton Fabric

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Abstract

Nosocomial infection is defined as an infection which develops 48 hours after hospital admission or within 48 hours after being discharged that was not incubating at the time of admission at hospital. Nosocomial infections (NI) are frequent complications of hospitalizations. The issue has been recognized for more than a century as a critical problem affecting the quality of health care and a principal source of adverse outcomes. Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain. The main objective of this research is to impart antimicrobial finish to cotton fabrics and to produce medical apparel from the finished fabrics. Thus the present study provided different products of medical importance, which could be used in different medical and healthcare applications. This work we investigated cost effective and environment friendly antibacterial properties of cotton fibers loaded with silver nanoparticles (AgNPs) synthesized from natural plant extracts as a reducing and capping agent.

Keywords: Green synthesis; *Hybanthusenneaspermus*; Nanofinished Antimicrobial Cotton Fabric; Nosocomial infections and Silver nanoparticles

Abbreviations: SEM: Scanning Electron Microscopy; TEM: Transmission Electron Microscopy; XRD: X-ray Diffraction; FT-IR: Fourier Transform Infra Red Spectroscopy; DLS: Dynamic Light Scattering.

Introduction

Nosocomial infections (NI) are frequent complications of hospitalizations. The issue has been recognized for more than a century as a critical problem affecting the quality of health care and a principal source of adverse outcomes [1]. Nosocomial infections can cause severe pneumonia and infections of the urinary tract, bloodstream and other parts of the body. Many types are difficult to attack with antibiotics, and antibiotic resistance is spreading to Gram negative bacteria that can infect people outside the hospital [2]. The risk factors for Nosocomial infections include: diabetes mellitus, intubation, persistent sounding, surgical drains, poor health status, lack of using gloves, irregular and inappropriate debridement and wound bandage. The Nosocomial infections are caused by bacterial, viral and fungal pathogens. The most common pathogens are *Staphylococci, Pseudomonas, E. coli, Mycobacteriumtuberculi, Candida, Aspergillus, Fusarium, Trichosporon* and *Malassezia.*

All are associated with increased morbidity and mortality. Precautions to prevent Nosocomial infection in ICU include use of hand hygiene before and after contact with patient and respiratory devices, aseptic technique during catheter insertion and care, and prompt removal of catheters that are no longer essential. Nosocomial infections are typically exogenous, the source being any part of the hospital ecosystem, including people, objects, food, water and air in the hospital. These infections are opportunistic

and microorganisms of low virulence can cause disease in hospital patients whose immune mechanisms are impaired. The outcome is that many antibiotics can no longer be used for the treatment of infections caused by such organisms and the threat to the usage of other drugs increases [3]. Prevention of Nosocomial infections is the responsibility of all individuals and services providing health care. Everyone must work cooperatively to reduce the risk of infection for patients and staff.

Infection control programs are effectively provided they are comprehensive and include surveillance and prevention activities, as well as staff training. An "Infection Control Committee" provides a forum for multidisciplinary input and cooperation, and information sharing. This committee should include wide representation from relevant disciplines, e.g. management, physicians, other health care workers, clinical microbiology, pharmacy, central supply, maintenance, housekeeping, training services. Nature has been a source of therapeutic agents for thousands of years and an impressive number of modern drugs have been derived from natural sources. Various active compounds (or their semi-synthetic derivatives) derived from medicinal plants have been assessed for their efficacy and tolerability in the treatment of different diseases. Beside the therapeutic potential of medical plant/drugs, therapeutic index also indicates the drug safety. The therapeutic index is a measure of the drug's beneficial affects at a low dose versus its harmful effects a high dose. A high therapeutic index is an indication of large safety margin between beneficial and toxic dose.

Medicinal plants are the important part of indigenous pharmaceutical systems. According to the World Health Organization (WHO), about 65-80% of the world's population in developing countries, due to the poverty and lack of access to modern medicine, depend essentially on plants for their primary healthcare [4]. Medicinal plants consist of components of therapeutic values and have been used as remedies for human diseases since long. Recently, due to the pathogens resistance against the available antibiotics and the recognition of traditional medicine as an alternative form of health care has reopened the research domain for the biological activities of medicinal plants [5].

Today about 1500 species of medicinal plants are being used in many countries including Albania, Bulgaria, Croatia, France, Germany, Hungary, Spain, Turkey and the United Kingdom [6]. Medicinal plants have played important role in the traditional and orthodox system of medicine in the curing of different types of diseases. Analysis of different species of medicinal plants for biologically active components known to have pharmacological properties have been conducted and most of the studied plants have shown antimicrobial property [7]. Nanotechnology is the emerging field in science. It is much clear that nanoscale materials can make huge difference than we experienced [8]. Nanotechnology extends the field of biological system which provides wide range of research in the field of genetics [9]. Nanotechnology which paved a way to understand and transform biosystem from nanoscale principal technology.

Silver plays a vital role in antimicrobial, catalytic and biological systems among the other metals and the synthesis of silver nanoparticles as an antimicrobial agent has gained more importance against the increasing threat posed by antibiotic resistant microbes. Though there are reports on the synthesis of silver nanoparticles with desirable size and shape exhibiting antimicrobial activity using physical and chemical methods, but their potential use in biomedical field is uncertain owing to their toxic nature [10]. In biological method synthesis of silver nanoparticles using microorganism, enzyme and plant or plant extract have been suggested as possible eco-friendly alternatives to chemical and physical methods. Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures. Silver shows an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes.

In addition, silver-containing consumer products such as colloidal silver gel and silver-embedded fabrics are used in sports related equipments [11]. *Hybanthusenneaspermus* (L.) F. Muell. (*H. enneaspermus*), belonging to the family Violaceae, is a herb or a shrub distributed in the tropical and subtropical regions of world, and occurs mostly in the warmer parts of Deccan peninsula in India. The plant is popularly called "Ratanpurus" by the local Yanadi and Santal tribes, villagers and herbalists. This ethno botanical herb is known to have unique medicinal properties [12]. It is also known as "hump back flower", and is a member of a genus of perennial herbs, often creeping, whose leaves are alternate or in clusters. The genus consists of 150 species found in different regions of the world, often seen in mountainous regions [13].

The main objective of this research is to import antimicrobial finish to cotton fabrics and to produce medical apparel from the finished fabrics. Thus the present study provided different products of medical importance, which could be used in different medical and healthcare applications. The present study was designed to synthesis and characterizes silver nanoparticles and to investigate the antibacterial activity and fabrication by *Hybanthusenneaspermus* extract. The obtained particles were analyzed by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD), Fourier Transform Infra Red Spectroscopy (FT-IR), Dynamic Light Scattering (DLS).This work we investigated cost effective and environment friendly antibacterial properties of cotton fibers loaded with silver nanoparticles (AgNPs) synthesized from natural plant extracts as a reducing and capping agent.

Materials and Method

Sample collection

The Nosocomial samples were collected from clinical laboratory at Namakkal. The specimens were transport immediately to the microbiology laboratory and processed without any delay.

Preparation of plant extract

Fresh leaves of *Hybanthusenneaspermus*, were collected from Kolli Hills, Tamilnadu and washed several times with

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water to remove the dust particles and then sun dried to remove the residual moisture and grinded to form powder. 100 g of *Hybanthusenneaspermus* powder was used for extraction with ethanol, hexane, chloroform, methanol and petroleum ether using a Soxhlet extraction apparatus at the boiling point of the solvent for 48-72 hours or until the extracted solvent become clear. After that extracts were filtered with the help of filter paper and solvent was evaporated from extract in rotary evaporator to get the syrupy consistency. Then extract was kept in refrigerator at 4°C for future experiments.

Phytochemical analysis of plant extracts

Qualitative chemical tests were carried out using extract from plant to identify the photochemical. A 10 mg/ml flower extract was used for the tests. The plant extract were analyzed for the Alkaloids, Flavonoids, Steroids, Terpenoids, Carbohydrate, Phenols, Tannins, Glycosides and Catachol.

Alkaloid

Mayer's test: Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in potassium Iodide). Formation of brown (or) reddish precipitate indicates the presence of alkaloids.

Flavonoids

Lead Acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Glycosides

Borntrager's Test: Extracts were treated with 3 drops of concentrated hydrochloric acid and heated on a boiling water bath for 10 minutes then cooled and add 1ml of chloroform and Ammonia. Formation of pink to red colour indicates the presence of glycosides.

Aqueous NaOH Test: A few drops of alcoholic neutral ferric chloride solution was added to the powdered flower sample previously dissolved in alcohol or distilled water. Formation of violet, Bluish green or bluish black colour indicated the presence of phenols.

Steroids

Salkowski Test: To a 0.5 gm of extract, 2 ml of chloroform was added and then concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colour formation at the interface was noted for the presence of steroids.

Terpenoids: About 5 ml of each extract was added to 2 ml of chloroform and 3 ml of concentrated H2SO¬4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.

Tannins: To 5ml of extracts were added to few drops of 1% lead acetate solution. A yellow precipitates indicated the presence of tannins.

Phenols

Ferric Chloride Solution: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of carbohydrate: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Catachol

To 2g of extract add Erlich's reagent and few drops of concentrated hydrochloric acid and the result was observed. The concentrated extracts were obtained as residues. After which the residues were transferred into pre-weighed sample containers and were stored and later used for phytochemical screening an antimicrobial activity.

Synthesis of silver nanoparticles: Four concentration ratios of plant and metal ions were prepared (30:1, 60:1,120:1& 240:1) by increasing the concentration of plant extract concentration in the solution. 0.17% of 1mM $AgNO_3$ metal ion was added in the prepared plant extract. Then the bio-reduced aqueous component was used to measuring UV-Vis spectra of the solution.

Characterization of silver nanoparticles

UV-Vis Analysis: The optical property of AgNPs was determined by UV-Vis spectrophotometer (Perkin-Elmer,Lamda 35,Germany). After the addition of $AgNO_3$ to the plant extract, the spectra's were taken in different time intervals up to 24 hours at 350to 500nm. Then the spectra were taken after 24hours of $AgNO_3$ addition.

Dynamic Light Scattering (DLS): DLS was used to determine the size of Brownian nanoparticles in colloidal suspensions in the nano and submicron ranges. Shining monochromatic light (laser) onto a solution of spherical particles in Brownian motion causes a Doppler shift when the light hits the moving particle, changing the wavelength of the incoming light. The average nanoparticles size and zeta potential of synthesized silver nanoparticles were determined by Dynamic light scattering was done using Malvern zetasizer version 2.2.

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Attenuated Total Reflection - Fourier Transform Infrared Spectrophotometery (ATR-FTIR): The Attenuated total reflectance (ATR) is a sampling technique used in conjunction with infrared spectroscopy. ATR is applicable to the same chemical (or) biological systems as the transmission method. A Fourier Transform Infrared Spectrophotometer, abbreviated as FTIR, can generate an infrared spectral scan of samples that absorb infrared light. A material's absorbance of infrared light at different frequencies produces a unique "spectral fingerprint" based upon the frequencies at which the material absorbs infrared light and the intensity of those absorptions (Bruker Tensor 27 Germany).

X ray Diffraction

X – Rays are electromagnetic radiations similar to the light, but with a much shorter wavelength. If an incident X – ray beam encounters a crystal lattice, general scattering occurs. Although most scattering interfere with itself and is eliminated, diffraction occurs when scattering in a certain direction is in phase with scattered rays from other atomic planes. Under these condition the reflection combine to form new enhanced wave fronts that mutually reinforce each other. The data of phase and structural analysis of the Ag nanoparticles was obtained with a Siemens x-ray diffractometer (Japan), and the target was CuKα (λ = 1.54 Å). The generator was operated at 30 kV and with a 20mA current. The scanning range (2θ) from 10 to 80°, scanning speed of 1°/min and a chart speed of 20mm/ min were selected.

Energy Dispersive X – ray Spectroscopy

Elemental images were obtained for the relevant surface elements and estimate their proportion at different position, thus giving an overall mapping of the sample. An electron beam strikes the surface of the conductive sample; the energy of the beam was typically in the range of 10 – 20 kV. This caused X-rays to be emitted from the material. The energy of X-ray emitted depends on the material under examination. The X – rays were generated in a region about two microns in depth. By moving the electron beam across the sample, an image of each element in the sample can be obtained. The surface elements of the synthesized Ag nanoparticles were analyzed using EDS.

High Resolution - Transmission Electron Microscopy (HR - TEM) and SAED analysis

Transmission electron microscope is a microscopy technique where by a beam of electron is transmitted through ultra thin specimen and interacts as passes through the sample. In TEM the crystalline sample interacts with the electron beam mostly by diffraction rather than by absorption. The intensity of the diffraction depends on the orientation of the planes of the atoms in a crystal relative to the electron beam; at certain angles the electron beam is diffracted strongly from the axis of the incoming beam, while at other angles the beam is largely transmitted. A small amount of liquid samples were redispersed by sonication on a copper grid and then air dried (TEM, JEOL-JEM 2100).

Antibacterial activity of biologically synthesized silver nanoparticles

A loop full of bacterial culture was taken from the stock culture and inoculated in nutrient broth kept for 12 hours incubation. The silver nanoparticles synthesized using *Hybanthusenneaspermus* plant extract was tested for antibacterial activity by agar well diffusion method against *E. coli*, *Pseudomonas aeroginosa*, *Staphylococcus aureus, Klebsiellapneumoniae, Serratiamarcescens*. Each stain was swabbed uniformly on Muller-Hinton agar plates using sterile cotton swabs. The disc was prepared with plant extract and synthesized silver nanoparticles extract in 30µg concentration and the disc were placed on Muller-Hinton agar plates with standard antibiotic tetracycline disc, then the plates were incubated at 24°C.

Finishing of fabrics with the silver nanoparticles

Fabric Used

The following test fabric was 100% Woven Cotton fabrics which was allowed for enzyme treatmen

Fabric pretreatment

The 100% bleached cotton fabric was desired using silver nanoparticles in a shaking water bath. Before finishing the fabric with the synthesized silver nanoparticles, the test fabric was allowed to enhance the easy absorption of SNPs by the fabric (Table 1).

Table1: Specifications of the fabric used for treatment.

Type	Pre Finishing	Warp Count	Weft Count	Ends Per Inch	Picks	Width
100% Woven Cotton Fabric	Bleached	40^sK	40^sK	80	65	165

Coating of Cotton Fabrics with Synthesized silver Nano particles by Pad Dry cure method

Biologically synthesized silver nanoparticles were applied on cotton using pad-dry-cure method. The cotton fabric cut to the size of 30 X 30 cm was immersed in the solution containing nanoparticles for 20 min and then it was passed through the padding mangle (R.B. Electronic and Engineering, Mumbai), running at a speed of 15 m/min with a pressure of 1 kg /cm^2 to remove excess solution. A 100% wet pick-up was maintained for all of the treatments. After padding, the fabric was air-dried and then cured for 3 min at 140°C and immersed for 5 min in 2 g/l of sodium lauryl sulfate to remove unbound nanoparticles and rinsed to remove the soap solution followed by air-drying [14].

Characterization of fabric after treatment

The fabric coated with synthesized silver nanoparticles was characterized for its antimicrobial, physical and chemical properties.

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Antimicrobial characterization of the finished fabrics

The biologically synthesized NPs were used as an antimicrobial finish for the cotton fabric. The antimicrobial efficacy of the fabric was assessed using the following tests.

- Antibacterial activity (AATCC 100)

Assessment of antibacterial activity

Antibacterial assessment of the biologically synthesized silver nano particles treated fabric was determined using following tests.

A. Qualitative tests

- Agar diffusion test (SN195920)

- Parallel streak method (AATCC TEST method 147-1988)

B. Quantitative tests

- Percentage reduction test (AATCC 100)

5.4.3. Agar diffusion method (SN 195920)

Sterile AATCC Bacteriostasis agar was dispensed in sterile Petri dishes. 24 hours broth cultures of test organisms used as an inoculums. Using sterile cotton swab the test organisms were coated over the surface of the agar plate. The test specimen was gently pressed in the center of the mat culture. The plates were incubated at 37oC for 18-24 hours and examined the zone of inhibition.

Parallel streak method (AATCC test method 147-1988)

Sterile AATCC bacteriostasis agar was dispensed in sterile Petri-dishes. 24 hours broth cultures were used as inoculums. Using sterile 4mm inoculating loop, one loop full of culture was loaded and transferred to the surface of the agar plate by making five parallel inoculums streaks approximately 60mm in length and spaced 10mm covering the central area of the petridish without refilling the loop. The test specimen was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with agar surface. The plates were incubated at 37oC for 18-24 hours and examined the zone of inhibition.

Zone of inhibition (mm) = (T-I)/2

T-width of zone of inhibition

I-width of specimen

Quantitative Bacterial Reduction Test (AATCC test method 100-2004)

Five sets of sterile AATCC Bacteriostasis broth were prepared each of 100ml quantity. The swatches to be treated were placed in sterile petridishes and 1.0 ml of the test inoculate were loaded using micropipette. The treated and untreated swatches were then transferred to the respectively labeled sterile AATCC Bacteriostasis broth. The flasks were then incubated in shaker at room temperature for 24 hours. After incubation, serial dilutions were made up to 10^{-7} for all the samples. About 0.1ml sample from each dilution were transferred to the sterile AATCC Bacteriostasis agar plates and spread plated. The inoculated plates were then incubated at 37oC for 24 hours and examined the percentage reduction.

100(B-A)/B=R

Where, R= % reduction

A= the number of bacteria recovered from the inoculated treated swatch

B= the number of bacteria recovered from the inoculated untreated swatch

Wash durability test

Washing was carried out as per test no: 1 of IS: 687-1979 by using a neutral soap (5gpl) at 40oC 2oC for 30 minutes, keeping the material: liquor ratio at 1:50, followed by rinsing, washing and drying. After drying, the test samples were assessed for antimicrobial activity using AATCC 100 procedure up to 40 laundering cycles.

Physical characterization

The biologically synthesized silver nano particles treated fabrics and untreated fabrics were physically characterized by ATR - FTIR, Air permeability test (ASTM D737-96), Tensile strength test, Stiffness test (D6828 – 02(2007), Abrasion test, Tear strength test,

Attenuated total reflection fourier transform infrared spectrophotometry (ATR FTIR)

The Attenuated total reflectance (ATR) is a sampling technique used in conjunction with infrared spectroscopy. ATR is applicable to the same chemical (or) biological systems as the transmission method. The surface of the treated and untreated fabrics was analyzed using FTIR Spectrophotometer (Bruker Tensor 27 Germany). A typical infrared scan was generated in the mid-infrared region of the light spectrum. The mid-infrared region was from 400 to 4000 wave numbers, which equaled wavelengths of 2.5 to 25 microns (10-3mm).

Air permeability: (ASTM D737-96)

This test method covers the measurement of the air permeability, the rate of air flow passing perpendicularly through a known area under a prescribed air pressure differential between the two surfaces of a textile material. Construction factors and finishing techniques can have an effect upon air permeability by causing a change in the length of airflow paths through a fabric. This test was done with an air permeability testing apparatus. Pressure gauge and flow meter were used to measure the air permeability. The samples were tested at a relative humidity 65% and at a temperature of 21°C. Five readings were taken for each sample and the average value was calculated.

Tensile strength

Tensile strength is the measure of the resistance of the fabric tensile load or stress in either warp or weft direction. It is the strength shown by a specimen subjected to tension as distinct from

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torsion, compression, or shear. Elongation defines the length to which a fiber may stretch before breaking. A sample of 12" X 2" was taken for the test. The tensile strength of the fabric was determined by cloth tensile strength tester. Tensile strength is performed using cut strip method. This test is used for coated or heavily sized fabrics. Four readings for every sample were taken and the average was calculated.

Stiffness tests: (D6828 – 02(2007)

This test method covers the determination of the stiffness of fabrics by measuring the force required to push a specimen into a slot of predetermined width with a metal blade working at a predetermined capacity. Four readings for every sample were taken and the average was calculated.

Abrasion tests: (AATCC 119-2004)

Abrasion test determine the ability of a fabric to withstand damage by friction. This is dependent on the fineness of the fiber, the amount of twist of the yarn and the weave structure of the fabric. Yarns that have a firmer and tighter twist are generally more resistant to abrasion. The fabric specimen was mounted over a foam rubber cushion and rubbed multi-directionally against a wire screen mounted on a weighted head. Abrasion was carried out for 75 revolutions for the fabrics. The initial and final weight of the fabric was noted. Four readings for every sample were taken and the average was calculated.

$$\text{Abrasion resistance \%} = \frac{\text{Weight before abrasion} - \text{weight after abrasion} \times 100}{\text{Weight before abrasion}}$$

Result and discussion

Identification of isolated organisms

The isolated organisms from Nosocomial infected patients were identified as Klebsiellapneumoniae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosa, and Serratiamarcescens based on their Morphology, Staining, Motility and Biochemical test.

Phytochemical analysis of Hybanthusenneaspermus

Phytochemical analysis of Hybanthusenneaspermus was carried out by the following solvents Methanol, Ethanol, Petroleum ether, Hexane and Chloroform. Petroleum ether extract of Hybanthusenneaspermus contains Steroids, Sugars, and Alkaloids, Phenolic groups, Flavones, Catachin, Saponins, Anthroquinone glycosides, Amino acids and Flavononones. Ethanol extract contain all the constituents except Alkaloids, Anthroquinone glycosides Amino acids. Methanol extract had all the components except Triterpenes, Anthroquinone glycosides Amino acids. Hexane extract found to have Steroids, sugars, Phenolic groups, Flavones Tannins and Flavononones all other constituents were absent. Chloroform extract consists of Steroids, Triterpenes, sugars, Phenolic groups, Flavones, Saponins, Tannins and Flavononones, others found to be absent. The predominant components present in all the extracts of Hybanthusenneaspermus are Sugars, Phenolic groups, Flavones, Tannins and Flavononones. The results were plotted in Table 2.

Table 2: Preliminary Phytochemical screening of various extracts of the leaves of Hybanthusenneaspermus

Constituents	Petroleum ether	Ethanol	Methanol	Hexane	Chloroform
Steroids	+	+	+	+	+
Triterpenes	-	+	-	_	+
Sugars	+	+	+	+	+
Alkaloids	+	-	+	-	-
Phenolic groups	+	+	+	+	+
Flavones	+	+	+	+	+
Catachin	+	+	+	-	-
Saponins	+	+	+	-	+
Tannins	+	+	+	+	+
Anthroquinone glycosides	+	-	-	-	-
Amino acids	+	-	-	-	-
Flavononones	+	+	+	+	+

Biosynthesis of silver nanoparticles (SNPs)

The reaction mixture, Hybanthusenneaspermus extract with aqueous solution of the silver nitrate, the reaction mixture started to changes its colour from yellow to brown after two hours of the reaction it is inferred in that AgNPs exhibit dark brown colour in an aqueous solution. Silver nanoparticles were synthesized using Tulsi leaf extract under static condition. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. As the extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from colorless to yellowish brown due to reduction of silver ion which indicated formation of silver nanoparticles [15].

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Physical characterization of silver nanoparticles

UV-Visible spectra analysis

The UV-Vis spectra analysis is a simpler and promising method for analyzing the formation of SNPs. UV-Vis spectrophotometer and expressed in Figure 1. The peaks in the absorption spectra were due to the light absorption. The organism synthesized silver nanoparticles showed a strong SPR absorption peak at around 400 to 412nm due to the formation of silver nano particles by Hybanthusenneaspermus. The peak at 440 nm was due to the excitation of longitudinal Plasmon Vibrations which resulted in the maximum absorbance at this wavelength. The presence of longitudinal Plasmon Vibrations in the synthesis of silver nanoparticles was also discussed by [16].

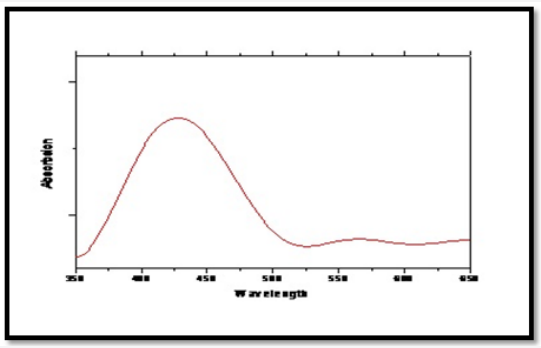

Figure 1: UV-Vis spectra of reduction of Silver nitrate to Silver Nanoparticles by Hybanthusenneaspermus.

Dynamic light scattering measurement (DLS)

The Dynamic light scattering was used for the measurement of small amount of large (>100nm) colloidal silver nanoparticles by the two electrodes. In order to remove the coarse particles and provide the reduction of silver ions present in the solution by ultra sonication. The particle size distribution showed high intensity of the maximum peak range from 20nm to 80nm and possessed an average size of 75.58nm with a zeta potential of -28.2. The particles were uniformly distributed (monodispersed) without significant agglomeration (Figure 2).

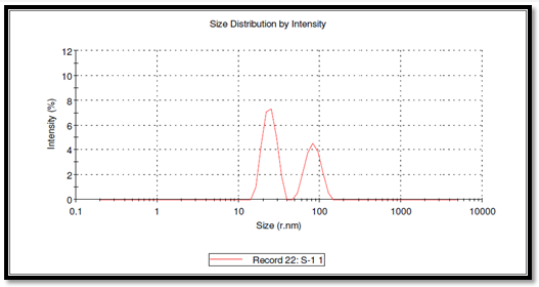

Figure 2: Particle size distribution of the silver nanoparticles synthesized by Hybanthusenneaspermus

Owing to their small size, the total surface area of the nanoparticles was maximized, leading to the highest values of the activity to weight ratio. The frequency distribution observed from the graph showed that almost 58% of the particles in the 24nm and 42% of the particles were in 84nm range. The test conducted had demonstrated that synthesized silver nanoparticles added to water showed a pronounced antibacterial/antifungal effect. It has been showed that the smaller silver nanoparticles have a greater effect (Figure 3). The particle size distribution of the silver nanoparticles synthesized by Hybanthusenneaspermus was evaluated using Dynamic light scattering measurements. The particle size distribution showed a high intensity in the maximum peak range from 5nm to 70nm and possessed an average size of 38.88nm with a zeta potential of -11 mV.

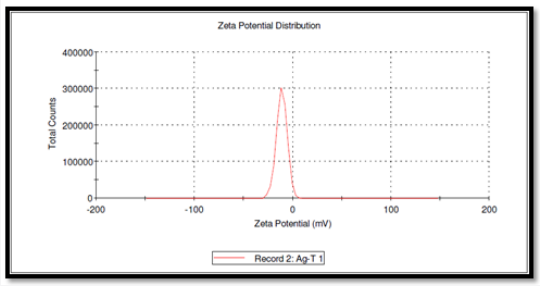

Figure 3: Zeta Potential of silver nanoparticles synthesized by Hybanthusenneaspermus.

The particles were uniformly distributed (monodispersed) without significant agglomeration. Owing to their small size, the total surface area of the nanoparticles was maximized, leading to the highest values of the activity to weight ratio. The frequency distribution observed from the graph showed that almost 100% of the particles in the 8nm to 70nm range. The test conducted had demonstrated that synthesized silver nanoparticles added to water showed a pronounced antibacterial/antifungal effect. It has been showed that smaller silver nanoparticles have greater effect [17] have concluded that at natural conditions.

X- Ray Diffraction (XRD)

The crystalline and phase purity of the synthesized Silver nanoparticles were examined via X- ray diffraction (XRD) analysis. The appearance of the corresponding peaks shows that the values ranging from 40 to 60°C in the spectrum of 2θ. An XRD spectrum of cubic phase crystalline silver structure was found. Diffraction Standards (Jcpdsfile no.04-0783). A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidence by the peaks at 2θ values of 4.086 corresponding to (111) and (200) planes for silver. Scherrer's equation for broadening resulting from a small crystalline size, the mean, effective, or apparent dimension of the crystal composing the powder isPhkl = kλ/β1/2cosθ, where θ is the Bragg angle, λ is the wavelength of the X-ray used, β is the breadth of the pure diffraction profile in radians on 2θ scale, and K is a constant approximately equal to unity and related both to the cubic shape and to the way in which θ is defined. The best possible value has been estimated as 0.89. Moreover,

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small insignificant impurity peaks are which may be attributed to the presence of other organic substances in culture supernatant (Figure 4).

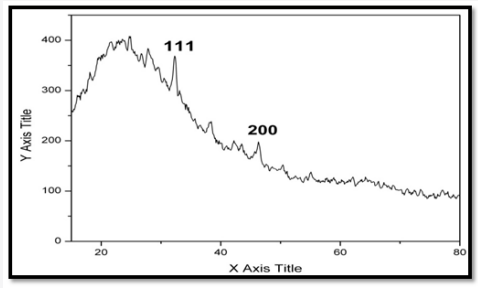

Figure 4: XRD Spectrum of the silver nanoparticles synthesized by *Hybanthusenneaspermus*.

HR - TEM Analysis

The HR–TEM analysis of the silver nanoparticles synthesized using Hybanthusenneaspermus were represented in Plate: 4. High Resolution Transmission Electron Microscopy (HR- TEM) has depicted the morphology and size details of the silver nanoparticles synthesized by Hybanthusenneaspermus. The morphology of the nanoparticles was roughly spherical in shape were found to be monodispersed. The nanoparticles were well separated and no agglomeration was noticed. These figures have shown that the nanoparticles were in the size range of 20 – 80 nm Hybanthusenneaspermus. The silver particles were crystalline, as can be seen from the selected area electron diffraction pattern (SAED) recorded from one of the nanoparticles in the aggregates. The results of TEM correlated with the results obtained in DLS analysis thus proving the size of the nanoparticles.

EDX

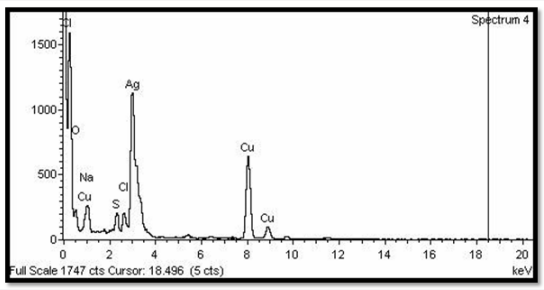

Figure 5: EDX spectrum of the silver nanoparticles synthesized by *Hybanthusenneaspermus*.

The elemental analysis of the silver nanoparticles synthesized using Hybanthusenneaspermus was recorded with an aid of secondary x-rays emerged from the scanning electron microscopic method and the results were plotted in Figure 5. The intensity of the peaks was directly proportional to the concentration of the atoms existed in the prepared nanoparticles. The quantitative analysis of the synthesized silver nanoparticles were analysed and the spectrum revealed that the sample had a high concentration of silver. The nanoparticles synthesized using Hybanthusenneaspermus synthesized nanoparticles had 68.75% of silver. Peaks of the Cu were from the grid used for TEM analyses [18] have opined that the silver nanoparticles spectrum showed 59% of silver atoms.

Attenuated Total Reflection - Fourier Transform Infrared Spectrophotometry (ATR - FTIR)

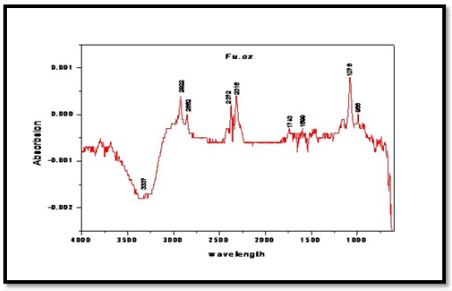

Figure 6: ATR - FTIR Analysis of silver nanoparticles synthesised by *Hybanthusenneaspermus*.

The liquid form of silver nano particles were subjected to ATR - FTIR instruments and it was carried out to identify the possible (protein) biomolecules responsible for the reduction of Ag+ ions and the bioreduced silver nanoparticles synthesized by Hybanthusenneaspermus. The representative spectra of nano particles obtained in the Figure 6 manifest absorption peaks located at about 1078, 2315 and 2922 cm^{-1} in the region of 1000 – 3000cm . The absorption peak at around 1078cm^{-1} can be assigned as near to the =C – H bend alkenes or C – N is assigned to the stretch aliphatic amines derived from the proteins. The peak at around 2315, 2852 and 2922 cm^{-1} corresponded to the presence of H – C= O : C – H stretch aldehydes and – C – H stretch alkanes regions that were characteristic of enzymes which were responsible for the reduction of metal ions when using fungi for the synthesis of metal nanoparticles. It also further indicated the binding of the nanoparticles with proteins.

The wavelength of light absorbed is characteristic of the chemical bond in the spectrum as the strength of the absorption is proportional to the concentration. Thus, the ATR - FTIR measurement indicated that the secondary structure of proteins was not affected because of its interaction with Ag + Ions or nanoparticles. The amide linkages between amino acid residues in polypeptides and proteins give rise to well known signatures in the infrared region of the electromagnetic spectrum. The positions of the amide I and II bands in the ATR - FTIR spectra of proteins were a sensitive indicator of conformational changes in the protein-secondary structure discussed by [19].

Antimicrobial Activity of biologically synthesized Silver nanoparticles

The antibacterial effects of biologically synthesized silver nanoparticles have been investigated against Escherichia coli,

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Staphylococcus aureus, Klebsiellapneumoniae, Serratiamarcescens and Pseudomonas aeroginosa. The disc was prepared with plant extract and synthesized silver nanoparticles extract in 30µg concentration and the disc were placed on Muller-Hinton agar plates with standard antibiotic tetracycline disc. The antibacterial activity of the standard antibiotic tetracycline disc at the 30µg concentration, the zone of inhibition (19mm, 14mm, 11mm, 21mm, and 10mm) against Staphylococcus aureus, Klebsiellapneumoniae, Pseudomonas aeroginosa, Escherichia coli and Serratiamarcescens respectively.

The antibacterial activity of the Hybanthusenneaspermus extract, at the 30µg concentration was high (11mm, 10mm) against Escherichia coli and S.aureus. Minimum activity was recorded (9mm, 8mm) against Klebsiellapneumoniae, Pseudomonas aeroginosa. There is no record found against Serratiamarcescens

The synthesised AgNPs plant extract in 30 µg concentrations was highly (13mm) acting against Staphylococcus aureus, Pseudomonas aeroginosa and Escherichia coli. Minimum activity was recorded in (11mm and 9mm) against Klebsiellapneumoniae and Serratiamarcescens respectively [20] reported biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and their antimicrobial activity was screened against both Escherichia coli and Staphylococcus aureus microorganisms [21] reported synthesis of silver phyto nanoparticles and their antibacterial efficacy against Staphylococcus aureus, Vibrio cholerae, Proteus vulgaris and Pseudomonas aeroginosa. From the zone of inhibition the synthesized AgNPs plant extract was highly inhibit the growth of the organisms. The levels of zone of inhibition for different kind of organism which are used in this experiment are showed in Table 3.

Table 3: Antimicrobial activity of biologically Synthesized Silver Nanoparticles with plant extracts

Organisms	Concentration of Extract and Zone of Inhibition (mm)		
	Tetracycline	Plant Extract	Synthesized AgNPs
Staphylococcus aureus	19	10	13
Klebsillapneumoniae	14	9	11
E. Coli	21	11	13
Pseudomonas aeroginosa	11	8	13
Serratiamarcescens	10	-	9

Antimicrobial Efficacy testing of SNP treated fabric

Agar Diffusion Method (SN 195920)

The antibacterial activity of silver nanoparticles coated cotton fabric was investigated against various Gram positive and Gram negative bacteria using disc diffusion technique. S. aureus and E. coli were used since they are AATCC standards. Bulk silver nitrate was maintained as the control against all the organisms in with the minimum zone of inhibition was observed. The diameter of inhibition zones around each disc with AgNPs and the control was represented in Table 4 and Plate: 6. The highest antimicrobial activity was observed against E. Coli and S. Aureus discussed by [22] in their work on In-Vitro evaluation of anti bacterial activity of Silver nanoparticles synthesized by using Phytophthorainfestans obtained Zone of inhibition of E. Coli, 17mm and for S. Aureus 13mm for a SNP concentration of 5µg/ml.

Table 4: Assessment of antimicrobial activity of SNPs treated fabrics by disc diffusion technique (SN 195920

S.No	Test Bacteria	Antibacterial activity (Zone of inhibition in mm)	
		Sample A	Sample B
1	E. Coli	30	21
2	S. Aureus	28	19

The maximum zone of inhibition obtained E. coli by SNPs treated fabrics using Hybanthusenneaspermus and S.aureus by SNPs treated fabric using Hybanthusenneaspermus Microorganisms contain a semi-permeable cell wall which maintains the integrity of cellular contents. Bactericidal agents cause the rupture of this cell membrane and damage the cells. Bacteriostatic agents only prevent the multiplication of bacteria, which may however remain alive, by inhibition of the synthesis of cell walls, alteration of cytoplasmic membrane permeability, alteration of the physical and chemical states of proteins and nucleic acids, inhibition of extract action, and inhibition of protein and nucleic acid synthesis. A chemical that is bactericidal at a particular concentration may only be bacteriostatic at a higher dilution.

Parallel streak method (AATCC test method 147-1988)

The results obtained in disc diffusion technique were further substantiated by parallel streak method. The antibacterial activity of silver nanoparticles treated cotton fabric was tested against Gram positive (S. aureus) and Gram negative strains (E. coli). Bulk silver nitrate was maintained as the control against all the organisms in which a minimum zone of inhibition was observed when comparatively biosynthesized silver nanoparticles treated fabric. The results of parallel streak method were represented in Table 5 and Plate: 5.

Table 5: Assessment of antimicrobial activity of SNPs treated fabrics by Parallel streak method (AATCC test method 147-1988)

S.No	Test bacteria	Antibacterial activity (Zone of inhibition in mm)	
		Sample A	Sample C
1	E. Coli	30	22
2	S. Aureus	26	20

when comparatively bacterial synthesised silver nano particles has the moderate antimicrobial activity was observed against E. Coli and the least was noticed against S. Aureus Similar results were reported by [23] who proposes that the AgNPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell, thereby leading to cell death. He reported a zone of inhibition of 27mm for S. Aureus and 30 mm for E. coli.

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Quantitative assessment (AATCC 100)

Table 6: Quantitative assessment of antimicrobial activity of SNPs treated fabrics (AATCC 100)

S.No	Test Bacteria	Antibacterial Activity (% reduction)	
		Sample A	Sample B
1	E. Coli	100	99.9
2	S. Aureus	96	94.5

The percentage reduction of bacteria by SNPs can be assessed by AATCC 100 method. The results were tabulated in Table 6. these results correlated with the results of SN 195920 and AATCC test method 147-1988.The obtained results suggested that the bio synthesized SNPs showed profound antimicrobial activity against Gram negative organism than on the Gram positive strain since the percentage reduction on gram negative is higher when compared with the Gram positive organism. Morones has also obtained similar results who justifies that the antimicrobial activity may be attributed by the interaction and penetration of SNPs into the microbial cells and interrupting in cellular functions.

Wash durability test

Table 7: Laundering durability of fabrics treated with SNPs synthesized using Hybanthusenneaspermus.

S.No	Test Fabric	No. of Washes	Antibacterial Activity (% Reduction)	
			Test organisms	
			E. coli	*S. aureus*
1.	Hybanthusenneaspermus synthesised nanoparticles treated fabric	5	99.9	94.5
		10	94	92
		15	89	89
		20	80	83
		25	70	73
		30	63	68
		35	55	58
		40	48	52

One of the most important factors to consider for the antimicrobial finish of cotton fabrics is its durability against repeated launderings, the silver-treated cotton fabrics were laundered for 0, 5, 10, 20, 25, 30, 35 and 40 cycles. The antibacterial activities of the silver-treated cotton fabrics after laundering were tested. The results were depicted in Table 7. the result showed excellent laundering durability of fabric treated nano silver. This has been the evidence of dynamic binding of the SNPs on the fabric surface. The higher resistance to laundering which results in significant bacteriostatis even after 40 laundering cycles may be due to the fact that nanoscaled materials have high ratio of particle number to volume as said by [24]. The results of the antimicrobial tests indicated that increasing laundering cycles only has a small negative impact on the retained antimicrobial activities of the silver-treated cotton fabrics as discussed by [25, 26] in his work on Antibacterial effect of nanosized Silver Colloidal solution on textile fabrics has obtained 80% and 83 % reduction respectively against *S. Aureus* and *E. Coli* after 20 laundering cycles.

Attenuated Total Reflection - Fourier Transform Infrared Spectrophotometry (ATR - FTIR)

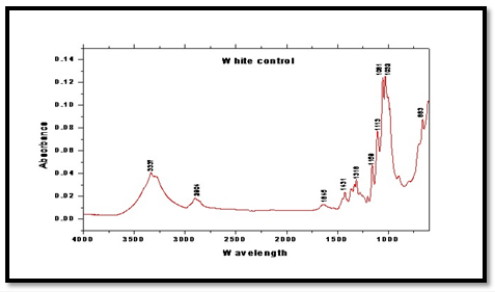

Figure 7: Untreated control Fabric (ATR - FTIR).

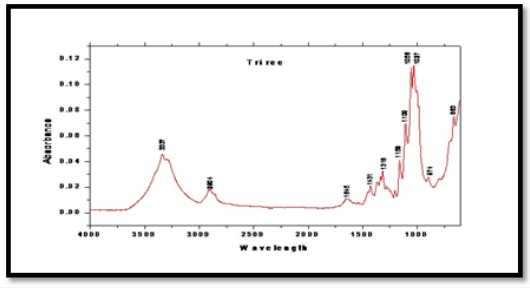

Figure 8: ATR – FTIR spectrum of the silver nanoparticles treated cotton Fabric synthesized by *Hybanthusenneaspermus*.

A comparative depiction of ATR – FTIR spectra of silver nanoparticles loaded fabric and control fabric without silver nanoparticles was shown in Figure 7 & 8. Spectrum of control fabric showed a characteristic peak in the range of 3500 – 3300 cm-1 that correspond to the - OH stretching of hydroxyl group of cellulosic cotton fabric. - OH bending of cellulosic fabric is observed at around 1645cm^{-1} and sharp peak of –OH in plane bending vibration occurred in the general region of 1000 to 1420 cm^{-1} in the plain fabric. The asymmetric C-H stretching 2904 cm^{-1} was found. Spectrum of Hybanthusenneaspermus synthesised silver nanoparticles treated fabrics shows a peak located at about 2361cm^{-1} corresponds to the presence of H – C= O : C – H stretch aldehydes, 2858 and 2922cm^{-1} corresponds to the presence of C – H stretch in the region of 2000 to 3000cm^{1}.

Tensile strength

Test data of the tensile strength on silver nanoparticles finished fabrics were shown in Table 8. The tensile strength of the silver nanoparticles finished fabric was higher than those of the untreated fabrics. When compared to the untreated fabrics the silver nanoparticles treated fabric showed significant difference for the tensile strength and breaking elongation. It was clear that the presence of silver nanoparticles also increased the breaking strength. Based on the studies of [27] the above result shows that the treatment of silver nanoparticles solution has no negative effect on the tensile strength of the fabric.

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Table 8: Assessment of Tensile strength of the fabrics.

S.No	Fabric Treatment	Tensile Strength (Kg)	Warp Elongation In Inches
1	Untreated fabric	20	1.6
2	Hybanthusenneaspermus synthesized silver nanoparticles treated fabric	22	1.7

Air permeability

The results of the air permeability of silver nanoparticles coated fabric have been illustrated in. The untreated fabric allowed 41.6 cm^3 /cm^2/sec of air to pass through the fibers where, the silver nanoparticles produced by Hybanthusenneaspermus treated fabrics allowed 34.7 cm^3 of the air to pass through. This decrease in air permeability was negligible and this decrease does not cause any interruptions regarding the comfort of the fabric as illustrated by [28]. When fabric allows some air to pass through the barrier, it helps in transferring high moisture levels and thus makes the wearer more comfortable

Abrasion

The experimental data on the abrasion resistance has been given in the Abrasion is the ability of the fabric to withstand loss of appearance through the destructive action of the surface and rubbing. It depended upon the construction of the yarn and the fabric. Silver nanoparticles produced by Hybanthusenneaspermus treated fabrics showed an abrasion resistance of about 58.5%. A slight decrease was observed in the abrasion resistance of untreated fabrics which was about 55%. It showed that the abrasion resistance of the silver nanoparticles produced by Hybanthusenneaspermus treated fabrics when compared with the untreated fabric was of higher order [29] also reported that the antimicrobial finished fabrics showed increased abrasion resistance.

Stiffness of the fabric

Table 9: Assessment of physico-chemical parameters of the fabrics.

S. No	Fabric Treatment	Air Permeability (cm^3/cm^2/sec)
1	Untreated fabric	18.2
2	Hybanthusenneaspermus synthesized silver nanoparticles treated fabric	34.7
S. No	**Fabric Treatment**	**Abrasion Resistance (%)**
1	Untreated fabric	55
2	Hybanthusenneaspermus synthesized silver nanoparticles treated fabric	58.5
S. No	**Fabric Treatment**	**Stiffness (in cm)**
1	Untreated fabric	2.1
2	Hybanthusenneaspermus synthesized silver nanoparticles treated fabric	2.4

The stiffness of the untreated and treated fabrics were measured and tabulated in Table 9. The bending length is a characteristic property of a textile fabric and was dependent upon the energy required to produce a given bending deformation under its own weight. It was evident from the table that there was an increase in stiffness of the Hybanthusenneaspermus synthesized silver nanoparticles treated fabrics. The increase in the stiffness of the treated fabric does not bring a drastic change in the air permeability. The results were similar to [30] that a mild increase in stiffness of the fabric due to antimicrobial finish had the properties which improved the functional abilities of the fabric without affecting the feel of the fabric. The antibacterial activity of the treated fabrics loaded with AgNPs was evaluated against Escherichia coli and Staphylococcus aureus bacteria. Results explored that, regardless of the concentration of AgNPs used, the bacterial reduction, in presence/absence of binder was always higher than 95% without washing. However, binder retains excellent antibacterial properties even after 20 washing cycles reflecting the significance of binder in fixation of AgNPs deposits on the surface of the fabrics [31].

Silver nanoparticles have been synthesized from the O. sanctum leaf extract. Structural analysis by XRD together with the chemical composition by EDS, strongly suggests the formation of elemental silver nanoparticles instead of their oxides. From the TEM analysis, the sizes of the nanoparticles are found to be 5 to 60 nm. FTIR measurements provided strong evidence for proteins to form a coat covering the silver nanoparticles to stabilize and prevent the agglomeration of the particles. This simple procedure for the biosynthesis of silver nanoparticles has several advantages such as cost effectiveness, compatibility and eco friendliness for biomedical and pharmaceutical applications. The phytomediated silver nanoparticles and Ag nanoparticles treated fabrics show durable significant antimicrobial activity against two common infectious bacteria, namely K. pneumoniae and E. coli. The synthesis of phyto-Mediated silver nanoparticles from O.sanctum is useful for application of dressing materials, delicate fabrics, knitted materials etc.

Summary and Conclusion

Totally five Nosocomial pathogens was isolated and identified as Escherichia coli, Staphylococcus aureus, Klebsiellapneumoniae, Serratiamarcescens and Pseudomonas aeroginosa based on their Morphology, Staining, Motility and Biochemical test. The synthesized AgNPs plant extract in 30 µg concentrations was highly (13mm) acting against Staphylococcus aureus, Pseudomonas aeroginosa and Escherichia coli. Minimum activity was recorded in (11mm and 9mm) against Klebsiellapneumoniae and Serratiamarcescens respectively. The organism synthesized silver nanoparticles showed a strong SPR absorption peak at around 400 to 412nm due to the formation of silver nano particles by Hybanthusenneaspermus. The particle size distribution showed high intensity of the maximum peak range from 20nm to 80nm and possessed an average size of 75.58nm with a zeta potential of -28.2.

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An XRD spectrum of cubic phase crystalline silver structure was found ranging from 40° to 60° in the spectrum of 2θ. From the HR-TEM analysis the nanoparticles size range of 20 – 80 nm Hybanthusenneaspermus.ATR - FTIR instruments and it was carried out to identify the possible (protein) biomolecules responsible for the reduction of Ag+ ions and the bioreduced silver nanoparticles synthesized by Hybanthusenneaspermus, the absorption peaks located at about 1078, 2315 and 2922 cm-1 in the region of 1000 – 3000cm-1.

The silver-treated cotton fabrics were laundered for 0, 5, 10, 20, 25, 30, 35 and 40 cycles. Antibacterial activity of cotton can be greatly enhanced by introducing green synthesized AgNPs into the fabrics. AgNPs loaded fabrics exhibit excellent antibacterial activity even after 40 washing cycles in the presence of binder. Results of durability to wash of the treated fabrics also showed long-lasting bactericidal effect. This simple procedure for the biosynthesis of silver nanoparticles has several advantages such as cost effectiveness, compatibility and eco friendliness for biomedical and pharmaceutical applications. The phytomediated silver nanoparticles and Ag nanoparticles treated fabrics show durable significant antimicrobial activity against two common infectious bacteria, namely Staphylococcus aureus, and E. coli. The synthesis of phyto-Mediated silver nanoparticles from Hybanthusennea sperms is useful for application of dressing materials, delicate fabrics, knitted materials etc. The present method of imparting antibacterial activity to the fabrics could be extended to different fabric structures for usage in hospital and related sectors.

The next stage was development of medical products such as surgical mask, medical napkin, and surgical cap from the silver nanoparticles finished antimicrobial cotton fabric from Hybanthusenneaspermus plant extract. The developed medical products were validated by three health care professionals after usage and the results showed that the products exhibited antibacterial activity even after washing and sterilization. Thus the present study provided different products of medical importance, which could be used in different medical and healthcare applications.

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