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COMPARATIVE STUDIES ON BIOSYNTHESIS OF SILVER NANOPARTICLES BY AZADIRACHTA INDICA, MENTHA ARVENSIS LEAF EXTRACT ITS CHARACTERIZATION AND APPLICATION FOR ANTIBACTERIAL AND ANTIMICROBIAL FINISH ON FABRIC

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Abstract:

Silver nanoparticles have unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaics to biological and chemical sensors. Examples include conductive inks, pastes and fillers which utilize silver nanoparticles for their high electrical conductivity, stability, and low sintering temperatures. An increasingly common application is the use of silver nanoparticles for antimicrobial coatings, and many textiles, keyboards, wound dressings, and biomedical devices now contain silver nanoparticles that continuously release a low level of silver ions to provide protection against bacteria. The comparative studies includes the characterization of silver nanoparticles by uv-visible spectroscopy, FTIR spectra. Identification of functional groups with IR spectroscopy. antibacterial, antimicrobial activity of azadirachta indica plant extract dyed cotton and silk fabrics. antibacterial, antimicrobial activity of mentha arvensis plant extract dyed cotton and silk fabrics , antibacterial, antimicrobial activity of silver nano cotton and silk fabrics, antibacterial, antimicrobial activity of silver nano cotton and silk fabrics. Silver nanoparticles synthesized by the green chemistry approach reported in this study using Azadirachtaindica and Menthaarvensis leaves extract shows potent applications . It has been demonstrated that use of a natural, renewable and low-cost biological reducing agent, such as Azadirachtaindica and Menthaarvensis leaves can produce metal nanostructures in aqueous solution at ambient temperature, avoiding the presence of hazardous and toxic solvents. The antibacterial, antimicrobial activity of SNP derived from Azadirachtaindica shows enhanced activity when compared to Menthaarvensis. By incorporating nano scale silver into textiles the manufactures can make materials that use a small amount of silver to kill the microbes present on the surface of the clothing material it helps to prevent spoilage from the microbial growth.

keywords:

Nanoparticles,uv-visiblespectroscopy,FTIR spectroscopy, Azadirachtaindica ,Menthaarvensis.
Nano particles.

Introduction:

Over the last decades silver nanoparticles have found applications in catalysis, optics, electronics andother areas due to their unique size-dependent optical, electrical and magnetic properties.[1-3] They are used as antimicrobial agents in wound dressings.[4]Silver nanoparticles have unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaics to biological and chemical sensors. Examples include conductive inks, pastes and fillers which utilize silver nanoparticles for their high electrical conductivity, stability, and low sintering temperatures.[5]Silver nanoparticles are nanoparticles of silver of between 1 nm and 100 nm in size.[6]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. Numerous shapes of nanoparticles can be constructed depending on the application at hand. Commonly used are spherical silver nanoparticles but diamond, octagonal and thin sheets are also popular.The most common methods for nanoparticle synthesis fall under the category of wet chemistry, or the nucleation of particles within a solution. This nucleation occurs when a silver ion complex, usually AgNO3 or AgClO4, is reduced to colloidal silver in the presence of a reducing agent. When the concentration increases enough, dissolved metallic silver ions bind together to form a stable surface. The surface is energetically unfavorable when the cluster is small, because the energy gained by decreasing the concentration of dissolved particles is not as high as the energy lost from creating a new surface.[7]Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There is formation of ‘pits’ on the cell surface, and there is accumulation of the nanoparticles on the cell surface.[8] The formation of free radicals by the silver nanoparticles may be considered

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to be another mechanism by which the cells die. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death.[9]Silver nanoparticles (NPs) have been the subjects of researchers because of their unique properties (*e.g.*, size and shape depending optical, antimicrobial, and electrical properties).[10]

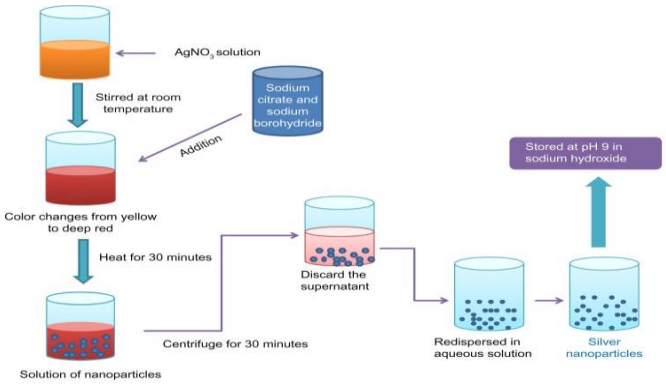

Figure-1 : SCHEME FOR PREPARATION OF SILVER NANO PARTICLES

Materials and Methods:

Requirements for Experiment:

Neem and Pudina leaves, microbial strains, viz. Two pure Escherichia coli and Staphylococcus aureus, Fresh fabrics of Cotton and Silk.

Requirements for Media:

Potato starch – 200gm
Agar – 25gms
Dextrose – 25gms
Distilled water-1000ml

Collection of Plant:

Azadirachta indica, leaves were collected from Nirmala College of Pharmacy and Mentha arvensis was purchased from local market .

Plant identification:

The aerial part like leaves of ***Azadirachta indica*** and ***Mentha arvensis*** plants were collected from nirmala college of pharmacy and local area market identified with the help of regional floras and taxonomists and finally confirmed with the department of botany acharya nagarjuna university.

Preparation of Bio extract:

Extraction by Infusion:

Twenty grams fresh leaves of neem and pudina were washed with tap water and then washed with distilled water, air dried and then they were finely cut and soaked in 100 ml boiling distilled water for 5–10 min and filtered through Whatman filter paper no. 42. This extract was used for generating silver nanoparticles. This bioextract is always used fresh.

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Figure-2: INFUSION EXTRACTION Figure-3: PUDINA, SILVER NITRATE, NEEM EXTRACT

PRELIMINARY PHYTOCHEMICAL SCREENING OF EXTRACT OF AZADIRACHTA INDICA
The extracted plant material is subjected to the following preliminary phytochemical screening:-

s.no	Chemical groups	Aqueous extract
1	Carbohydrates	+
2	Alkaloids	_
3	Glycosides	+
4	Steroids	+
5	Saponins	+
6	Proteins	_
7	Tannins	+
8	Flavones	+
9	Starch	_

Table :1 PRELIMINARY PHYTOCHEMICAL SCREENING OF EXTRACT OF AZADIRACHTA INDICA

PRELIMINARY PHYTOCHEMICAL SCREENING OF EXTRACT OF MENTHA ARVENSIS
The extracted plant material is subjected to the following preliminary phytochemical screening:-

s.no	Chemical groups	Aqueous extract
1	Carbohydrates	+
2	Alkaloids	+
3	Glycosides	+
4	Steroids	_
5	Saponins	_
6	Proteins	_
7	Tannins	_
8	Flavones	+
9	Starch	_

Table :2 PRELIMINARY PHYTOCHEMICAL SCREENING OF EXTRACT OFMENTHA ARVENSIS

Preparation of Silver Nanoparticles (snp):
5ml of leaves extract was added into 45 ml 0.002 M AgNO3 solution in 100 ml conical flasks at room temperature in dark. After 1 h, formation of silver particles started to appear in the flask.

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Figure no: 4 SNP OF PUDINA AND NEEM LEAF EXTRACTS

PLATING POTATO DEXTROSE AGAR:

PDA was accurately weighed and dissolved in distilled water, then kept in conical flask and plugged with cotton before keeping it for sterilization. After sterilization of about 20 ml Agar was poured in each sterilized Petri plates then these plates were allowed to cool so that agar gets solidified and then inoculation was done.

DYEING OF COTTON AND SILK BY SLIVER NANOPARTICLES*:*

Pre-washed cotton and silk fabrics dyed with lemon leaf extract were used as control fabric whereas silver nanoparticles-treated cotton and silk pieces were used as sample fabrics to assess durable textile finishing by subsequent washing method and further for antifungal activity. The control samples were prepared by dipping fabrics in 20% aqueous extract of lemon leaves at 65–70C for 2 h keeping material to liquor ratio 1:25. Then it was dried in shade without squeezing. Similarly pre-washed cotton and silk were dipped in silver nanosolution generated by lemon leaves, for 4 h and then taken out and dried in shade. The cotton-treated fabric was grayish brown and silk-treated fabric was greenish brown in color.

DURABLE TEXTILE FINISH TEST:

The pieces of cotton and silk dyed/coated with silver nanoparticles having dimensions of 4x 3cm were used for wash sustainability to assess the results of durable textile finish. Five subsequent washings were carried out. Washings were carried out by thorough wetting of treated fabrics in distilled water where samples were left for 4 h at room temperature. After drying, changes in sample color and bleeding to white fabric were determined.These samples were further used for estimation of antibacterial and anti microbial activity.

ANTIMICROBIAL, ANTIMICROBIAL ACTIVITY ASSESSMENT OF TEXTILE MATERIAL:

PARALLEL STREAK METHOD:

In this method as well, control and sample fabric pieces were placed with intimate contact of the media, i.e., PDA which had been previously streaked with an inoculums (0.05 ml) of test organism. After 18–24 h, a streak of uninterrupted or low colony area were counted along the side of fabric, indicating antifungal, antimicrobial effectiveness of the fabric.Plant extracts of Azadirchat indica and Mentha arvensis were checked against two different bacteria, namely Staphylococcus aureus, Escherichia coli. Four different concentrations of plant extract were tested for anti-bacterial and anti-microbial activity using potato dextrose agar medium.The micro-organisms were inoculated in 100ml flask containing potato dextrose agar broth. These flasks were incubated at 37 for 24hrs. Media was prepared using N-agar, test microorganisms were then spread over the solidified plates and A Bacterial positive control and antibiotic control were kept for comparative study.According to optimum temperature required for bacterial species anti-bacterial activity was obtained by determining the zone of inhibition around the cloth.Zone of inhibition was measured in cm.

Results and Declaration:

BIO SYNTHESIS OF SILVER NANO PARTICLES AND CHARATERISATION BY UV–VISIBLE SPECTRAL ANALYSIS:

UV- Visible spectra was recorded for monitoring the reaction. The plant extract of Neem shows 220 nm absorption maxima before the reaction. Silver nanoparticles exhibit yellowish brown colour in aqueous solution after 1hr of reaction and shows the absorption maxima at 541nm. The plant extract of Pudina shows 230nm absorption maxima before the reaction. Silver nanoparticles exhibit yellowish brown colour in aqueous solution after 1hr of reaction and shows the absorption maxima at 543nm.

Characterization of silver nanoparticles by uv-visible specrtoscopy:

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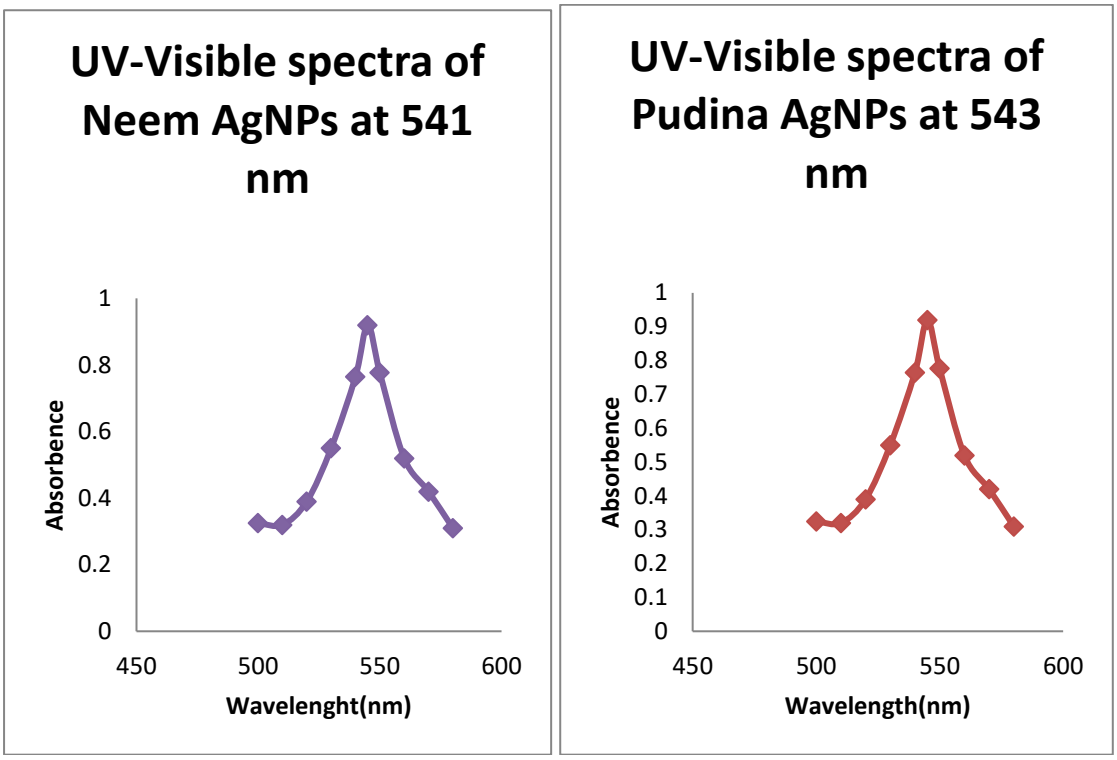

UV-visible spectra of Ag np's with Azadirachta indica:

ABSORBENCE	WAVELENGHT(nm)
500	0.325
510	0.32
520	0.39
530	0.55
540	0.765
545	0.92
550	0.777
560	0.52
570	0.42
580	0.31

Table : 3 absorption maxima by uv spectra

Observation: The plant extract of Neem shows 220 nm absorption maxima before the reaction. Silver nanoparticles exhibit yellowish brown colour in aqueous solution after 1hrof reactionandshows the absorption maxima at 541nm.

UV-visible spectra of Ag np's with Mentha arvensis:

wavelength	Absorbance
500	0.325
510	0.320
520	0.390
530	0.550
540	0.765
545	0.890
550	0.777
560	0.520
570	0.420
580	0.310

Table : 4 absorption maxima by uv spectra

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Observation: The plant extract of Pudina shows 230nm absorption maxima before the reaction. Silver nanoparticles exhibit yellowish brown colour in aqueous solution after 1hr of reaction and shows the absorption maxima at 543nm.

CHARACTERISATION BY FTIR SPECTRA:

Identification of functional groups with IR spectroscopy:

IR spectroscopy is one of the major tools used for obtaining information on functional groups on a solid surface. The principle of IR spectroscopy is based on molecules vibrating with specific frequencies associated with internal vibrations of groups of atoms. These frequencies occur in the IR region of the electromagnetic spectrum, i.e. ~ 200 to ~ 4000 cm '1. When a sample is placed in a beam of IR radiation, the sample absorbs all radiations corresponding to those of molecular vibrational frequencies, and transmits all other frequencies. Identification of functional groups is possible because differences in the chemical structure give rise to characteristic vibrations and yield unique IR spectra, known as the 'fingerprint' spectra. The Fourier transform infrared spectrometer (FTIR) employs an interferometer instead of a monochromator**.**In the present work, FTIR measurements were taken with a Perkin Elmer Spectrum RX I spectrometer (range 4000 - 400 cm'1) using 'nujol' method for sample introduction. The NLP samples were kept in an oven at 333 - 343 K overnight for getting rid of moisture, allowed to cool to room temperature in a dessiccator and then, a tiny amount was spread on a nujol film between two KBr windows.It is to be noted that the IR spectra show the presence of the following functional groups on the NLP surface in Azadirachta indica as follows:

a) -O H (3644 cm"1),
b) -^NH2 (3387.00 cm'1),
c) X > N - (1593.33cm"1),
d) =CM >, =C~N< and = C -0 - (1021 -1158.97 cm"1),
e) > C O, >C=C< (1642-1698.7cm"1),
f) >C=S (1021 -1 584 cm"1).

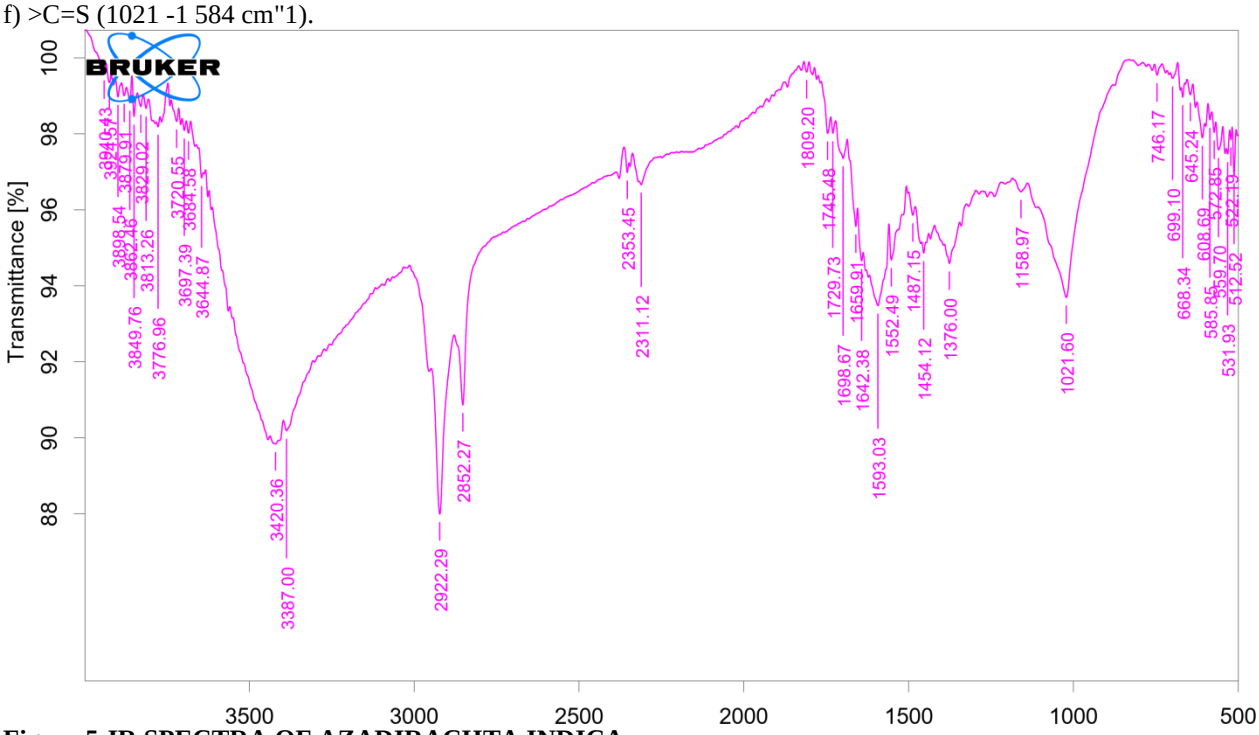

Figure 5:IR SPECTRA OF AZADIRACHTA INDICA

IR spectra show the presence of the following functional groups on the NLP surface in Mentha arvensis as follows:

a) -C-C- (1127 cm^{-1})
b) -C-H- (2922.74 cm^{-1})
c) -C-C- (1318.18 cm^{-1})

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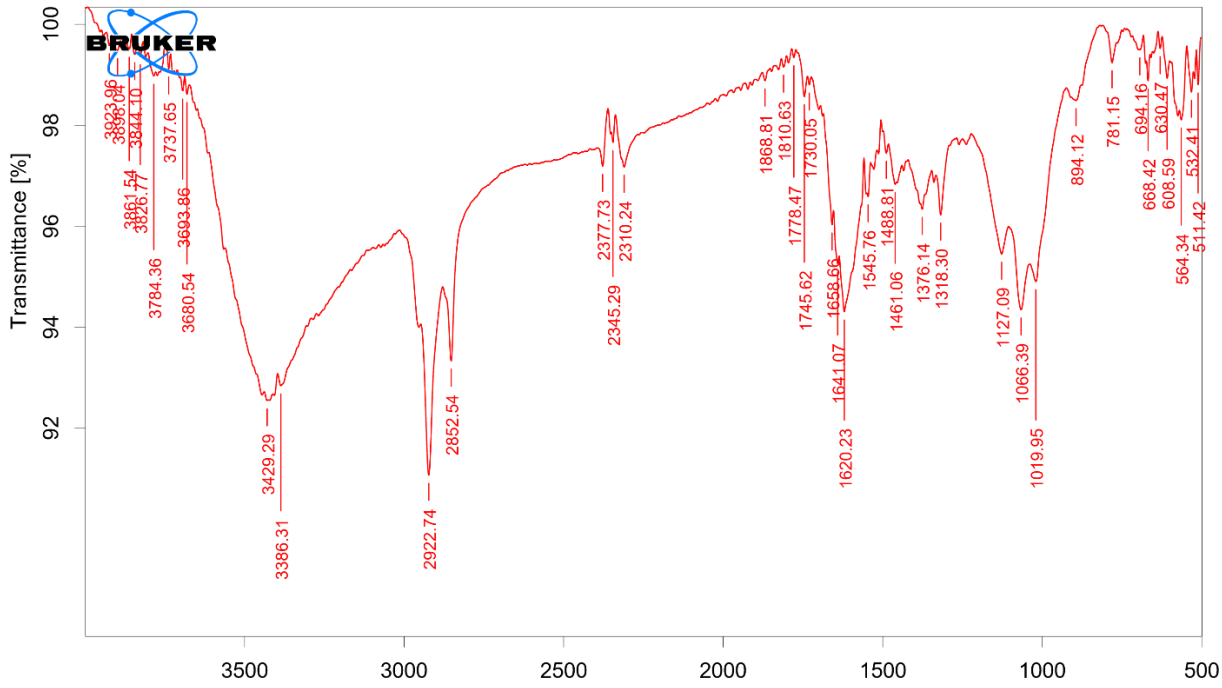

Figure 6: IR SPECTRA OF MENTHA ARVENSIS:

Durable Textile Finish Test:

The pieces of cotton and silk dyed/coated with silver nanoparticles having dimensions of 4x 2.5cm were used for wash sustainability to assess the results of durable textile finish. Five subsequent washings were carried out. Washings were carried out by thorough wetting of treated fabrics in distilled water where samples were left for 4 h at room temperature. After drying, changes in sample color and bleeding to white fabric were determined. Theses samples were further used for estimation of antibacterial and antimicrobial activity.

ANTIBACTERIAL, ANTIMICROBIAL ACTIVITY OF AZADIRACHTA INDICA PLANT EXTRACT DYED COTTON AND SILK FABRICS:

In this experiment neem dyed cotton and silk fabrics were put on E. coli, Staphylococcus aureus and the zone of inhibition was checked for every 3hrs and compared with the control plate. Zone of inhibition of both the fungal species was obtained in terms of growth restrictions in both the fabrics.

S.NO	TIME (HRS)	ZONE OF INHIBITION FOR PLANT EXTRACT DYED SILK FABRIC(cms)	ZONE OF INHIBITION FOR PLANT EXTRACT DYED COTTON FABRIC(cms)
1	23	2.5	2.2
2	26	2.2	2.0
3	29	2.0	1.9
4	46	1.9	1.7
5	49	1.8	1.5

Table 5: ZONE OF INHIBITION OF DYED SILK AND COTTON FABRIC (AZADIRACTHA INDICA)

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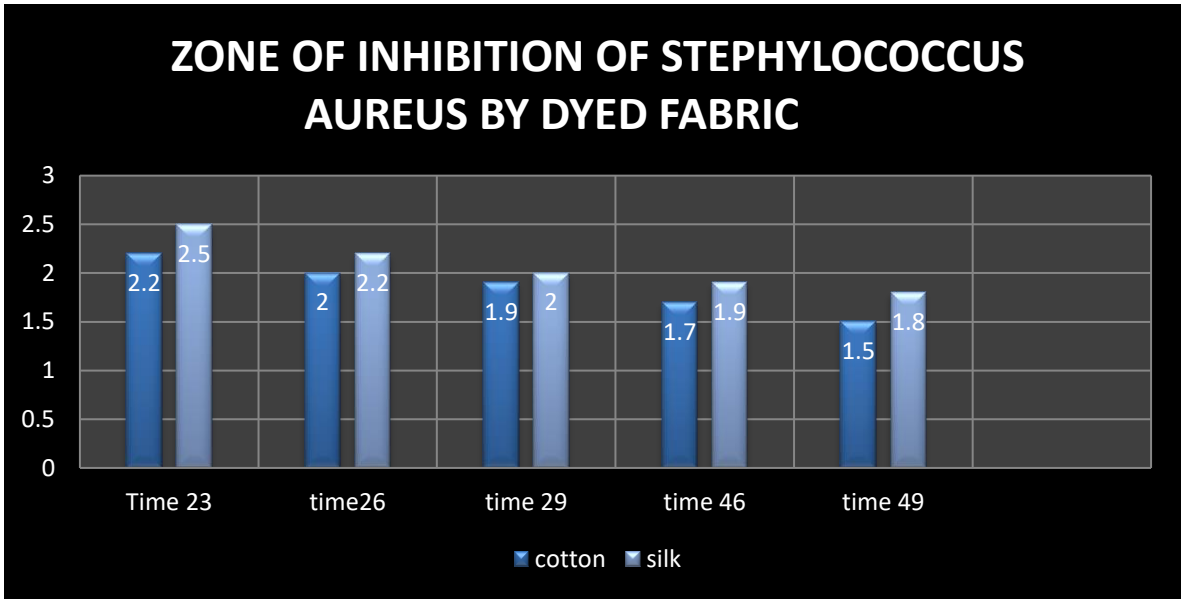

Graph no: 1: ZONE OF INHIBITION OF STEPHYLOCOCCUS AUREUS BY AZADIRACHTA INDICA DYED FABRIC

Observation: Zone of inhibition was observed for Azadirachta indica dyed fabric on Stephylococcus aureus was at Conc. of 50 ug/ml at Time 49 hrs is 1.8cm for silk and 1.5cm for cotton.

ANTIBACTERIAL,ANTIMICROBIAL ACTIVITY OF MENTHA ARVENSIS PLANT EXTRACT DYED COTTON AND SILK FABRICS:

In this experiment neem dyed cotton and silk fabrics were put on E. coli, Staphylococcus aureus and the zone of inhibition was checked for every 3hrs and compared with the control plate. Zone of inhibition of both the fungal species was obtained in terms of growth restrictions in both the fabrics.

S.NO	TIME (HRS)	ZONE OF INHIBITION FOR PLANT EXTRACT DYED SILK FABRIC(cms)	ZONE OF INHIBITION FOR PLANT EXTRACT DYED COTTON FABRIC(cms)
1	23	2.3	2.5
2	26	2.1	2.3
3	29	2.0	2.2
4	46	1.9	2
5	49	1.7	1.8

Table 6: ZONE OF INHIBITION OF DYED SILK AND COTTON FABRIC (MENTHA ARVENSIS)

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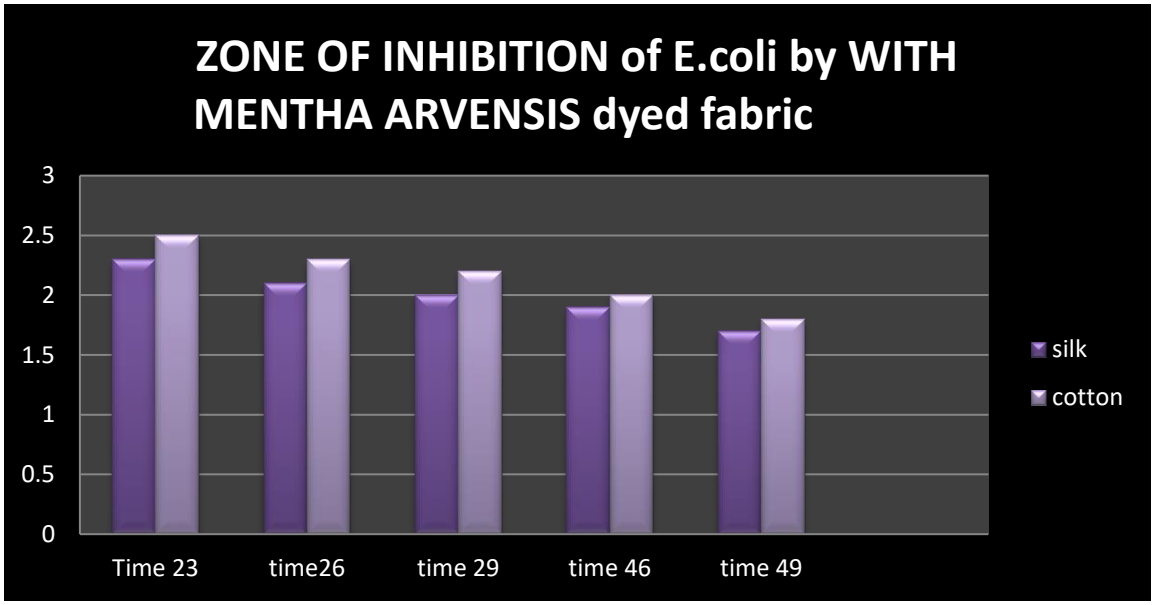

Graph no: 2: ZONE OF INHIBITION OF E.COLI BY WITH MENTHA ARVENSIS DYED FABRIC
Observation: Zone of inhibition was observed for Mentha arvensis dyed fabric on E.Coli was at concentration of 50ug/ml at time 49 hrs is 1.8cm for cotton and 1.7cm for silk fabric.

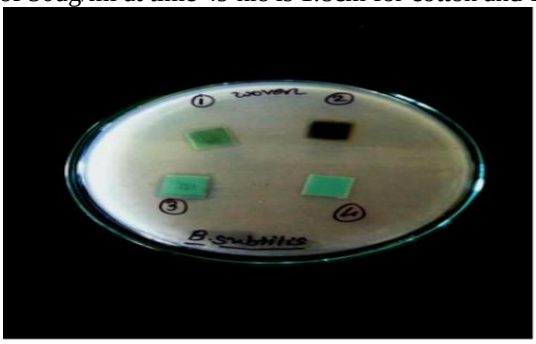

Figure 7 : ZONE OF INHIBITION FOR MENTHA ARENSIS AND AZADIRACHTA INDICA PLANT EXTRACT DYED FABRIC USING E.COLI

ANTIBACTERIAL,ANTIMICROBIAL ACTIVITY OF SILVER NANO COTTON AND SILK FABRICS:

In this experiment neem silver nano dyed cotton and silk fabrics were put on E. coli, Staphylococcus aureus and the zone of inhibition was checked for every 3hrs and compared with the control plate. Zone of inhibition of both the fungal species was obtained in terms of growth restrictions in both the fabrics.

S.NO	TIME (HRS)	ZONE OF INHIBITION FOR PLANT EXTRACT SNP DYED SILK FABRIC(cms)	ZONE OF INHIBITION FOR PLANT EXTRACT SNP DYED COTTON FABRIC(cms)
1	23	2.2	2.0
2	26	2.9	2.7
3	29	3.2	3.1
4	46	3.5	3.3
5	49	4.2	3.7

Table 7: ZONE OF INHIBITION OF DYED SILK AND COTTON FABRICS (AZADIRAKTHA INDICA)

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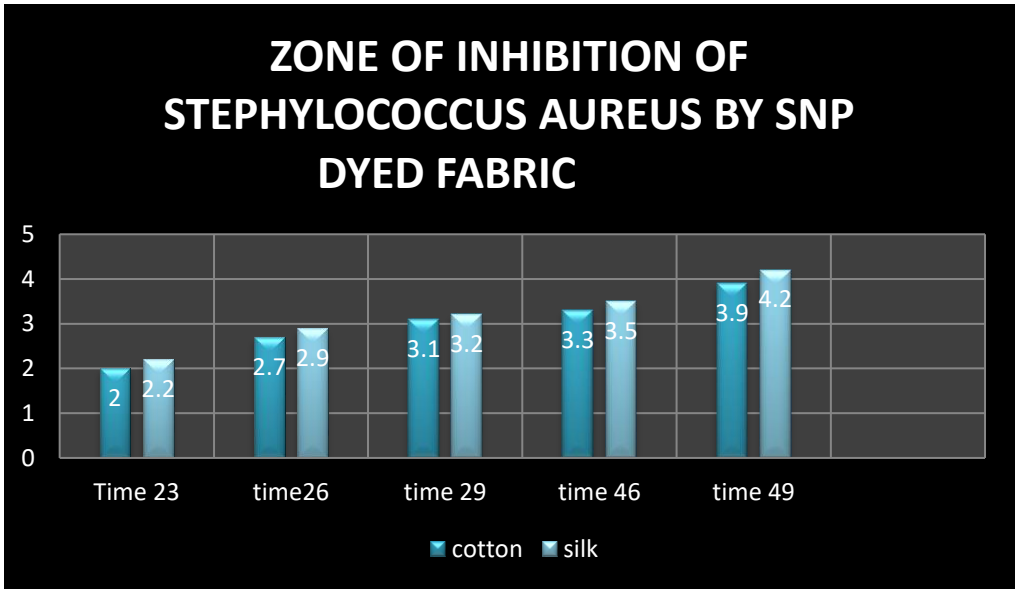

Graph : 3 ZONE OF INHIBITION OF STEPHYLOCOCCUS AUREUS BY SNP AZADIRACHTA INDICA DYED FABRIC

Observation: Zone of inhibition was observed for Azadirachta indica SNP dyed fabric on Stephylococcus aureus was at Conc. of 50 ug/ml at Time 49 hrs is 4.2 cm of silk and 3.9 cm of cotton.

ANTIBACTERIAL,ANTIMICROBIAL ACTIVITY OF SILVER NANO COTTON AND SILK FABRICS:

In this experiment neem silver nano dyed cotton and silk fabrics were put on E. coli, Staphylococcus aureus and the zone of inhibition was checked for every 3hrs and compared with the control plate. Zone of inhibition of both the fungal species was obtained in terms of growth restrictions in both the fabrics.

S.NO	TIME (HRS)	ZONE OF INHIBITION FOR PLANT EXTRACT SNP DYED SILK FABRIC(cms)	ZONE OF INHIBITION FOR PLANT EXTRACT SNP DYED COTTON FABRIC(cms)
1	23	2.0	2.2
2	26	2.3	2.5
3	29	3.3	3.5
4	46	3.5	3.7
5	49	3.7	3.9

TABLE : 8 ZONE OF INHIBITION OF DYED SILK AND COTTON FABRICS (MENTHA ARVENSIS)

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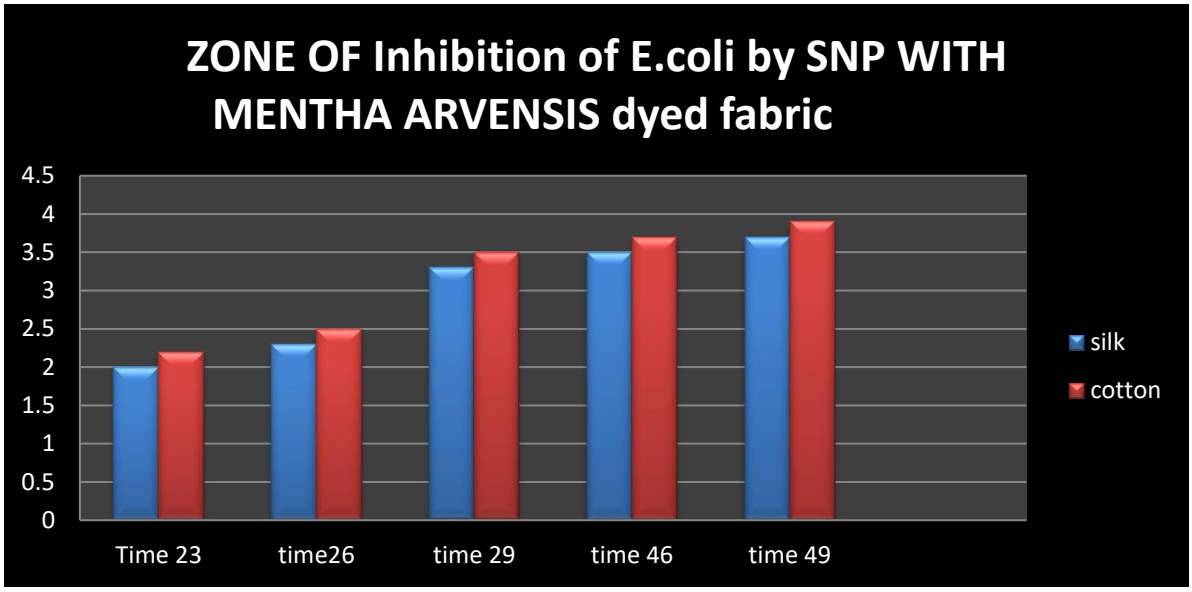

Graph : 4 ZONE OF INHIBITION OF E.COLI BY SNP WITH MENTHA ARVENSIS DYED FABRIC

Observation: Zone of inhibition was observed for Mentha arvensis SNP dyed fabric on E.Coli was at concentration of 50ug/ml at time 49 hrs is 3.9cm for cotton and 3.7cm for silk fabric.

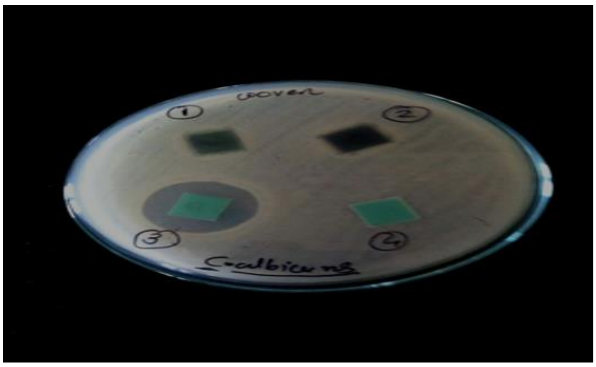

Figure:8 ZONE OF INHIBITION OF MENTHA ARVENSIS AND AZADIRACHTA INDICA SNP DYED FABRIC BY USING STEPHYLOCOCCOUS AUREUS

Conclusion:

Silver nanoparticles synthesized by the green chemistry approach reported in this study using Azadirachta indica and Mentha arvensis leaves extract shows potent applications it has been demonstrated that use of a natural, renewable and low-cost biological reducing agent, such as Azadirachta indica and Mentha arvensis leaves can produce metal nanostructures in aqueous solution at ambient temperature, avoiding the presence of hazardous and toxic solvents. The antibacterial, antimicrobial activity of SNP derived from Azadirachta indica shows enhanced activity when compared to Mentha arvensis. By incorporating nano scale silver into textiles the manufactures can make materials that use a small amount of silver to kill the microbes present on the surface of the clothing material it helps to prevent spoilage from the microbial growth.

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