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RESEARCH ARTICLE

"SYNTHESIS, CHRACTERIZATION AND EVALUTION OF ANTIMICROBIAL EFFECT OF CHITOSAN CONJUGATED SILVERNANOPARTICLES AGAINST PATHOGENIC BACTERIA AND THEIR BIOMEDICAL APPLICATION ''.

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Abstract

The study is aimed to determine the antimicrobial evaluation of chitosan conjugated silver nanoparticles againist pathogenic bacteria. Pathogenic bacteria samples were collected from SMS hospital and then subjected to identification of bacteria according to their m characterstics and morphology. Chitosan conjugated silver nanoparticles synthesized by adding NaOH and $AgNo_3$ solution to chitosan solution at 45ºc and were characterized by UV, SEM, TEM and FTIR. The antimicrobial activity was determined by kirbuy stokes method. Antibacterial effect of chitosan-silver nanoparticle was increased by increasing the concentration of the (ch-AgNPs). The presence of small concentration of silver nanoparticles in the composite was enough to significantly enhance antibacterial activity.

Introduction

Nanotechnology has developed to be a considerable research field in all disciplines including medicinal chemistry.[1] The physical properties, size and orientation of nanoparticles have allegedly shown to alter the performance of any material.[2] Chitosan is a linear polymer composed of glucosamine and N-acetyl glucosamine linked with a b-1-4-glycosidic linkage . chitosan is a biopolymer, biocompatible, and can be degraded with the help of enzymes in human body, the degradation products of chitosan are non-hazardous.[3] Chitosan is semi-crystalline polymers and its crystallinity plays a vital role in adsorption effectiveness. Chitosan is a biopolymer which shows a broad spectrum of antimicrobial potency by binding to the negatively charged bacterial cell wall attached to the DNA, inhibiting its replication.[4] Silver nanoaparticles has an important application in the area of biology such as a DNA sequencing and antimicrobial agent. Silvernanoparticles has been well-known to show strong toxicity towards a wide range of microorganisms.[5] Silver nanoparticles, typically size in the range of 1 -10 nm append to the surface of cell membrane and considerably disturb its appropriate functions like permeability and respiration [6]. Silver nanoparticles (AgNPs) have been proven to acquire immense importance and thus, have been widely studied. The use of AgNps in various applications such as electrical conducting, sensing, catalytic, optical and antibacterial applications[7] In the last some years, there has been an rise in studying AgNPs on account of their essential antbacterial efficacy[8]. Chitosan silver nanoparticles can be used in future generation as therapeutic agents against numerous drug-resistant microbes[9].

Chitosan has various applications in many areas, mainly pharmaceutical and biomedical fields, because to its specific properties Among these properties chitosan has excellent biocompatibility; high bioactivity; reactivity of the group amino deacetylated; biodegradability; selective permeability; polyelectrolyte action; antimicrobial activity; ability to form gel and film; chelation ability and absorptive capacity. These specific properties provide a wide variety of applications to the chitosan, such as: anti-bacterial drug carrier of controlled release, both chitosan and silvernanoparticles are antibacterial agents so chitosan conjugatedAg nanoparticle has exhibited strong antibacterial effect. Comparative studies showed that chitosan-Ag nanoparticle is much more effective against bacteria than pure chitosan. Chitosan can be used as a stabilizing agent instead of chemical reducing agent for protecting Ag

nanoparticles from agglomeration. "Because" of these peculiar properties chitosan-Ag nanoparticle has taken attention in the recent years.[10].

Scanning electron microscope (SEM), Transmission electron microscopy (TEM), UV spectrophotometer, and Fourier transform infrared (FTIR) techniques were used for the physio- chemical characterization of the size and structure of chitosan-Ag nanoparticles. The study aimed to determine the antimicrobial efficacy of chitosan conjugated silver nanoparticles. Chitosan-Ag nanoparticle was synthesized by mild method in the aqueous sodium hydroxide. Antimicrobial efficacy of chitosan-Ag nanoparticles against *E.Coli, S. aureus, S.typhii, P.aeroginosa and C.Albicans* were observed.

Materials and methods:-

All chemicals used in this study were purchased from sigma aldrich . The bacterial culture media were obtained from HiMedia Laboratories Pvt. Ltd., India. *Staphylococcus auras, Escherichia coli, Pseudomonas aeruginosa Salmomella typhi and candida albicans* clinical isolates were provided by the SMS hospital Jaipur, department of microbiology and used for in vitro antimicrobial study. These test microorganisms were subcultured in MRS broth for further activity. After this these sample were subjected to biochemical test for further identification The bacterial culture was maintained in brain heart infusion agar slant and stored at 4º c.

Conjugation of chitosan- silvernanoparticles:-

High molecular – weight chitosan (85% deacetylated) was used for synthesis.Chitosan solution 2mg/ml was prepared in 1% acetic acid. Mixture was stirred at 45ºc to obtain a homogenous solution. The chitosan solution (40ml) was then mixed with 0.1N sodium hydroxide (NaOH) solution (80ml) and 10 Mm AgNO3 solutions 2ml was added to the resulting solution. Change to the yellow color appearance indicated the formation of ch- AgNps.

Identification of pathogenic bacteria:-

Identification of pathogenic bacteria was performed according to their morphological, physiological and biochemical characteristics. The biochemical tests carried out were Gram reaction, motility test, production of Catalase test, coagulase test, citrate test, oxidase test.

Gram staining test:-

Staining of the bacteria forms the primary and important step in the identification of gram positive and gram negative bacteria. The isolated bacteria were examined using gram staining procedure and were observed under compound microscope.

Catalase test:-

Catalase test was performed by isolated single colony was streaked on a glass slide. One drop of 3 % hydrogen peroxide was added on to the slide. Oxygen effervescence shows the positive reaction of the bacteria to a catalase test.

Oxidase test:-

Oxidase test was performed kovac's reagent was added on filter paper with loopul isolated colonies of bacteria. After sometime the color changes to dark purple. It shows that isolate is oxidase positive, and if there is no color change on filter paper. It shows negative oxidase test.

Coagulase test:-

Coagulase is an enzyme that causes plasma to clot by activating prothrombin to form thrombin which then catalyzes the activation of fibrinogen to form fibrin. Coagulase test was performed one drop of citrated human plasma was added on a slide by using a plastic loop or wooden stick. Mix well and clumping was observed within 10-15 seconds indicate a coagulase positive test.

Citrate test:-

Fresh (16- to 18-hour) pure culture was used as inoculation source. A single isolated colony was slightly streak on the surface of the simmon citrate agar slant. Incubate at 35ºC for 18 to 48 hours. Growth was observed at the slant surface and the color of medium was changed from intense green to a deep Prussian blue.

Determination of antibacterial efficacy of ch-AgNps:-

The antimicrobial activity of pathogen was determined by kirbuy stokes method. Different concentration of ch-AgNps such as 20µl, 40µl, 60µl, 80µl and 100µl were taken. Muller hinton agar plate was prepared for the test. 500µl of each culture suspension was added in sterile MH agar media plate. 50µl of standard was loaded into the well of plates. 20, 40, 60, 80 and 100µl concentration of ch-agnp was added into the respective wells. . *S.aureus E.coli, s.typhii, P.aeroginosa,* was incubated at 37º c for 24 hrs. *C.albican* was incubated at 22 c at 72 hrs.

Result and Disscusion:-

TEM Analysis:-

TEM microscopy illustrated that chitosan conjugated silver nanoparticles were typically in spherical shape with an average size of 20 nm. The size of Ag nanoparticles particles showed that loaded Ag particles on chitosan matrix were achieved to be nanosized. Silver nanoparticles were well detached in chitosan matrix with the average diameter of around 3–8. Small particles exhibit greater antimicrobial activity than big particles. This result is due to the greater number of silver particles are penetrated because of smaller size as compare to larger size ag particles because they can cover more surface area. The antibacterial efficacy is related to the total surface area of the nanoparticles. Smaller particles with larger surface to volume ratios have higher antibacterial activity.

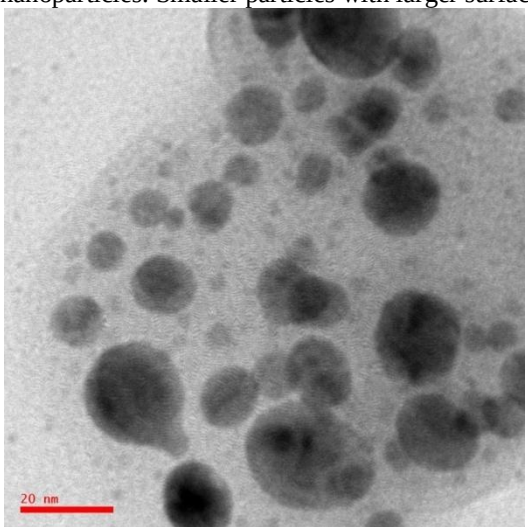

Fig 1:- TEM images of chitosan-Silver nanoparticles synthesized material with the size of 20nm.

UV –VIS Analysis:-

The uv- spectroscopic observation specifies that the chrome yellow solution of silver nanoparticles was not aggregate because of the stable position of the absorbance peak. The silver colloidal particles obtain a negative charge because of to the adsorbed citrate ions. The absorption peak was observed at about 420nm, which is the typical characteristic absorption peak for Ag nanoparticles. UV absorption peak of chitosan-Ag nanoparticles was observed by other researchers in the range 410–450 nm.

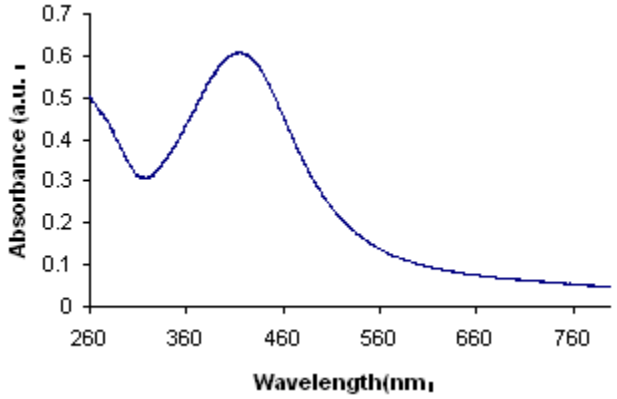

Fig2: uv**:-**visible graph of chitosan conjugated silver nanoparticles.

SEM Analysis:-

The result of scanning electron microscopy study reveals that chitosan conjugated silver nanoparticles were spherical in shape with the size of 1µm. The 1 µm size of chitosan conjugated silver nanoparticles confirmed their nano size structure.

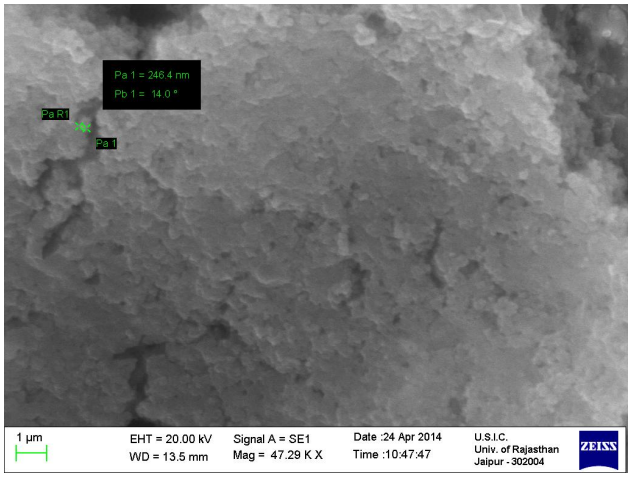

Fig3: SEM images of ch-AgNps with the size of 1µm.

FTIR Analysis:-

The profile of FTIR spectrum the main absorption of ch-agnps as 3345, 2123.71, 1639.43, 1045.09cm^{-1}. The FTIR spectrum of the ch-agnps show OH stretch at 3345.78 and N-H bending at 1639 cm^{-1}. In the FTIR spectrum of ch-agnps, the shift of chitosan peak is observed which shows amplification in the intensity of c-o stretch.

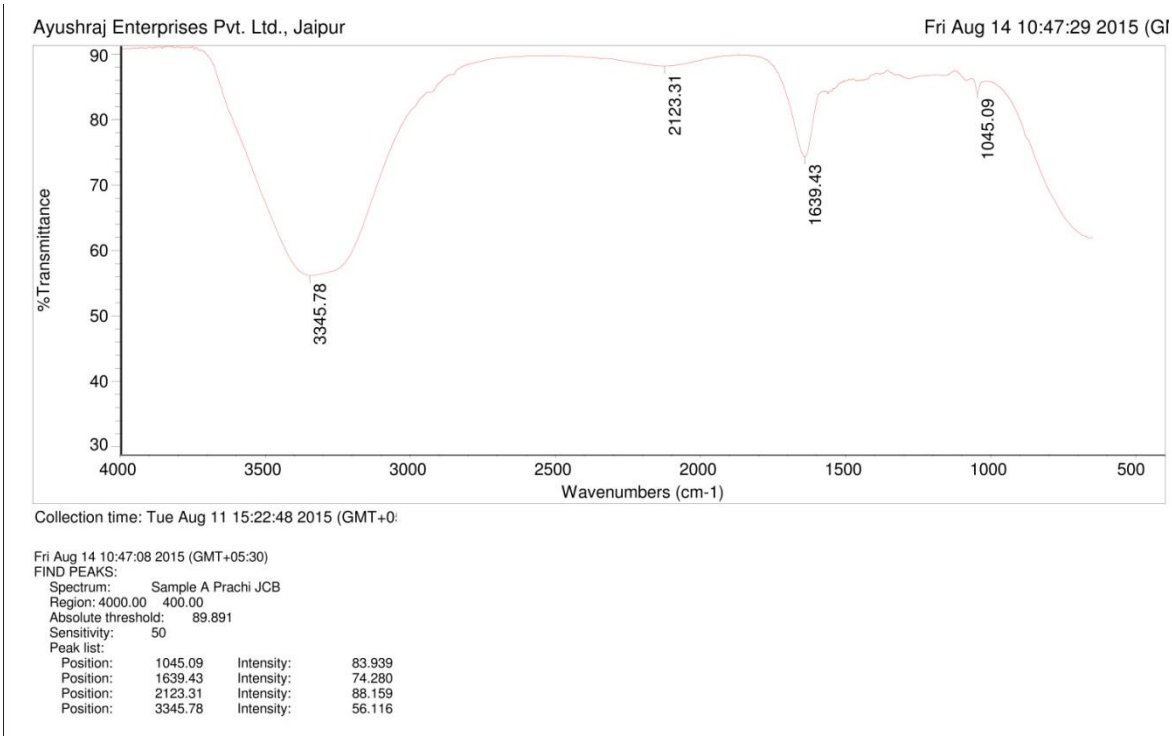

Fig 4: FTIR Graph of chitosan conjugated silver nanoparticles.

Table 1:- Culture characterstics of pathogenic bacteria.

Isolates	**Gram staining**	**Shapes of isolates**	**Color isolates colonies**
E.COLI	Gram (-)	Rod shape	Shiny mucoid colonies which have entire margins
S.typhii	Gram (+)	Rod shaped	Smooth,low convex, circular colonies
S.AUREUS	Gram (+)	cocci	Yellow to white
P. AEROGINOSA	Gram (-)	Rod shape	Mucoid colonies with ubonate elevate

Table 2:- Result of biochemical test

Isolates	**catalase**	**Oxidase**	**Coagulase**	**Citrate test**	**staining**
E.COLI	+	_	_	_	Gram (-)
L.BACILLUS	-	-	-	-	Gram (+)
S.typhii	-	_	_	_	Gram (-)
S.AUREUS	+	_	+		Gram (-)
P.AEROGINOSA	_	+	_	_	Gram (-)

Table 3:- Zone of inhibitions result. Antimicrobial activity of ch-ag nanoparticles against identified pathogenic bacteria.(gentamycin was used as standard)

Sample 1 con. in µl	*E.Coli*	*S.typhii*	*S.Aureus*	*P.Aeruginosa*	*C.Albicans*
Control	6mm	8mm	5mm	6mm	5mm
20µl	12mm	13mm	11mm	10mm	0mm
40 µl	13mm	17mm	16mm	12mm	0mm
60 µl	19mm	17mm	20mm	16mm	12mm
80 µl	20mm	20mm	23mm	18mm	16mm
100 µl	23mm	24mm	25mm	20mm	18mm

Disscusion:-

In the present study all the clinical isolates were identified by observed their colony Morphology,s and biochemical characterization, as *E.coli, P.aeruginosa, S.typhii and S. aureus and C.Albicans.* The results of this antibacterial activity of ch-ag np suggested that the presence of a small percentage of Ag nanoparticles in the composite was enough to enhance antibacterial activity significantly. Chitosan conjugated silver nanoparticles exhibit strong bacteriocidal activity at different concentration as compared to gentamycin. According to the result we can use chitosan conjugated silver nanoparticles to treat various bacterial infection caused by pathogenic bacteria. Ch-AgNps can also help to reduce the problem of toxicity and to avoid the problem of multi drug resistance. The broad spectrum of bioactivity of AgNPs makes them promising agents not only to fight. Both chitosan and silver nanoparticles are antibacterial agents so chitosan-Ag nanoparticle composite material has exhibit strong antibacterial effect not only fight infections, but in many other biomedical areas.

Conclusion:-

Antibacterial effectiveness of chitosan-Ag nanoparticle materials was investigated against e.coli, s.aureus, p.aeruginosa, s.typhi, and c.albicans. Antimicrobial activity of pathogen was checked at different concentration of 20µl, 40µl, 60µl, 80µl and 100µl. gentamyin is used as standard. Antimicrobial effectiveness of gentamycin (control) against pathogenic strain was not so effective as compared to ch-Ag Nps. The result of zone of inhibition suggests that antimicrobial efficacy of ch-AgNp was increased by increasing the concentration of ch-AgNps. So, we can use chitosan conjugated silvernanoparticles and to overcome the problem of multidrug resistance problem. Multidrug resistance is growing problem in the treatment of infectious diseases and the widespread use of broad spectrum antibiotics has produced antibiotics resistance mechanisim against many bacterial pathogens.

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