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Synthesis of Chitosan - Silver Nanoparticles from Peneaus Indicus Shell Waste and Their Antimicrobial Efficiency

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ABSTRACT

Green synthesis of noble metal nanoparticles has recently received a lot of attention because of the vast range of novel uses in numerous industries. Silver nanoparticles, in particular, are gaining popularity in medicinal applications as very efficient antibacterial agents with minimal hazardous side effects. The current study describes an environmentally acceptable strategy for combating bacterial infections using multi-potency silver nanoparticles synthesized from shrimp shell bio-waste. The bio-fabricated chitosan silver nanoparticles (Cs-AgNPs) were validated by UV-visible spectroscopy, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy, energy dispersive X-ray spectroscopy, X-ray powder diffraction, and zeta potential analysis. The SEM analyses revealed that the produced Cs-AgNPs were predominantly spherical in shape. Chitosan and Cs-AgNPs at low dosages demonstrated strong antibacterial activity against bacterial pathogens such as E.coli, Klebsiella sp, Pseudamonas sp, and Streptococci sp. The antibacterial investigations revealed that Cs-AgNP inhibited the investigated bacterial pathogens significantly. Overall, this work demonstrates that chitosan derived from shrimp shell wastes has the potential to be exploited in the management of bacterial infections.

I. INTRODUCTION

Marine bio-nanotechnology is a rapidly emerging sector with great potential in biological research. The marine environment's diverse micro and macro species offer opportunities for nano science and nanotechnology [4]. Nanotechnology, defined as "the development and

utilization of nano-scale materials"[2], is important in the development of innovative materials in various sectors of research [26]. Green synthesis of organic coated inorganic nanomaterials is a developing topic of nanoscience and nanotechnology because to their long-term uses in the biopharma, food, leather, and textile sectors [41].

Nanotechnology has attracted a lot of attention over the last decade since it creates unique nanoparticles that have become the subject of interdisciplinary research due to the dramatically different and intriguing qualities they have when compared to their bulk forms [9] [13]. Nanoparticles (NPs) are particles of any shape with size ranging from 1 to 100 nm. The 100 nm limit is based on the fact that physical and chemical features that distinguish nanoparticles from bulk materials often appear at lengths less than 100 nm. Other phenomena, such as transparency and steady dispersion, can occasionally expand the upper limit, and the term "nano" is used for dimensions less than 500 nm [46]. The unusual features of nano-scale materials have prompted extensive study into NP manufacturing, characterization, and applications [31] [23]. Nanoparticles exhibit distinct physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical, and biological properties [49]. Because of their high surface-to-volume ratio, NPs are appealing prospects for a wide range of applications [6] [3]

Chitosan is a natural, harmless copolymer of glucosamine and N-acetyl glucosamine derived from chitin through deacetylation, which is a major component of crustacean shells. Recently, biocatalysis and sustainable chemistry researchers have been directed to develop new green approaches aimed at reducing and preventing pollution at its source [33] [17]. Chitosan is a naturally occurring polysaccharide with numerous unique features, including biocompatibility, biodegradation, biological activity, and low toxicity [34]. Chitosan's nontoxicity, biodegradability, and antibacterial characteristics make it suitable for a wide range of applications. It is employed in biomedical industries, agriculture, genetic engineering, food processing, environmental pollution management, water treatment, paper manufacturing, photography, and other fields [7]. Chitosan, a polysaccharide biopolymer produced from natural chitin, has distinct polycationic, chelating, and film-forming capabilities due to the presence of active amino and hydroxyl functional groups. Chitosan also has a number of fascinating biological actions, such as antibacterial activity, induced disease resistance in plants, and a variety of stimulating or inhibitory effects on various human cell types. [47] [46].

Silver and silver salt-based compounds have been known for their antibacterial properties since antiquity. AgNPs are commonly utilized in the textile, cosmetics, and food industries to prevent microbial contamination [37] [27]. Silver nanoparticles (AgNP) have received a lot of interest because of their potential in a variety of biological and medical disciplines, including biosensors, wound healing, and burn treatment [36][29][21][10]. Silver (Ag) nanoparticles have a broad range of antibacterial actions and operate well even at low concentrations, therefore, they have been utilized for many years to prevent and treat a variety of diseases and infections [15]. Ag nanoparticles have been shown to offer promising cancer-treatment properties [42].

II. MATERIAL AND METHODS

2.1. Collection of shrimp shell

The shells of *Peneaus indicus* shrimps were obtained from Mudasalodai landing centre (Lat.11°27'N; Long.79°45'E), and the attached tissues were removed. The waste shrimp shells are cleaned in tap water before being properly washed in distilled water. The washed shells are then dried for 15 minutes in a hot air oven. The dried shells are ground to a fine powder.

2.2. Extraction of chitosan

This process was followed by [28], with the first three steps (Demineneralization. Deproteination and Decolorization) producing chitin and the final step doing deacetylation to produce chitosan from chitin.

2.2.1. Demineralization

At room temperature, 20 g of shrimp powder was introduced to 300 mL of 1 N HCl. The solution was then agitated for two hours. The powder was then filtered and rinsed with distilled water to eliminate any excess acid.

2.2.2. Deproteination

The filtered powder was then mixed with 200ml of 15% NaOH solution. The solution was agitated at 600 degrees Celsius for three hours. The powder was then filtered and rinsed with distilled water.

2.2.3. Decolorization

The powder was mixed with 200ml of 15% sodium hypochloride solution and agitated for 15 minutes at room temperature. The powder was then filtered and rinsed with distilled water. The powder was dried on a hot plate at 800 degrees Celsius for 12 hours.

2.2.4. Deacetylation

A 65% NaOH solution was added to the powder and heated for 1 hour at 1000 degrees Celsius. The powder was then rinsed with distilled water. The powder was then allowed to cool for 30 minutes at ambient temperature before being dried in an oven at 101 degree Celsius for 6 hours.

2.5. Biosynthesis and characterization of CS-Ag NPS

Two grams of the powered chitosan were placed in a 250 mL Erlenmeyer flask with 100 mL of double distilled water, and the mixture was heated for 20 minutes. The extract was filtered through Whatman filter paper n.1 and kept at -4°C. In an Erlenmeyer flask, the filtrate was treated with an aqueous solution of 1mM silver nitrate and incubated at room temperature. A pale-yellow solution showed the development of Cs-Ag NPs, as aqueous silver ions were reduced by the chitosan extract, resulting in a stable Cs-Ag nanocomposite in water. UV-Vis spectral measurements validated the synthesized Cs-Ag NP. The absorption spectra of synthesized Cs-NPs were obtained at 200-800 nm with a UV-3600 Shimadzu spectrophotometer. The size and shape of the Cs-AgNPs were investigated using Scanning Electron Microscope. To identify potential biomolecules present in chitosan extract, FTIR was done using a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer. Furthermore, the presence of elements in the sample was determined using EDX.

2.6. Bactericidal efficacy of Chitosan-Ag NPs

The antibacterial activities of the synthesized Chitosan-Ag NPs against human pathogenic bacteria (10^{-5}) of *E.coli*, *Staphylococcus* Spp, *Bacillus* Spp, *Pseudomonas* Spp, *Salmonella* Spp, and *Vibrio* Spp were investigated using the Agar Well Diffusion method (AWD). In the assay, 100 µl of pre-grown bacterial samples were dispersed on the Nutrient Agar medium (NA) plate separately. Cut 6 mm wells in the Nutrient agar plate and load with chitosan-Ag NPs at varied concentrations (25, 50, 75, and 100 µg/ml). The plats were incubated at 37^{0}C for 24 hours. The antibacterial activity was measured and the zone of inhibition (mm) was found out (Li et al. 2022).

III. RESULT

3.1. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS OF CHITOSAN

Chitosan's FTIR analysis revealed peaks at 3430 cm-1, 3389 cm-1, 3253 cm-1, 3105 cm-1, 3090 cm-1, 2876 cm-1, 1995 cm-1, 1653 cm-1, 1619 cm-1, 1543 cm-1, 1419 cm-1, 1375 cm-1, 1307 cm-1, 1260 cm-1, 1203 cm-1, 1152 cm-1, 1111 cm-1, 1064 cm-1, 1007 cm-1, 951 cm-1, 894 cm-1, 658 cm-1, and 667 cm-1 (Figure 6). The peak at 3253 cm-1 and 3389 cm-1 indicates O-H stretching vibration. 3253cm-1 exhibits N-H stretching vibration. 3105 cm-1 displays O-H stretching vibration, while 3090 cm-1 and 2876 cm-1 show C-H stretching, and 1995 cm-1 and 1653 cm-1 show amide stretching vibration. The vibration of amide II is shown at 1619 cm-1. Peak 1543cm-1 exhibits N-H deformation of amide II, peak 1419cm-1 displays OH and CH ring vibrations, peak 1375cm-1 shows C-O stretching of the primary alcoholic group, peak 1307cm-1 shows CH2 wagging vibrations, and peak 1260cm-1 reveals complicated NHCO group vibrations. Peaks 1202cm-1 and 1152cm-1 reveal the C-O-C bond, peak 1111cm-1 shows C=O stretching, peak 1064 cm-1 shows the stretching vibration of the C-O bond, and peak 1007cm-1 shows C-O stretching in acetamide. Peak 951cm-1 shows a C-H bond peak 894cm-1 exhibit pyranose ring skeletal vibrations, and peak 667cm-1 and 658cm-1 show N-H out of plane.

Fig:1 FTIR spectrum of Chitosan

3.2. SCANNING ELECTRON MICROSCOPY (SEM) OF CHITOSAN

The morphology of chitosan nanoparticles is investigated using a scanning electron microscope (Figure 2). The surface study provides useful information regarding their structure. The morphology of chitosan was practically uneven in shape. The chitosan had average diameters of less than 100nm.

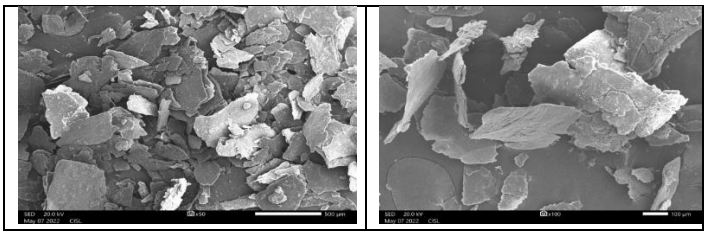


Fig:2 SEM image of chitosan

3.3. ELEMENT -X RAY DIFFERECTION (EDAX) OF CHITOSAN

The EDAX method was used to analyze the elements (Figure 3). Chitosan exhibits a pronounced peak due to C and O, confirming its formation. The weight and atomic weight determined from the peak heights confirm the expected proportion of C and O, whereas the spectrum shows the detection of expected components. Table 1 shows that elements C and O have atomic percentages of 47.43±0.14% and 52.57±0.32%, respectively, and mass percentages of 54.58±0.16% and 45.42±0.28%. The observed and theoretical atomic/mass percentages for pure chitosan agree well.

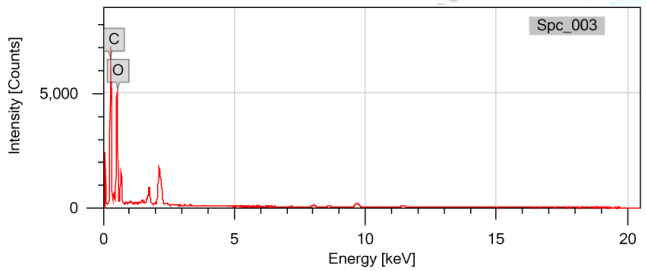


Fig:3 EDAX spectrum of Chitosan

Element	Line	Mass%	Atom%
C	K	47.43±0.14	54.58±0.16
O	K	52.57±0.32	45.42±0.28
Total		100.00	100.00
Spc_003			Fitting ratio 0.3678

Table :1 Atomic percentage of present element

3.4. Ch-Ag NPs SYNTHESIS

The synthesis of chitosan-silver nanoparticles (Ch-Ag NPs) was initially characterized by colour alterations. The production of Ch-Ag NPs causes the hue to change from white to brown (Figure 4).

Fig: 4 Formation of Ch-Ag NPs

3.5. UV- SPECTRUM ANALYSIS OF Ch-Ag NPs

Furthermore, the generation of Ch-Ag NPs was validated utilizing an important system of UV-spectra from the 372nm range (Figure 5). To synthesize nanoparticles, Ag NO3 is reduced by adding chitosan as a reducing agent.

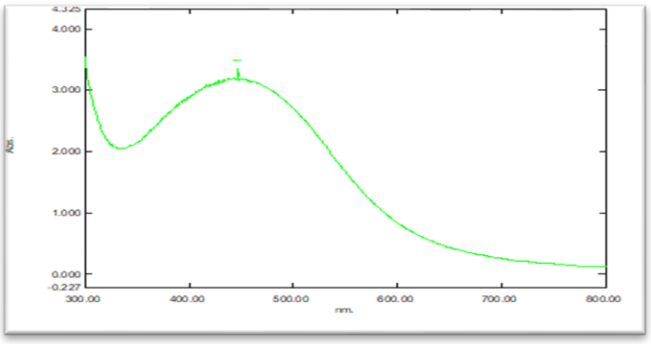


Fig: 5 UV Spectrum of Ch-Ag NPs

3.6. FTIR ANALYSIS OF Ch-Ag NPs

The FTIR spectra were utilized to assess the probable interaction between the nanoparticle and the bioactive chemicals responsible for the creation and stabilization of silver nanoparticles derived from chitosan extract. Figure 6 shows the FTIR spectrum of produced Ch-Ag NPs. They identified strong peaks at 3444.22 cm-1, 2074.32 cm-1, 1633.39 cm-1, 1383.03 cm-1, and 684.22 cm-1. The peak at 3444.22 cm-1 corresponds to the O-H stretch, H bound vibration of alcohol and phenol groups. The spectral peak at 2074.32 cm-1 was identified as C (triple bond) C- stretching vibrations in alkynes compounds. The peak at 1633.39 cm-1 corresponds to the N-H bend of primary amines. The signal at 1383.03 cm-1 was allocated to alkane-containing C-H rocks. The peak at 684.22 cm-1 corresponds to the -C (triple bond) C-H:C-H bend of an alkyne.

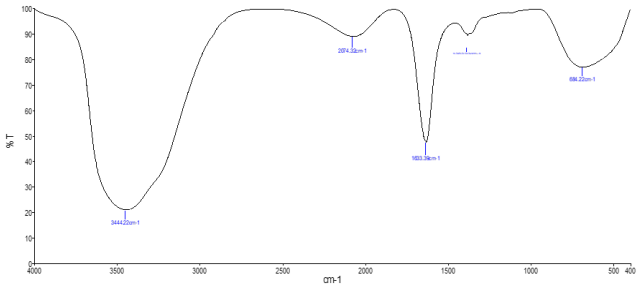


Figure 6. FTIR analysis of chitosan-silver oxide nanoparticles

3.7.X-RAY DIFFRACTION (XRD) OF Ch-Ag NPs

X-ray diffraction analysis was used to confirm the crystalline structure of the produced Ch-Ag NPs. The

XRD spectrum of Ch-Ag NPs showed distinct diffraction peaks at 24.88, 29.52, 32.41, 46.71, 58.63, 65.87, 68.43, 68.09, 69.28, and 76.98, which correspond to the (100), (111), (111), (200), (211), (220), and (310) planes and confirmed the (hcp) pattern based on the comparison with the standard as given by JCPDS (file no.00-076-1393) (Figure 7).

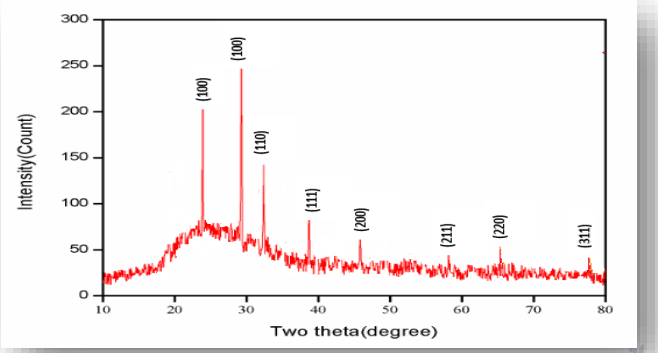


Figure7. XRD analysis of chitosan-silver nanoparticle

3.8. SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS OF Ch-Ag NPs

A scanning electron microscope (SEM) is used to examine the morphology of produced silver nanoparticles (Figure 8). The surface study provides useful information regarding their structure. The morphology of silver nanoparticles was virtually hexagonal and spherical. The average silver nanoparticle size ranged from 25 to 32 nm.

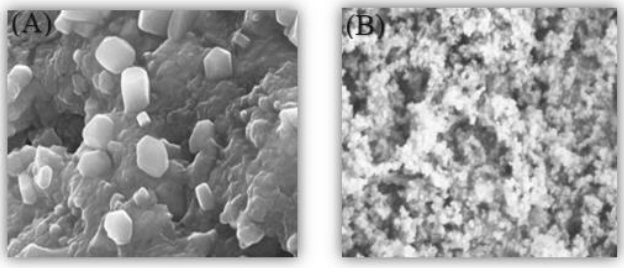
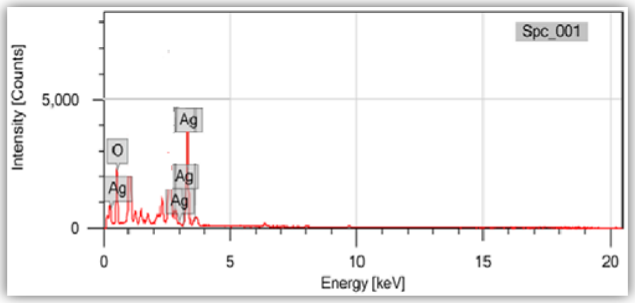


Fig :8 SEM image of Ch-Ag NPs

3.9. ELEMENT -X RAY DIFFERECTION (EDAX) ANALYSIS OF Ch-Ag NPs

The element analysis was performed using the EDAX approach. The Chitosan-silver nanoparticle shows a strong peak due to Ag and O, indicating the development of silver oxide. The weight and atomic weight determined from the peak heights confirm the expected proportion of Ag and O, and the spectrum demonstrates the identification of expected components. The current atomic and mass percentages of the elements Ag and O are 26.56±0.55% and 73.44±0.78%, respectively. The observed and theoretical atomic/mass% values for pure Chitosan-silver nanoparticles accord well.



Element	Line	Mass %	Atom %
O	K	37.63±0.12	73.44±0.78
Ag	L	62.37±0.05	26.56±0.55
Total		100.00	100.00
Spc_001			Fitting ratio 0.248

Fig 9: EDAX spectrum and Atomic percentage of elements present

3.10. ANTIBACTERIAL ACTIVITY STUDIES

The antimicrobial efficacy of chitosan-silver against pathogenic bacteria (*E.coli, Klebsiella sp, Pseudamonas sp, Streptococci sp*) was validated by the inhibition zone produced around the well impregnated with various concentrations (25, 50, 75, and 100µg/ml). The anti-biofilm effects vary depending on the species and concentration of nanoparticles. The *E.coli* concentration of 100µg/ml resulted in the highest zone (17.4mm).

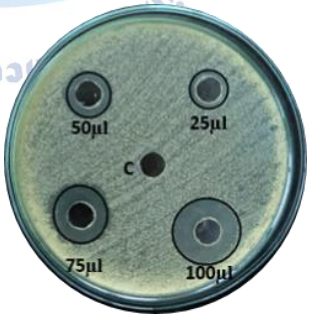


Fig 10 : *E.coli*

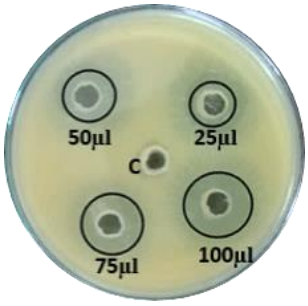


Fig 11: *Klebsiella sp*

Fig 12 : *Pseudamonas sp*

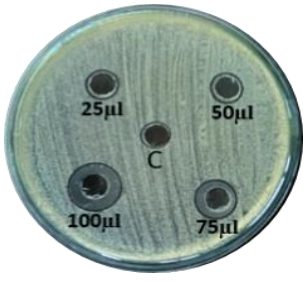


Fig *13 : Streptococci sp*

IV. DISCUSSION

Nanotechnology has attracted a lot of attention over the last decade since it produces unique nanoparticles that have become the subject of interdisciplinary research due to the drastically different and intriguing qualities they exhibit when compared to their bulk forms. (Durán et al.2016). Nanoparticles are well-known because to their remarkable optical, magnetic, electrical, and catalytic properties, as well as their distinctive size and shape (Dubey et al. 2010; Das and Marsili et al. 2010). According to Singh et al. (2008), there have been methods for producing nanoparticles; however, the majority of these procedures required expensive chemicals, resulting in pollution from dangerous leftovers deposited in the ecosystem and on living things. Bio-fabrication is the process of generating nanoparticles from bacteria, fungi, algae, and plants. This approach is affordable, safe, and environmentally friendly (Abdel et al.2014). According to Saif et al. (2016), this method allows for large-scale production of nanoparticles with few impurities. Chitin is the most abundant natural polysaccharide after cellulose, and it may be found in the shells of crustaceans such as shrimp, crab, and lobster. Chitin can also be extracted from mushrooms and mushroom. Many investigations have found chitin sources in mushroom cell walls, coral, algae, and nematodes (Synowiecki and Al-Khateeb, 1997; Trung et al. 2006). Researchers in biocatalysis and sustainable chemistry are developing green approaches to reduce pollution at its source (Rinki et al. 2011; Gavrilescu and Chisti, 2005). However, the current study focuses on investigating chitosan-silver nanoparticles derived from *Penaeus indicus* shrimp shell waste. The present study successfully extracted chitosan from *Penaeus indicus* shell waste. Boudouaia et al. (2019) also describe the synthesis of chitosan from shrimp waste. FTIR study of chitosan indicated the presence of various kinds of chemical compounds, including amines, aromatics, amides, alkynes, acetamide nitro groups, and alkyl halides. A similar report was noted in the prior report. A scanning electron microscope is used to study the morphology of chitosan nanoparticles. The surface analysis provides significant information regarding their structure. Chitosan morphology was almost uneven in shape (Ghadi et al. 2014). Chitosan has a strong peak in the EDAX due to C and O, confirming its creation. The weight and atomic weight estimated from the peak heights confirm the expected percentage of C and O. The spectra detected the expected elements (Eddya et al. 2020). Kalaivani et al. (2020) reported that the creation of chitosan-silver nanoparticles (Ch-Ag NPs) was first characterized by color alterations. The production of Ch-Ag NPs causes a colour change from white to brown. The FTIR analysis of crude extract and green produced chitosan-silver NPs revealed the presence of many kinds of chemical compounds, including amines, aromatics, alkynes, nitro groups, and alkyl halides. The biomolecules included in the crude extract of *Penaeus indicus* are responsible for the creation of chitosan-silver particles. Similarly, Regiel et al. (2012) used biological synthesis to create Ag2O NPs. Scanning and transmission electron microscopes were used to explore the structure and size of nanoparticles. The sperical structure of chitosan-silver oxide is the focus of this study. Previously, Karthik et al. (2021) reported that chitosan-silver oxide was physiologically synthesized in a spherical shape.

In the current study, the antibacterial activity of green produced chitosan-silver oxide NPs was investigated against harmful microorganisms. It is important to note that examined bacteria shown high sensitivity to biosynthesized chitosan-silver oxide NPs, and the reduction of harmful bacterial growth was proportional to nanoparticle concentration. The highest antibacterial activity was observed in chitosan-silver against *E.coli.* Recently, Potara et al. (2011) found that biosynthesized chitosan-silver nanoparticles have good inhibitory efficacy against harmful microorganisms. The antibacterial effects were mostly caused by the interaction of NPs with thiol groups (-SH) in bacterial respiratory enzymes (Slavin et al.2017).

V. CONCLUSION

As a result, this paper reports a quick and environmentally friendly synthesis of chitosan-silver nanoparticles. The respective NPs were formed by the participation of several functional groups present in chitosan. In the current study, chitosan-silver was biologically manufactured, which is both cost effective and environmentally friendly. It also possesses an exceptional nanostructure and promising antibacterial effects against harmful bacteria. Nonetheless, ongoing research in this field may lead to the discovery of novel pharmaceutical techniques.

Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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