AGAR PLATE PREPARATION

Firstly, 28 g of nutrient agar powder was added to 1 L of DI water and mixed with a magnetic stirrer for 10 min before being sterilised in an autoclave at 121°C and 1.5 atm/cm^2 for 45 min. A sterilized glass pipet was used to create a uniform 4 mm deep agar layer in the sterile petri dishes. After the agar had set, the petri dished were put in a sealed plastic container and stored at 4°C until used (within 1 week).

NUTRIENT BROTH PREPARATION

A 25 g of Lauria-broth powder was added to 1 L of distilled water and mixed with a magnetic stirrer for 10 min before being sterilised in an autoclave at 121°C and 1.5 atm/cm^2 for 45 min. The broth was left to cool at room temperature and stored in a sealed glass bottle at 4°C until used (within 1 week).

SUB-CULTURING THE BACTERIA

To avoid contamination of the commercial *E. coli* (O157:H7) culture a 0.33 mm sterilised nichrome wire was used to inoculate a petri dish with the gram-negative bacteria. The inoculated plate was incubated at 36 °C for 24 hrs. Cells from the petri dish were transferred to 200 mL nutrient broth in a sterilized Erlenmeyer flask using an inoculation loop and incubated at 36°C in an incubation oven with a mixer plate at 50 rpm for 24 hours.

DISC DIFFUSION METHOD

100 µL of the inoculated nutrient broth was added to 150 mL of fresh nutrient broth and incubated. The optical density (OD_{600}) was continuously monitored until an OD_{600} value of 1 (McFarland half standard) was reached. 100 µL of the inoculated solution was then pipetted into the petri dishes, using sterile pipet tips. After flame sterilising a glass lazy-L-spreader and allowing to cool, the inoculum was uniformly spread over the entire surface of the agar. The glass spreader rod was submerged in a 70% ethanol solution and flame sterilised in between each plate to avoid cross contamination. Following the Dr Kirby Bauer disk diffusion method [55,56], the petri dishes were sub-divided and labled on the bottom before inoculation. After inoculation 3 equally spaced blank discs from Davis Diagnostics (Pty) Ltd., SA, were placed onto the agar and gently pressed using a flame sterilised tweezer. Each blank disc was then impregnated with 15 µL of the antibacterial medium (CG capped AgNPs of different dilutions). Each of the AgNPs solutions was diluted to obtain a 1:0, 1:1, 1:2 and 1:3 dilution with distilled water. The discs had a diameter of 6 mm, and any inhibition was measured as a diameter where any measurement larger than 6 mm indicated inhibition. The inoculated petri dishes were incubated at 36°C for 20 hours. As a positive controls 2 commercial antibiotic disks, 100 µg Carbenicillin and 30 µg Vancomycin, were used. Chemical structure of (b) carbenicillin and (c) vancomycin are shown in **Fig.1**

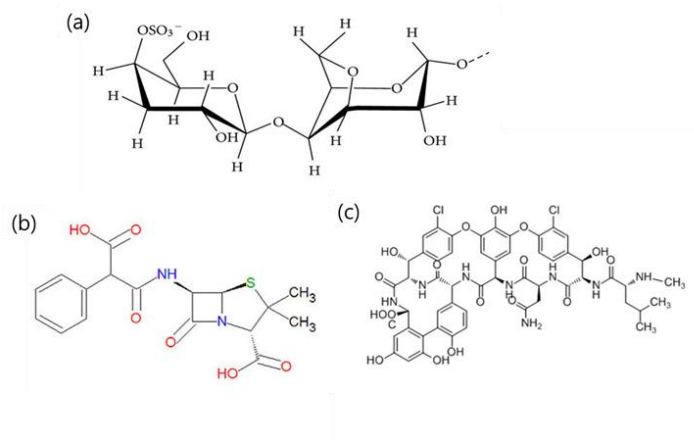

Fig. 1 Chemical structure of (a) *k*-Carrageenan gum; (b) Carbenicillin and (c) Vancomycin.

III. RESULTS AND DISCUSSION

The reduction of Ag^+ to Ag^0 was primarily monitored by visual inspection of the reaction mixture. The color of the reaction mixture changed from colourless to dull yellow to brown colour within few seconds of microwave irradiation [57] **(Fig.2a).** This gave the preliminary confirmation of formation of CG capped AgNPs. In order to know the size, shape and distribution, SPR plays a very important role. AgNPs SPR properties with their morphology is a fast and easy way for in situ monitoring of the synthesis by UV–visible spectroscopy [58]. This is very useful, for instance, in the early stages of wet chemistry synthesis, when many different chemicals are present in solution and sample preparation for transmission electron microscopy (TEM) analysis poses serious concerns about particles modification.
The change in color has been attributed to excitation of surface Plasmon resonance (SPR) of AgNPs [57]. The SPR of different combination of CG capped AgNPs was observed in the wavelength region between 420 nm-430 nm which confirms the synthesis of AgNPs. SPR also shows the spherical shape of formed AgNPs **(Fig.2b).**

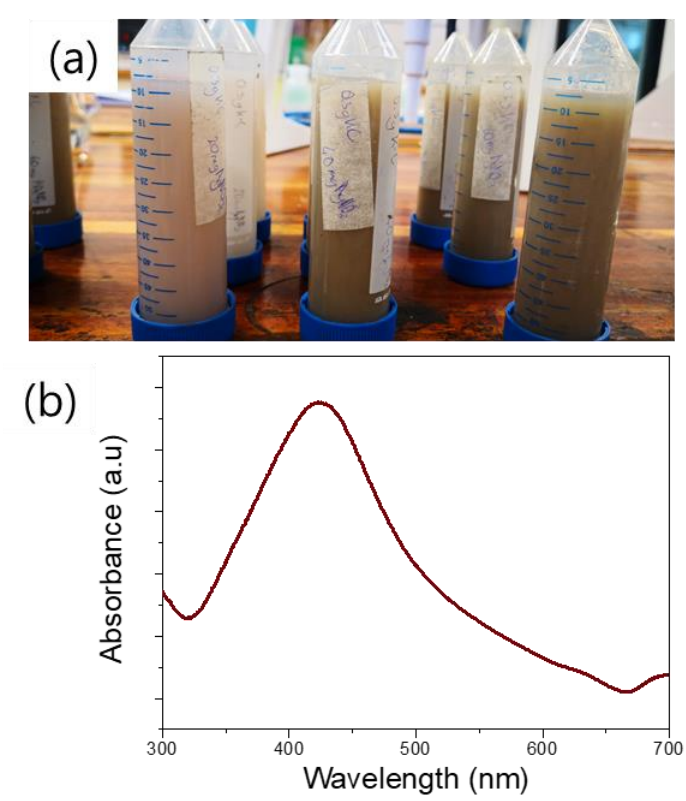

Fig. 2: Visual observation of the formation of CG capped AgNPs synthesis of different ratio of $AgNO_3$/CG; (b) UV visible spectra of CG capped AgNPs showing SPR at 424nm.

The CG-capped AgNPs synthesized in this method are characterized using powder XRD to confirm the particles as silver and to know the structural information. **Fig.3a** shows the XRD pattern of CG-capped AgNPs. The pattern clearly shows the main peaks at (2θ) 38.56, 44.70, 64.78 and 77.42 corresponding to the (111), (200), (220) and (311) planes, respectively [57]. By comparing JCPDS (file no: 89-3722), the typical pattern of CG-capped AgNPs is found to possess a face centred cubic (fcc) structure. The surface morphology of CG-capped AgNPs was performed by using SEM micrograph, at two different magnifications (Figure .3b, c). The CG-capped AgNPs showed homogenous dispersion of AgNPs in the matrix of biopolymer (CG). No agglomeration was observed in it. These are our preliminary work, for the confirmation of shape, size and distribution we will perform the TEM in the near future. So those details are not included in this script.

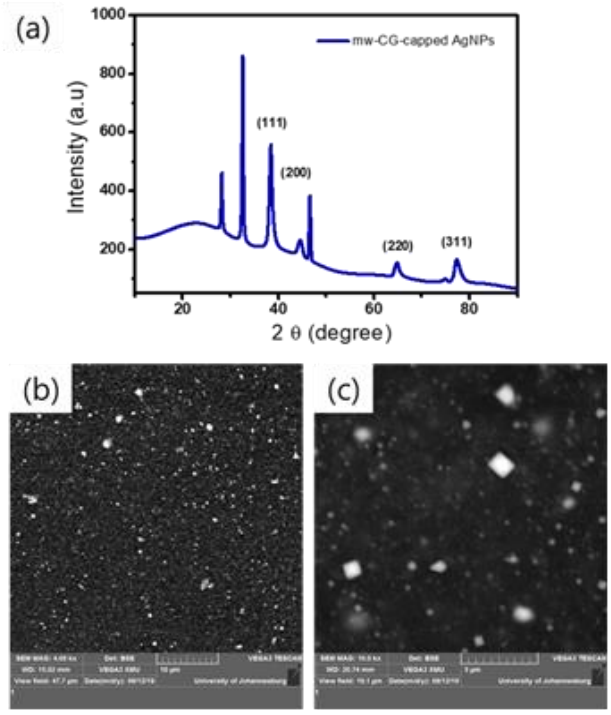

Fig. 3**:** (a) XRD pattern of CG-capped AgNPs; (b.c) SEM images at two different magnifications.

PHYSICAL CHARACTERISATION RESULTS

A zeta potential was used to determine the surface potential of the silver nanoparticles. It also provides the information link to stability of our CG-capped AgNPs. The zeta potential analysis results reveaedl that the change in zeta potential was observed with increasing silver nitrate concentrations and by increasing κ-carrageenan concentration. All the silver nanoparticle solutions had zeta potentials that were negative and smaller than -30 mV. It was already stated that zeta potential value smaller than -30 mV is indicative of a stable particle indicating that the particles formed have high stability [57] and the zeta potential values did not change after several days clearly confirming the high stability of our CG-capped AgNPs **(Fig.4).** The compound with the best antimicrobial results had a zeta potential of -38.17mV.

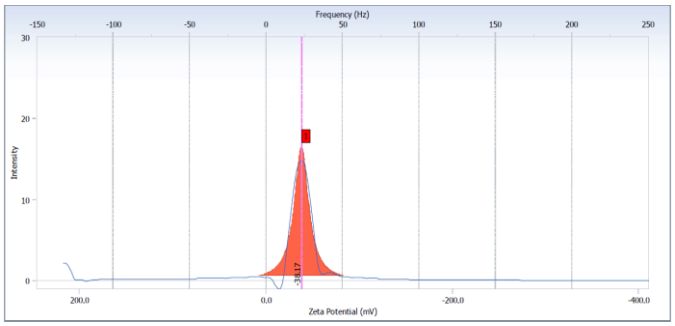

Fig.4 Shows the zeta potential of CG-capped AgNPs-38.17mV and no change was observed after several days

ANTIMICROBIAL RESULTS

The growth inhibited zones surrounding the impregnated discs had a max diameter of 7 mm with the largest inhibition zone being 11 mm in diameter. This measurement was made on the (0.3 g CG -60 mg silver nitrate)/50 mL solution diluted to a 1:1 dilution. This size of inhibition zone for an experimental compound compares well to the 21 mm inhibition zone measured for the carbenicillin commercial antibiotic. In this case the dilution of the CG-capped AgNPs solution is 33% as effective as this commercial antibiotic. The vancomycin discs did not significantly inhibit any growth of the *E. coli*. In **Fig. 5(a)** the inhibition zone can clearly be seen as a clear growth free circle around the white disc. **Fig. 5 (b)** is for comparison and represents the carbenicillin control. Some petri dishes showed initial inhibitions were after the bacteria grew further. This was indicated by a set of 2 concentric inhibition zones where the outer zone had some bacterial growth. This can be attributed to resistance to the antimicrobial compound [59,60].

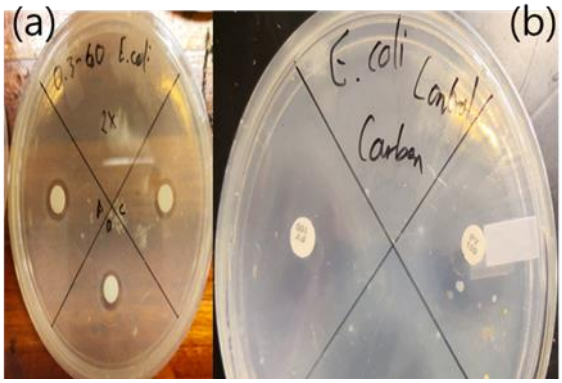

Fig. 5**:** Inhibition of *E. coli* by (a) CG-capped AgNPs, (b) carbenicillin

IV. CONCLUSION

We have successfully synthesised simple, fast, low cost eco-friendly CG-capped AgNPs by using biopolymer, kappa carrageenan gum. The XRD results clearly depict the FCC structure of silver. The zeta potential results confirm the high stability of CG-capped AgNPs. From this study, it was found that CG-capped AgNPs have promising bactericidal activity, although the actual mechanism of antimicrobial action remains to be discovered, the antimicrobial activity exhibited by CG-capped AgNPs makes it a superior nominee for use as bactericidal agents that can be used for several medical applications.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

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