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# Antibacterial activities and *In-Vitro* Anticancer on human breast and liver cell cancer based on Silver Nanoparticles Biosynthesized from Marine Crab.

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**ABSTRACT**

AgNPs were synthesized from the whole body of marine crab crude extract and analysed by UV-Visible spectrophotometric. Antibacterial study was assayed by biochemical properties while cytotoxicity assay was completed using cancer cell lines. Biosynthesized AgNPs was attained by the addition of silver nitrate (1mM) to the marine crab crude extract supernatant and monitored for colour change after incubation. AgNPs formation was characterized by UV–Visible spectrophotometer analysis which displayed absorbance peak of 420nm to 440nm. Transmisson Electron Microscopy (TEM) revealed that AgNPs were spherical and ranged in size from 5nm to 25nm, having an average size of 15nm. Fish pathogen testing showed that biosynthesized AgNPs inhibited growth of all tested pathogens including; *Streptococcus agalactiae, Edwardsiella tarda, Streptococcus iniae*, *Encherichia coli* and *Vibrio parahaemolyticus.* AgNPs application at 250µg/mL displayed greater prevalence than Oxytetracycline in inhibiting *Streptococcus agalactiae-1* and *Edwardsiella tarda*. The cytotoxicity study of AgNPs exhibited anticancer activity against MCF-7 cells, human breast adenocarcinoma cell-line at $IC_{50}$ of 46.77 µg/mL and HepG2, human liver carcinoma cells at $IC_{50}$ of 30.19µg/mL, after 72h. This study indicates the great potential AgNPs has as an antibacterial and anticancer agent. Marine crab crude extracts could be used efficiently as a greener route in AgNPs synthesis.

**Keywords:** Marine Crab, UV-Vis Spectroscopy, Cytotoxicity, Silver nanoparticles AgNPs, Antibacterial activity, MCF-7 and HepG2 cells

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## INTRODUCTION

Crabs have high health benefits due to the presence of proteins, vitamins and unsaturated essential fatty acids and mineral elements which is of great importance on human health[1]. Organisms existing in oceans have adapted to thrive in marine environments, a mechanism owing to the production of biologically active secondary metabolites. A variety of secondary metabolite compounds were isolated from marine crabs with potential medial and agricultural interests[2]. Nanotechnology is an emerging scientific field involving the synthesis and application of nanoparticles. The majority of metallic nanoparticles are able to be synthesized by various chemical, physical and biosynthetic approaches, mediated by several microorganisms and plants[3]. Silver nanoparticles (AgNPs) are used in biomedical applications such as antibacterial properties against gram-negative and gram-positive microorganisms: yeast, fungi, moulds and viruses [4,5]as well as drug delivery vectors including anticancer agents[6] and against different cancer cell lines[7]. The synthesis of silver nanoparticles (AgNPs) using chemical methods allows AgNPs to synthesize in solution with ease[8]. In the synthesis of well-defined nanoparticles, both physical and chemical methods are considered suitable. Recent studies have found an increase in the green synthesis of NPs using natural products as a source of reagents[9]. The biological synthesis of AgNPs have been widely reported using fungus *Trichoderma viride*[10], the lactic acid bacteria (LAB) *Lactobacillus spp*. ,[11] *Pediococcus pentosaceus* and *Enterococcus faecium* [12], the exopolysaccharide of *Leuconostoc lactis* [13] ,the pathogenic bacteria *Klebsiella pneumonia*[14], the marine bacteria *Vibrio alginolyticus and* pathogenic yeast *Candida albicans*[15], the marine worms (polychaeta) [16] and from crab [17]. Bacterial disease is causing major threats to the sustainable development of aquaculture resulting in millions of dollars in loss, annually. The use of antibiotics produce residue related issues and drug resistance from bacterial fish disease can be transferred to humans, contributing to one of the world's most significant healthcare problems [18]. For this reason, the control of biological disease in aquaculture is the most successful approach to infectious diseases [19]. Antimicrobial activity has been detected in several crustaceans, including lobster, crab and shrimp [20]. The antimicrobial activity of crude haemolymph extract have been investigated from *O.macrocera* shore crab [21], *Carcinus maenas* fresh water crab [22], *Paratelphusa hydrodromous* blue crab [23], *Callinectes sapidus* blue crab and *Scylla serrata* mud crab [21]. Chitosan is a natural polysaccharide present in the exoskeleton of crustaceans such as crab and shrimp [24]. Chitosan-synthesized AgNPs demonstrated excellent antibacterial effects against *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris* and *Bacillus subtilis* [17]. Other studies of silver nanoparticles demonstrated antibacterial effects against *Vibrio harveyi* [25], *Aeromonas hydrophila* [26], *Aeromonas salmonicida* [27].

Cancer is a group of disease where uncontrolled growths of abnormal cells generate a range of metabolic and pathological changes in cellular environments including cell proliferation, angiogenesis and metastasis [28]. The toxicity of nanoparticles (NPs) plays an important role for application in biomedical theranostics [29]. These toxicity properties, including cytotoxicity and genotoxicity, of AgNPs reveal anticancer activity against different forms of cancer cells [30]. Apoptosis is a common cell response to NP treatment. A comparative study using albumin-coated silver NPs (AgNPs) revealed that the value of ($LD_{50}$) was lower for breast cancer cells than that of normal white blood cells at the tested concentrations and time of exposure [31]. The microalgal chloroform crude extracts in co-application with the AgNPs exhibit the $IC_{50}$ against MCF-7 cells of 17.78 µg/ml and against 4T1 cells of 52.7 µg/ml after 72 h treatment, without affecting the normal Vero cells [11]. The potential of crab as a source of biologically active products is largely unexplored. Marine drugs have demonstrated great potential of marine natural products; however, many of these drugs do not go beyond the pharmaceutical pre-clinical trials.

There are 7 pharmaceutical products, mostly antitumor drugs, manufactured from marine organisms the market today [32] such as orally active b-lactone, made up of actinomycete *Salinispora tropica* for the treatment of various solid tumors, hematological malignancies and more commonly, multiple myeloma [33].

This study synthesized silver nanoparticles from marine crab using bio-reduction methods to evaluate the antibacterial bioactivity of AgNPs and to assess the cytotoxicity of synthesized nanoparticles AgNPs against MCF-7 cells, human breast adenocarcinoma cell-line and HepG2, human liver carcinoma cells.

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## MATERIALS AND METHODS

### Chemicals

The chemical silver nitrate ($AgNO_3$) was purchased from Sigma Aldrich (St. Louis, USA). Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) were purchased from (Difco, USA). The MCF-7 cells and the HepG2 cell lines were obtained from Sigma-Aldrich Chemical Company, USA. Fresh, healthy species of marine crab, *Portunus pelagicus*, was collected from Pulau Kambing jetty in Kuala Terengganu, Malaysia.

### Silver nanoparticles synthesis

Whole-crab crude extracts, *Portunus pelagicus*, were taken from the stock of a previous study [2]. Briefly, 10 g of crab powder was extracted using 100 ml of methanol with soxhlet apparatus at 45 ºC for 72 h until complete exhaustion of the material. The solvent was evaporated using a rotator evaporator. 25 ml of dried extracts were added into 225 ml of aqueous solution of 1 mM silver nitrate ($AgNO_3$) prior to incubation at 37 ºC in a rotary shaker at 200 rpm in the dark for a period of 3 days to avoid other biological changes. After reducing silver nitrate into Ag+ ions, nanoparticles formation was observed. The reduction of silver ions to metallic silver can be visualized by a colour change from colourless to brown. The solutions were filtered and centrifuged at 15000 x g for 15 min at 4 ºC. The pellets were collected to be washed with sterile deionised water 3 times and left to air dry at room temperature [34].

### Characterization of AgNPs

### UV/vis spectrophotometer

The reduction of Ag+ ions with crab crude extracts to form silver nanoparticles AgNPs was monitored by scanning UV/vis spectrophotometer (Perkin-Elmer, Lambda-19, USA) for optical absorption spectra of AgNPs in the range of 300 nm and 700 nm. All UV-Vis spectra measurement samples were prepared by centrifugation of pellet solution supernatant (1.5ml) at 10,000 rpm for 10 min at 25 ºC.

### Transmission electron microscopy (TEM) analysis of AgNPs

A total of 1 0µl of silver nanoparticles sample was placed onto carbon coated copper grid and allowed to dry at room temperature. The particle size distribution was achieved using TEM (JEOL-JEM-1200 EX Japan) operating at 120 kV.

### Antibacterical activity of AgNPs

### Bacterial strain

The five bacterial isolates used in the present work were kindly provided by Professor Najiah from Fish Disease Laboratory, University Malaysia Terengganu (UMT) Malaysia. These were local clinical isolates from diseased fish and identified by 16-S-rRNA molecular technique in previous studies of [35,36]. The stock cultures of gram-negative bacteria consisted of *Vibrio parahaemolyticus, Encherichia coli* and *Edwardsiella tarda,* where gram-positive bacteria included *Streptococcus agalactiae and Streptococcus iniae*. The cultures were stored at – 20 ºC in Tryptic soy broth (TSB) (Merck, Germany) supplemented with 15% (v/v) sterile glycerol (Merck, Germany). Test organisms were activated by transfers in TSB at 30 ºC for 24 h then streaked on Trypticase Soy Agar (TSA) (Difco, USA) prior to incubation at 30 ºC for 24 h. Several pure colonies of bacteria were inoculated into a 0.85% physiological saline solution and mixed with vortex. The turbidity of the bacteria suspension was adjusted to 0.5 McFarland Standard $1.2 \times 10^8$ CFU $mL^{-1}$. The bacterial suspension were used in the Hole plate diffusion method for antibacterial assay of silver nanoparticles.

### Hole plate diffusion method

The Hole plate diffusion method used in determining the antagonistic activity of the biosynthesized AgnPs was as reported by [37]. Muller Hinton Agar (Difco, USA), with and without supplementation of 1.5% NaCl (MHA–1.5 % NaCl), were prepared according to manufacturer's instructions. The medium were sterilized by

autoclaving at 121ºC for 15 min and allowed to solidify in sterilized petri dish. A sterile cork borer was used to create 6 mm holes in the agar. The bacterial suspensions 1.2 x $10^8$ CFU $mL^{-1}$ were swabbed evenly onto the MHA using sterile cotton buds and 50 µL at concentration of 250 µg/mL of the synthesized AgNPs were pipetted into the respective hole. The zone of inhibition was measured after incubation at 30 ºC for 24 h. This test was done in duplicate.

**Determination of Cytotoxic effects**

**Cell culture and maintenance**

MCF-7 and HepG2 cell lines were well maintained in Mammary Epithelial cell growth medium (MEGM) supplemented with 10% Fetal Bovine Serum (FBS), 1% Sodium Pyruvate, 1% Pencillin Streptomycin and 1% Non-essential Amino acid. The cell-lines were developed in 25 $cm^3$ and 75 $cm^3$ tissue culture flasks in a humidified atmosphere containing 5% $CO_2$ and 95% air, at 37 ºC. Old media was replaced with fresh media every 2-3 days after washing cells with Phosphate Buffered Saline (PBS) (pH 7.4).

**Cytotoxic Assay (MTT assay)**

Cytotoxic assay is a colorimetric assay that relies on the reduction of a yellow tetrazolium salt (MTT) by the viable cell mitochondrial enzyme (dehydrogenase) cleaving the tetrazolium rings of the pale yellow MTT to form purple colored formazan crystals. Consequently, the number of surviving cells is proportional to the level of the formed formazan [38]. The cytotoxicity of the methanolic extracts of crab on MCF-7 and HepG2 cancer cells were assayed after 48 h and 72 h. 100 µl cell suspension at 5 x $10^4$ cells/ml was pipetted into each well of a 96-well plate and incubated overnight to allow cell attachment and proliferation. Following the overnight incubation, cells were treated with various concentrations (3.125-100 µg/ml) of extracts. MTT labelling reagent (final concentration 5mg/ml) was added into each well then incubated prior to undergo solubilisation.

Spectrophotometric absorbance of the samples was measured by using a microliter plate (ELISA) reader at the wavelength of 570 nm. The cytotoxicity percentage was calculated as follows[39]:

$$\text{Cell viability (\%)} = [(\text{OD sample} - \text{OD blank}) / \text{OD control}] * 100 \quad \ldots\ldots\ldots\ldots (1)$$

Where; OD sample = Absorbance of the treated sample, OD blank = Absorbance of media + DMSO and OD control = Absorbance of non-treated sample.

The sample concentration that inhibits 50% of the cell viability or half the maximal inhibitory concentration ($IC_{50}$) was determined by using GraphPad Prism, Version 6 software.

**Morphological characterization**

MCF-7 and HepG2 cells were seeded for 24 h before replacing the media with a fresh media containing crab extracts at $IC_{50}$ levels for 72 h. An inverted microscope was used to observe the morphology of cells. Negative control did not receive treatment.

## RESULTS

**Visual observation of AgNPs**

Silver nanoparticles formation synthesized by extracts of whole-body crab is shown in Table 1. After 8 minutes of reaction with the crab crud extract, the sample underwent a colour change from colourless to brown (Fig.1), indicating the formation of silver nanoparticles.

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**Table 1. Silver nanoparticles formation synthesized by extracts of whole crab body.**

| SAMPLE | WEIGHT OF CRAB (g) | VOLUME OF WATER(ml) | VOLUME OF FILTRATE(ml) | CONC OF $AGNO_3$ (M) | VOL OF $AGNO_3$ (ml) | TIME REACTION(min) |
|---|---|---|---|---|---|---|
| **WC1** | 0.1 | 500 | 5 | 0.01 | 90 | 8 |
| **WC2** | 0.1 | 500 | 10 | 0.01 | 90 | 8 |
| **WC3** | 0.1 | 500 | 15 | 0.01 | 90 | 8 |
| **WC4** | 0.1 | 500 | 20 | 0.01 | 90 | 8 |
| **WC5** | 0.1 | 500 | 25 | 0.01 | 90 | 8 |
| **WC6** | 0.1 | 500 | 30 | 0.01 | 90 | 8 |
| **WC7** | 0.1 | 500 | 35 | 0.01 | 90 | 8 |
| **WC8** | 0.1 | 500 | 40 | 0.01 | 90 | 8 |
| **WC9** | 0.1 | 500 | 45 | 0.01 | 90 | 8 |

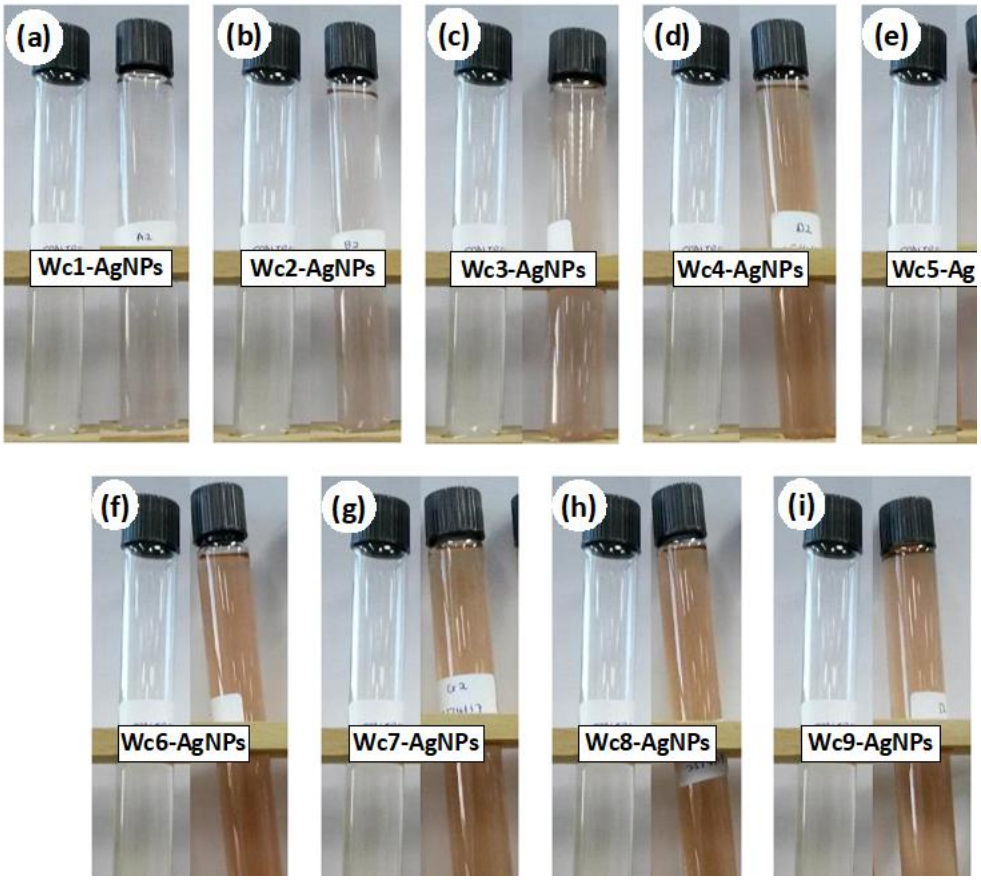


**Fig.1: Visual observation for colour changes of silver nanoparticles for all samples : (a) Wc1-AgNPs, (b) Wc2-AgNPs, (c) Wc3-AgNPs, (d) Wc4-AgNPs, (e) Wc5-AgNPs, (f) Wc6-AgNPs, (g) Wc7-AgNPs, (h)Wc8-AgNPs, (i) Wc9-AgNPs after 8 minutes incubation reaction.**

**Scanning spectrophotometry (UV/vis*)***

Silver nanoparticles formation was confirmed using UV-Vis spectrophotometers. The biologically synthesized silver nanoparticles showed maximum absorbance at 420 nm to 440 nm (Fig.2).

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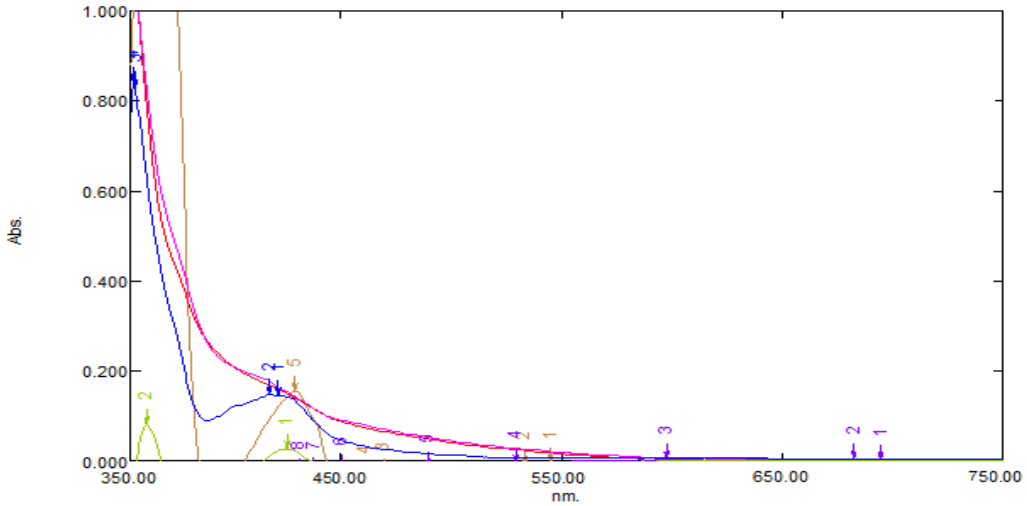


**Fig. 2: UV-Vis spectrum of synthesized silver nanoparticles showing absorbance peak at 420.00 nm to 440.00 nm.**

**Transmission Electron Microscopy (TEM) Analysis**

Transmission Electron Microscopy (TEM) images showed that nanoparticles morphology were mostly spherical in shape (Fig.3) and the size distribution ranged between 10 nm to 50 nm, with the highest dispersion at 25 nm (Fig.4).

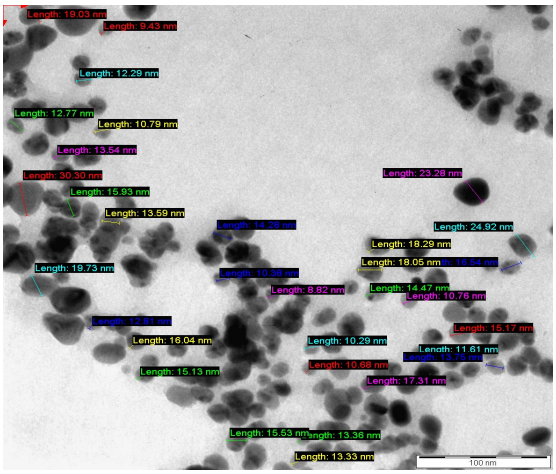

**Fig. 3: TEM image of synthesized AgNPs.**

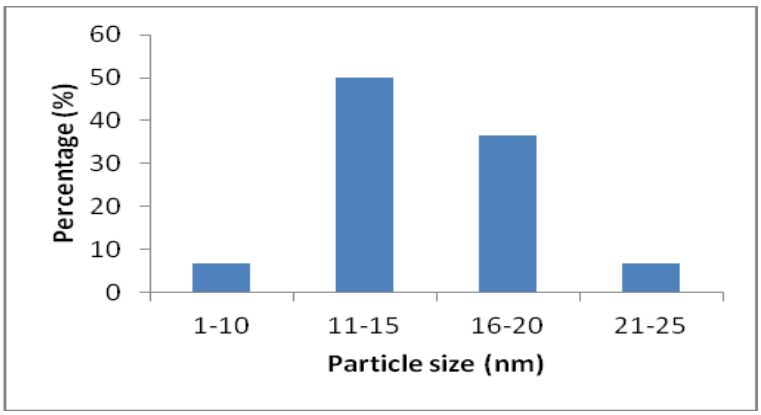


**Fig. 4: Particle size distribution of AgNPs.**

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**Evaluation of antimicrobial activity of the bio-synthesized silver nanoparticles**

The antibacterial activity results of AgNPs determined by Hole plate diffusion method were reported in (Fig.5) and (Table 2). All samples from Wc1-AgNPs to Wc9-AgNPs were successful in inhibiting the growth of all bacterial isolates; *Streptococcus agalactiae, Edwardsiella tarda, Streptococcus iniae*, *Encherichia coli* and *Vibrio parahaemolyticus.* Interestingly, synthesized AgNPs of all samples from Wc1-AgNPs to Wc9-AgNPs have the greatest antibacterial activity against *Streptococcus agalactiae-1* and *Edwardsiella tarda* isolates compared to Oxytetracycline.

**Table 2. Antibacterial activity (means of inhibition zone) of AgNps against pathogenic bacteria isolates. SA=*Streptococcus agalactiae*, ET=*Edwardsiella tarda*, SI=*Streptococcus iniae*, *E.coli*=*Encherichia coli*, VP=*Vibrio parahaemolyticus*, Control=Oxytetracycline 30ug.**

| Samples | Inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| | SA-1 | ET | SI | EC | SA-2 | VP |
| CONTROL | 9.7 | 0.0 | 30.7 | 14.3 | 13.3 | 14.3 |
| WC1 | 14.3 | 15.0 | 15.0 | 9.0 | 8.3 | 11.3 |
| WC2 | 15.3 | 14.3 | 15.3 | 10.0 | 9.0 | 11.3 |
| WC3 | 15.0 | 13.3 | 16.0 | 10.3 | 8.7 | 12.7 |
| WC4 | 14.7 | 14.0 | 16.0 | 10.0 | 9.0 | 11.0 |
| WC5 | 14.3 | 14.0 | 15.3 | 9.0 | 8.3 | 10.7 |
| WC6 | 14.0 | 14.0 | 15.0 | 9.0 | 8.0 | 12.0 |
| WC7 | 14.7 | 14.0 | 15.3 | 11.3 | 9.0 | 11.7 |
| WC8 | 15.7 | 14.0 | 15.3 | 10.7 | 7.3 | 10.7 |
| WC9 | 15.0 | 14.0 | 15.3 | 9.7 | 8.0 | 12.7 |

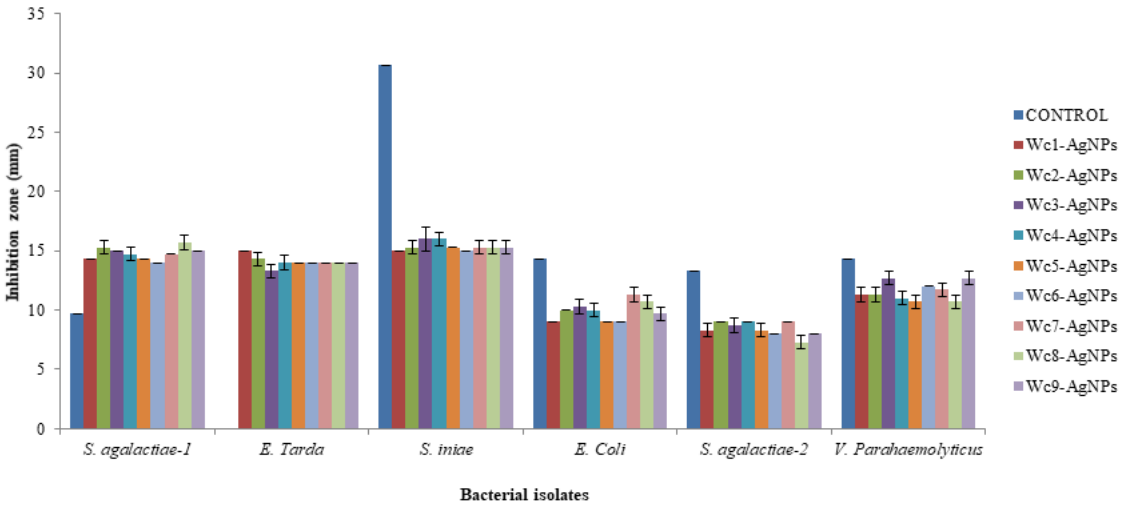


**Fig. 5: Antibacterial activity (inhibition zone) of AgNps against pathogenic bacteria isolates. *S. agalactiae*=*Streptococcus agalactiae*, *E. tarda*=*Edwardsiella tarda*, *S. iniae*=*Streptococcus iniae*, *E.coli*=*Encherichia coli*, *V. parahaemolyticus*=*Vibrio parahaemolyticus* Control=Oxytetracycline 30ug. The values are with mean ±SD (n=3).**

**Cytotoxic activities**

The cytotoxic effects of crab crude extracts extracted by methanol on MCF-7 and HepG2 are shown in Fig.6 and Fig.7, respectively. The crab crude extracts exerts cytotoxicity towards MCF-7 cell lines at $IC_{50}$ of 51.29 and 46.77 µg/ml after 48h and 72 h, respectively; whereas HepG2 cell lines were 74.13 and 30.19 7 7µg/ml after 48 h and 72 h, respectively (Table 3).

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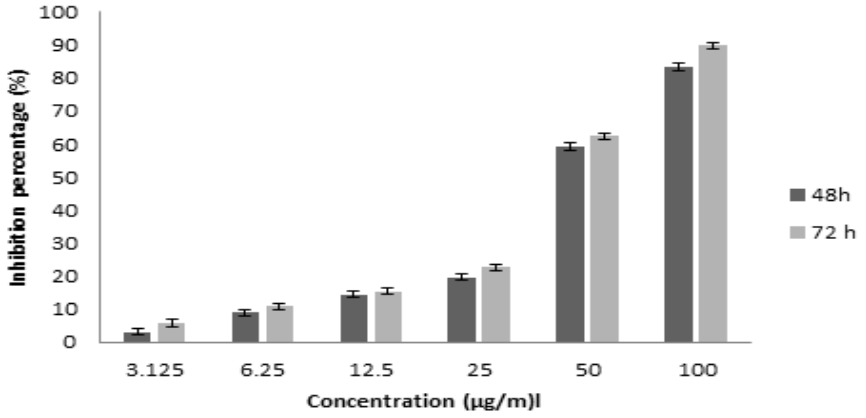


**Fig. 6: Cytotoxic effect of crab crude extracts on MCF-7 cancer cells line.**

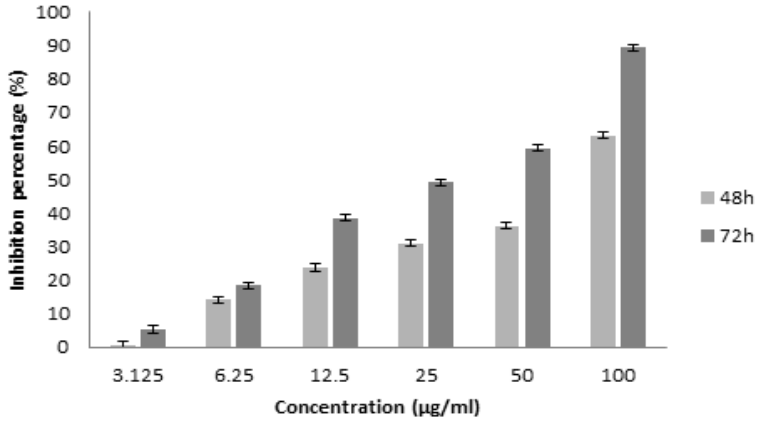


**Fig. 7: Cytotoxic effect of crab crude extracts on HEPG2 cancer cells line.**

**Table 3. $IC_{50}$ values (µg/ml) of methanol crab extract on MCF7 and HEPG2 cancer cell line.**

| Cell lines | 48 h | 72 h |
|---|---|---|
| MCF7 | 51.29 | 46.77 |
| HEPG2 | 74.13 | 30.19 |

**Morphological characterization**

The morphological characteristics of MCF-7 and HepG2 cells treated with crab extracts at the $IC_{50}$ values were studied under light microscopy. The control (untreated) cells were large, round, healthy-looking and attached to each other without apoptotic bodies or necrotic cells observed. The treated cells, however, were rounded up, decreased in size and detached from the monolayer surface of the wells. The total number of cells had diminished when compared to the control (Fig.8 and Fig.9).

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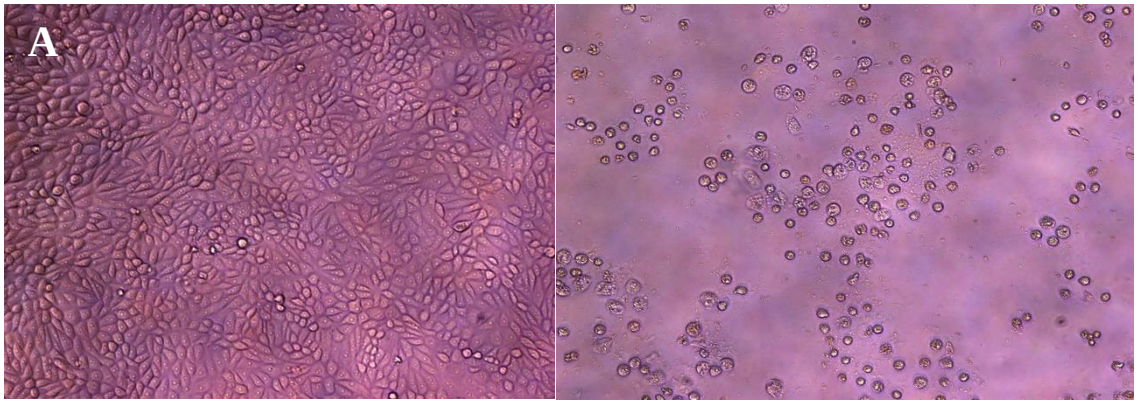


**Fig. 8: Morphology of MCF-7 cells for (A) Control; (B) Treated cells with crab crud extracts for 72 h.**

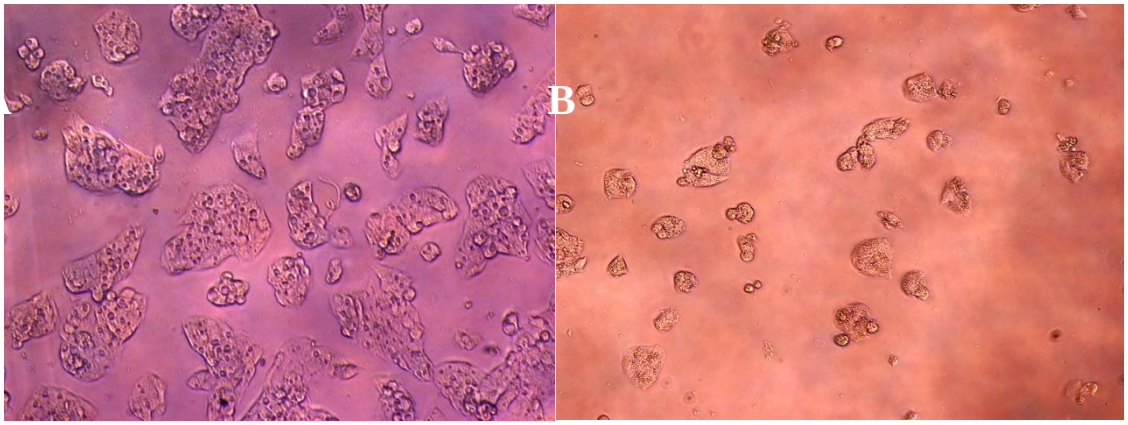


**Fig. 9: Morphology of HEPG2 cells for (A) Control; (B) Treated cells with crab crud extracts for 72 h.**

## DISCUSSION

Silver nanoparticles AgNPs synthesis is an eco-friendly technology widely used in biosynthetic methods which utilize medicinal plants or biological microorganisms. Research lacks in the study of marine crabs and other aquatic animals capable of taking on the role as reducing or capping agents. Marine animals have the benefits of being easily available, safe to handle and posses' variable metabolites that may be considered. In this study, biological synthesis of AgNPs from whole-body marine crab, *Portunus pelagicus,* extract revealed great antibacterial activity against various species of pathogenic fish bacteria including; *Streptococcus iniae, Streptococcus agalactiae, Encherichia coli, Edwardsiella tarda* and *Vibrio parahaemolyticus*. The AgNPs have potential to be a new antimicrobial drug, owing to the high ability to inhibit bacterial growth. [40] study reported silver nanoparticles exhibiting a broad bactericidal activity against both gram-positive and gram-negative bacteria. [41] effectively used silver nanoparticles biosynthesized using plant extract against *Staphylococcus aureus.* A related result was observed with silver nanoparticles synthesise from the haemolymph of two marine crabs, *Carcinus maenas* and *Ocypode quadrata,* that determined AgNPs possess antimicrobial activity on human and fish pathogens [34]. In another reported study, scientists conducting experiments on silver nanoparticles (AgNPs) synthesized from Streptomyces rochei proved successful in inhibiting the growth of medically important pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, , *Vibrio damsela*, *Staphylococcus aureus, Vibrio fluvialis*, *Bacillus cereus, Bacillus subtilis* and *Salmonella typhimurium* [42]. The mechanism of AgNPs against bacterial isolates may be due to the interaction of positively charged Ag+ ions on the negative charge of the microorganism cell membrane causing bacterial cell death [41]. Morones et al. 2005[43] reports that adherence to bacterial cells of the cell wall is effortlessly made by smaller particles, obstructing the cells permeability, respiration and other normal behaviour with the release of Ag ions from the AgNPs particle. In agreement, Sondi and Salopek-Sondi, 2004[44] have also observed AgNPs adhered to the cell wall of the gram-negative bacteria (*E. coli*), causing destruction of the bacterial cell, while the study of Vaseeharan et al. 2010 [25] agrees on silver nanoparticles mechanism of action as a bactericidal agent by disturbing the integrity and functionality of the bacterial cell membrane. In addition, silver ions were

able to inactivate the protein in the bacterial cell and block the nutrient for bacteria growth and lead to the death of the bacteria cell Meng et al. 2016[45]. Finding a new molecule to combat cancer encourages several researches to identify interesting drug candidates from a specific resource. Crabs are rich in polysaccharides and those derived polysaccharides possess a wide range of pharmacological activities. It is rich in chitin, chitosan, selenium and natural carotenoids that have been shown to have an anti-cancer activity [46]. The crab polysaccharides and those derived polysaccharides posses a wide range of pharmacological activities [47].

Previous studies reported minor antitumor effect of unburned crab shell extracts that can be explained by presence of chitin and its derivatives as reported by Kimura and Okuda 1999[48], however, this antitumor compound appears less effective when compare with burned crab shell extracts which is more effective. Presence of antitumor compounds in crab, such as Tachyplesin, which is present in leukocytes of the horseshoe crab [49,50] besides the crab shell, there is chitin that is mentioned in some research as antitumor compound [51]. Experiments carried out by Jeon and Kim, 2002 [52] observed antitumor activities *in vitro* and *in vivo* from oligomers of chitosan. Low molecular weight chitosan has been proven to contain significant antimetastatic effects in mice with lung cancer[53]. Low molecular weight chitosan was also studied by Maeda and Kimura, 2004 [51] where the antitumor effects of low-molecular-weight chitosan was investigated and reported a weakening of tumor growth and a decrease in the final weight of the tumor in mice with sarcomas. In addition to being used in medicine, nano-chitosan can also be used as a nanocapsule for slow release of vaccines and cancer treatment [54]. In recent years, the viability of cancer cells has remarkably decreased in number of cell lines, regardless to whether the chito-oligosaccharide derivatives were positively or negatively charged [55]. Nakahara et al. 2002[56] evaluated the effects of cooked and uncooked crab shell extract and its derivative carotenoids on a liver tumor cell line (AH109A) and observed that the inhibitory effect has been attributed to the antioxidant effect of the extract. However, the inhibitory effect of crab shell extract on MCF-7 cells was found to be associated with a decrease in Nitric oxide (NO) and apoptosis induction. The hemolymph of the *D. dehani* crab has the potential to be a good source to inhibit the HepG2 cells at $IC_{50}$ 75 µg $mL^{-1}$ and it would be a great source for anticancer compounds which can be useful for human welfare. There has been an increase of interest for AgNPs for their therapeutic applications in cancer diagnostics, probing and anticancer agents. Silver nanoparticles (AgNPs) synthesized from *Streptomyces rochei* demonstrated a significant degree of anticancer activity against assorted tumour cell lines including hepato-cellular carcinoma cells (HepG2) and breast carcinoma cells (MCF-7) [42], normal human lung fibroblast cells (IMR-900), human glioblastoma cells (U251) [57]. Significant toxicity was observed against MCF-7 cancer cells [58].

Silver nanoparticles assist in gathering and transporting drug into the cancer cells [59] and they also obstruct with metabolism of cancer and tumour proliferation [60]. The AgNPs anticancer property was researched in normal human glioblastoma cells (U251) and human lung fibroblast cells (IMR-90). AgNPs stimulated alterations in metabolic activity and cell morphology, reduced viability of cells and increased oxidative stress, causing damage to the mitochondria and increased production of reactive oxygen species (ROS), resulting in DNA damage [61]. This work detects the capability of Ag NPs in cancer theranostics of breast and liver cell cancer as well as alternative to Oxytetracycline in the near future.

## CONCLUSION

The biosynthesis and characterization of AgNPs exhibiting antibacterial activities on the fish pathogens and the cytotoxicity against human breast adenocarcinoma MCF-7 and the liver carcinoma HepG2 cell-lines were successfully achieved. The AgNPs synthesized from the whole body of the Marine Crab crude extract supernatant and silver nitrate, were by the UV–Visible spectrophotometer at the absorbance peak of 420.00 to 440.00 nm. The AgNPs were spherical in shape, having a size range of 5-25 nm with the average size of 15 nm. The AgNPs application at 250 µg/mL exhibited greater activities than Oxytetracycline against *S. agalactiae-1* and *E. tarda*. The cytotoxicity against MCF-7 cell lines was shown at $IC_{50}$ of 46.77 µg/mL and against HepG2 cell lines at $IC_{50}$ of 30.19 µg/mL, after 72 h, treatment. The Marine Crab crude extracts could be used in the biosynthesis of AgNPs with great potential for applications as antibacterial and anticancer agents.

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**Conflict of Interests**

The authors declare no conflict of interests regarding the publication of this paper.

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