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Activities of pomegranate and nanoparticles as antimicrobial and anticancer

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ABSTRACT

Pomegranates have been recognized as valuable resources of natural antimicrobial compounds. Pomegranate extracts selectively inhibit the growth of breast, prostate, colon and lung cancer cells in culture. Cancer is the third leading cause of death in developed countries and the second leading cause of death in the United States. The results in the current study revealed antimicrobial efficiency against Enterohaemorrhagic *Escherichia coli* O157:H7 (ATCC 51659) *Yersenia enterocolitica*, *Bacillus cereus (*EMCC 1080) and *Staph. aureus* and *Aspergillus flavus* (ATCC 16872) with disk diffusion sensitivity. Antitumor efficiency Cytotoxicity of tested sample was measured against HepG2, MCF-7 and Caco-2 cells and activities effect of the pomegranate and its extract with copper nanoparticles. In addition, the present study built on three consequent ideas; the first one is concerned with pomegranate juice and synthesis copper nanoparticle by green method using pomegranate juice as antioxidant and Ethylene Glycol as a capping agent. The second is characterization and identification of prepared materials. Biosynthesized nanoparticles were characterized by UV-VIS, FT-IR, SEM and TEM analysis. Results indicate that the highest antimicrobial sensitivity was recorded against *Escherichia coli* O157:H7 using 10% from initial concentration of copper nanoparticles supernatant and against *Yersenia enterocolitica* using 10% from initial concentration of copper nanoparticles pellet and against *Aspergillus flavus* (ATCC 16872) using 4% from initial concentration of copper nanoparticles supernatant. Among the various concentration ratios of pomegranate juice and copper nanoparticles, anticancer activity is estimated for an optimized pomegranate lyophilized juice concentration using human colorectal adenocarcinoma cells (Caco-2) cancer cell lines. The synthesized Cu Nps has IC50 equals 81.6 µg/ml against human colorectal adenocarcinoma cells (Caco-2) cancer cell lines.

***Keywords*:** Antimicrobial, anticancer, pomegranate, copper Nano particle, UV, FT-IR, SEM and TEM.

Introduction

Pathogenic microorganisms have become a major problem not only in the developing countries but also in the developed countries. Antibacterial materials are an area of prime interest today in the light of the need to control methicillin resistant *Staphylococcus aureus* (MRSA) infections in hospitals and other health care facilities as well as *campylobacter* in food preparation and *Aspergillus niger* in HVAC systems, among others (CDA, 2004). The expanding bacterial resistance to antibiotics has become a growing concern worldwide (Bezalwar *et al*., 2017). Intensive care physicians consider antibiotic resistant bacteria a significant or major problem in the treatment of patients (Wilke, 2010). Treatment of these infections is often very difficult due to cross-resistance of these bacteria with a large group of antibiotics, so it seems reasonable to explore new sources of natural compounds with antibacterial and antifungal activities (Atai *et al*., 2009 and Walter *et al*., 2011). Recent researches have shown that pomegranate extracts selectively inhibit the growth of breast, prostate, colon and lung cancer cells in culture (Adhami *et al.,* 2009). Natural products and herbal medicines with antimicrobial effects have been recognized with increasing interest be clinical pharmacologists. For millions of people, traditional medicine serves as the only opportunity for health care (Atai *et al*., 2009; Upadhyay, 2011 and Walter *et al*., 2011). A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds (Hemaiswarya *et al*., 2008; Alviano, 2009; Upadhyay, 2011 and Abedini *et al.,* 2014). A number of medicinal plants have been screened for antimicrobial activity in recent years (Wikaningtyas and Sukandar, 2016). Popular

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observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always proven completely for known mechanisms (Budzinski *et al*., 2011). Studies demonstrate broad-spectrum antimicrobial activity of pomegranate against both bacteria and fungi (Mathabe, 2005 and Mahady, 2005). More importantly, the results indicated that methanolic extracts of pomegranate are more effective against bacteria and fungi than the aqueous extracts. The presence of phytocompounds in the extracts including phenols, tannins and flavonoids as major active constituents may be responsible for these activities. In addition, it was reported that the bark, leaves, flowers and fruits of pomegranate are widely used as phytotherapeutic agents in Brazil (Mathabe, 2005 and Prashanth *et al.*, 2001). Cancer is the third leading cause of death (after heart disease and stroke) in the developed countries and the second leading cause of death (after heart disease) in the United States (Weibo *et al.,* 2008). Normally, the division of cells in the body is strictly controlled; if the balance of cell division and death is altered, a tumor may form causes include problems with the body's immune system, which can lead to tumors. Benzene and other chemicals and toxins, drinking excess alcohol, excessive sunlight exposure, genetic problems, inactivity (sedentary lifestyle), obesity and radiation, many plant extracts have shown to have therapeutic values with respect to diseases (Singhai *et al*., 2009). In preclinical animal studies, oral consumption of pomegranate extract inhibited growth of lung, skin, colon and prostate tumors. An initial phase II clinical trial of pomegranate juice in patients with prostate cancer reported significant prolongation of prostate specific antigen doubling time (Adhami *et al.,* 2009). The bactericidal effect of metal nanoparticles has been attributed to their small size, and high surface to volume ratio, which allow them to interact closely with microbial membranes and it is not merely due to the release of metal ions in solutions (Morones *et al*., 2005). The metal nanoparticles such as Ag, Cu etc., are found to have antibacterial activity. (Wei, 2010). The efficiency of the copper nanoparticles as antifungal agents is also proved by the results of experiments where these particles were mixed with commercial acrylic paints (Wei, 2010). Because of the high death rate caused by cancer, plenty of research is going on in the field of Nano medicine for Cancer diagnosis and therapy. (Weibo *et al.,* 2008). Infectious diseases and drug resistance to human pathogenic agents have become widespread problems. Therefore, therapeutic compounds that can inhibit the growth of pathogens more, kill them and have no or the least toxicity to host cells are considered as candidates for developing new antimicrobial and anticancer products (Abedini *et al*., 2014). As previously stated, many medicinal plants have antimicrobial activities (Mehta *et al*., 2014). The aim of study focuses on the research and development of new antimicrobial and anticancer materials from pomegranate and nanoparticles product.

Materials and methods

Plant and Nano materials:

Pomegranate samples were purchased from the local supermarket during summer time. Initial concentration solid 0.1g sample /9mL Phosphate Buffer Solution (PBS) and liquid 1mL sample /9mL (PBS) were used. Then, by sequencing concentrations of 1=1%= 100µL from Initial /9mL (PBS), 2=2%= 200µL from Initial /9mL (PBS), 3=3%= 300µL from Initial /9mL (PBS), 4=4%= 400µL from Initial /9mL (PBS) and 10=10%= 1mL from Initial /9mL (PBS) were employed.

Test Microorganisms:

Enterohaemorrhagic *Escherichia coli* O157:H7 (ATCC 51659**)** *Yersenia enterocolitica*, *Bacillus cereus (*EMCC 1080**)** and *Staph. aureus* and *Aspergillus flavus* (ATCC 16872) from fungi. All tested microorganisms were kindly provided from the Microbiology Laboratory, the Dairy Science Department of the National Research Center.

Preparation of Pomegranate sample:

Pomegranate fruits were peeled by hand and the seeds were kept in refrigerator at 4^0C. Seeds of pomegranate were collected from refrigerator, red in color, and separated. All seeds were mixed and then blended with high speed 2000 rpm for 15min. The last juice was filtrated by two sieves. The first one included two layers the first layer pours have diameter of 1mm^2 and the second pours 2 mm^2.

The second sieve had one layer by pours 1mm^2. Mechanical pressure with using metal spoon were used upon juice and ash to gain juice equals 1980.44 ml and ash equals 400.20g (Hernández, 1999).

Synthesis of Copper nanoparticles from Pomegranate sample

The method recommended by Caroling *et al.* (2015) was fallowed as 100 mL of 0.02 molar $CuSO_4$ solution was heated to 80 ˚C with magnetic stirring at 800 rpm, the solution had blue color. A 150 ml of Ethylene Glycol solution was added drop wise into a beaker while stirring, the color changed, to white. Amount of 800 mL of Pomegranate juice was added to solution heated to 80 ˚C with magnetic stirring at 800 rpm till the solution color changed to reddish-brown. Quantity of 450 mL of de-ionized water was added to complete the volume to 1500ml. Quantity of 500 mL of 0.1 molar NaOH solution was added to adjust the solution pH values from 3 to be 4. The mixture was kept at 80 ◦C with magnetic stirring at 800 rpm for 3h and 20 min until a dark solution was obtained (from reddish brown to dark brown). The resulting liquid was centrifuged at 8000 rpm for 15 min. The precipitate had dark brown color and washed by deionized water at 8000 rpm for 15 min while the color was still dark brown but with a black color spot in center. The preparation was lyophilized at -50 ºC, 0.1mp for three days. Then, it was left for 3 days in a glass containing CaO to absorb humidity. The precipitate had the same color (dark brown). A mortar was used to grind the dig matter to be converted from solid to powder. The powder was stored at ambient conditions inside a closed tightly falcon tube at 50ml capacity. (Caroling *et al.,* 2015).

Characterization and identification of materials

Fourier Transform-Infrared (FT-IR) Characterization:

Thin films were prepared from the resulting products by compression-moulding with KBr pellets. The FTIR spectra were recorded on (JASCO FTIR 6100 spectrometer, 64 scans with 4 cm^{-1} resolution) and employed to demonstrate the chemical composition of nanomaterials in the range of 4000–400 cm^{-1} (Caroling *et al.,* 2015, UMER *el al*., 2014 and Jing *et al.,* 2011).

UV–Visible spectrophotometer

UV–Visible spectrophotometer was carried out using a double beam spectrophotometer Model JASCO V-670, Japan instrument. Absorbance spectra were determined in the wavelength range 190-1000 nm. The specimens were dissolved in ethanol and measured in a quartz cuvette (Caroling *et al.,* 2015, UMER *et al.,* 2014 and Jing *et al.,* 2011).

Scanning electron microscope (SEM)

The morphologies of samples were studied by using Field emission scanning electron microscope (FE-SEM) on a Quanta FEG 250 (Czech Republic). An electronic microscope was used to investigate the morphology analysis cooped (TEAM –EDAX Model). The samples were characterized without any further coating by gold sputtering (Caroling *et al.,* 2015, UMER *et al.,* 2014 and Jing *et al.,* 2011).

Transmission electron microscope (TEM)

The size and shape of the nanotubes were performed with a high-resolution transmission electronic microscope (HRTEM) JEOL–JEM-1011, Japan. Images were recorded at a rate of 200 kV. for each sample, low concentration of suspension dispersion was deposited on a carbon copper grid and left to dry at room temperature. (**Caroling *et al.,* 2015, UMER *et al.,* 2014 and Jing *et al.,* 2011).**

Antimicrobial activities Agar well diffusion Method

Antimicrobial activities were carried out using the modified agar well diffusion method described by Nair and Chando, (2005). A sterile 5mm^2 cork borer was used. The prepared nutrient agar plates were rubbed with the test organisms using sterile swab sticks. A cork borer was used to bore a hole on the agar film in the Petri dish and filled with each of the different extract prepared. This was then transferred to the incubator at 37 ºC for 24 hours. After absorption, the cork borer was used to bore. The dishes were incubated for five days at 25 °C. (Visible inhibition zone around bore) was measured according to Freire *et al.* (2001).

Anti-tumour activity

Cytotoxicity of the tested sample was measured against HepG2, MCF-7 and Caco-2 cells using the MTT Cell Proliferation and viability assay. MTT (3-[4,5-dimethylthiazole-2-yl]-2,5 diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals which are largely impermeable to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm (Hansen *et al.,* 1989).

Results and Discussion

Synthesis of Copper nanoparticles from Pomegranate sample

Results in photo 1, (a) Illustrate the preparation sequencing of Copper nanoparticles from Pomegranate sample with heating and mixing continued until the color changed from blue to white, reddish-brown and finally dark brown. The whole process was completed within 4h and 26 min. (b) The resulting from (a) was centrifuged at 8000 rpm for 15 min. Precipitate was dark brown but with a black color spot in center while the filtrate supernatant was clear light brownish pink color solution, (c) The resulting from (b) was lyophilized at -50 ^{o}C, 0.1mp for three days and then it was left for 3 days in glass containing CaO to absorb humidity.

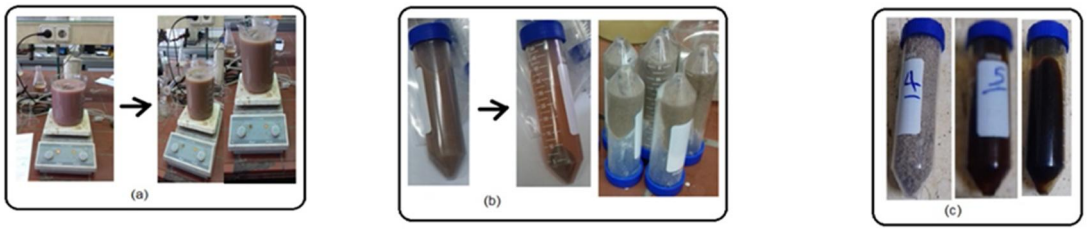

Photo 1: Synthesis of Copper nanoparticles from Pomegranate sample

Visual characterization:

In current results, the preparation of copper nanoparticles from pomegranate extract involves a four-stage process. The color change of the solution changed from blue to white, reddish-brown and finally dark brown. The mixture was kept at 80 $^{\circ}$C with magnetic stirring at 800 rpm for 3h and 20 min until a dark solution was obtained (from reddish brown to dark brown). This is indicated by Caroling *et al.* (2015), Guru Prasad, 2013 and Mathammal, 2015) whom explain the change of color to brown indicates the reduction of copper sulphate and formation of copper nanoparticles. Brown color was noted for the optimum amount of precursor_and extract producing greatest number of copper nanoparticle in aqueous medium. Also, the ratio of volume of extract: CuSO4 the formation of Copper nanoparticle depends on the volume of extract to $CuSO_4$ ratio are 1:3, according to this, the ratio was found to be ideal as the biosynthesized nanoparticles showed maximum absorption at 294nm, which is in agreement with the values reported in the literature (Caroling *et al.,* 2015).

Fourier Transform-Infrared (FT-IR) Characterization

Result in Figures 1, 2 and 3 Illustrate IR spectrum and the reading as follows:
The strongest peaks of hydroxyl at 3419 cm-1, α, β- unsaturated ketone band at 1710 cm-1, olefinic band at 1610 cm-1, primary and secondary alcohols functionalities bands at 1043 cm-1 as well as the peaks around 3000 and 1400 cm-1 attribute to aliphatic C-H stretching and bending modes. By comparing the spectrum of copper nanoparticles with that of the pomegranate extract, we can observe that the two spectra are similar in their spectral features. Therefore, the compound on the surface of

copper nanoparticles has a very close chemical composition to that of pomegranate extract but them not identical.

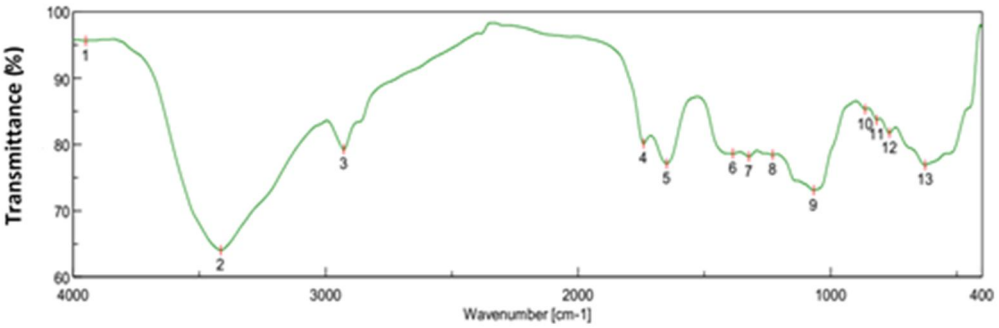

Fig. 1: FT-IR spectrum of Pomegranate lyophilized juice.

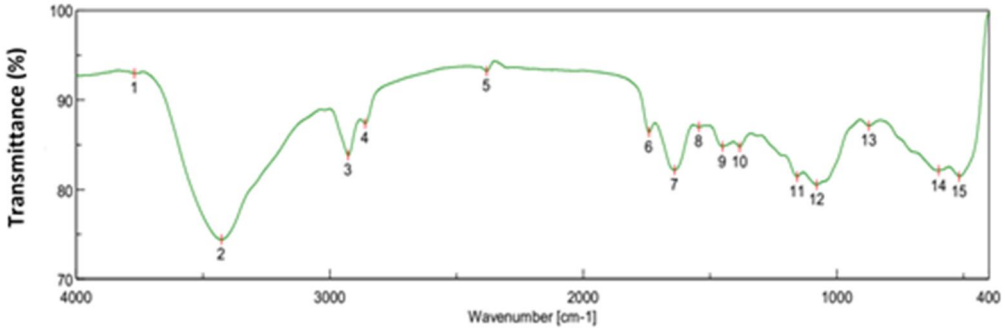

Fig. 2: FT-IR spectrum of Copper nanoparticles from Pomegranate pellet.

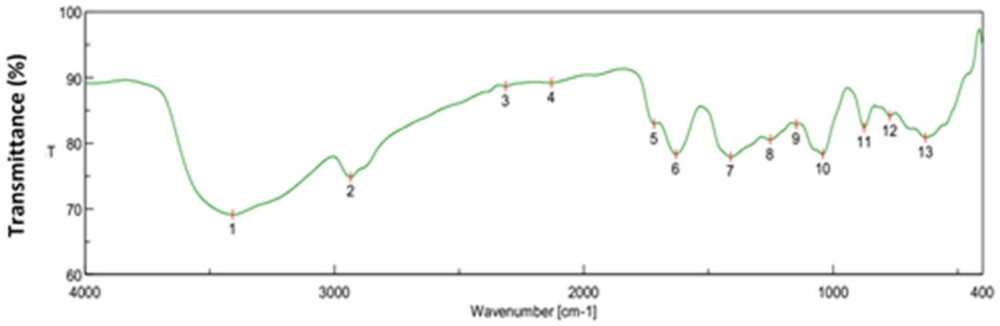

Fig. 3: FT-IR spectrum of Copper nanoparticles from Pomegranate supernatant.

It was found that many peaks obtained by the pomegranate extract have been repeated in the FT-IR spectrum of copper nanoparticles with changes in the position as well as the intensity of absorption. The absorption peaks at 3419, 1604, 1107 and 1053 cm-1 corresponding to OH, C=C and C-O observed in the plants extract get narrower and shifted to higher frequently regions, while those at around 3000 and 1400 cm^{-1} are attributable to aliphatic C-H stretching and bending modes deceased in intensity and shifted to low frequency regions. In addition, the disappearances of γ C=O stretching vibration of the α, β- unsaturated ketone at 1710 cm-1 confirm that the reduction and the stabilization of copper nanoparticles proceed via these groups. This confirm that water-soluble compounds such as terpenoids are present in pomegranate extract has the ability to perform dual functions of reduction and stabilization of copper nanoparticles. Several works as, that of Magne *et al.,* (2005) and Abboud, (2013), have reported a similar observation. Peaks were observed at 3408 cm^{-1}, 1717 cm^{-1}, and 1629 cm^{-1}. Correspond to the hydroxyl, oxidated ester carbonyl groups, and conjugated carbonyl groups, respectively. These results indicate the presence of the polyhydroxyl structure on the surface of copper nanoparticles. The polyhydroxyl structure has an excellent dispersion effect on copper nanoparticles and this agree with (Jing *et al.,* 2011). The presence of ascorbic acid (natural vitamin

C), polyphenols and other phytonutrients present in pomegranate extract are mainly responsible for the bio reduction process, because of the scavenging ability of their –OH groups. The antioxidant property of polyphenolic compounds is mainly due to the redox property that allows them to act as reducing agents (Rodriguez, 2007).

UV–Visible spectrophotometer

Results in Figure 4, Illustrate UV-Visible spectrum of (a) pomegranate lyophilized juice, (b) The formation of Copper nanoparticles from pomegranate pellet and (c) The formation of Copper nanoparticles from pomegranate supernatant, sample 3.

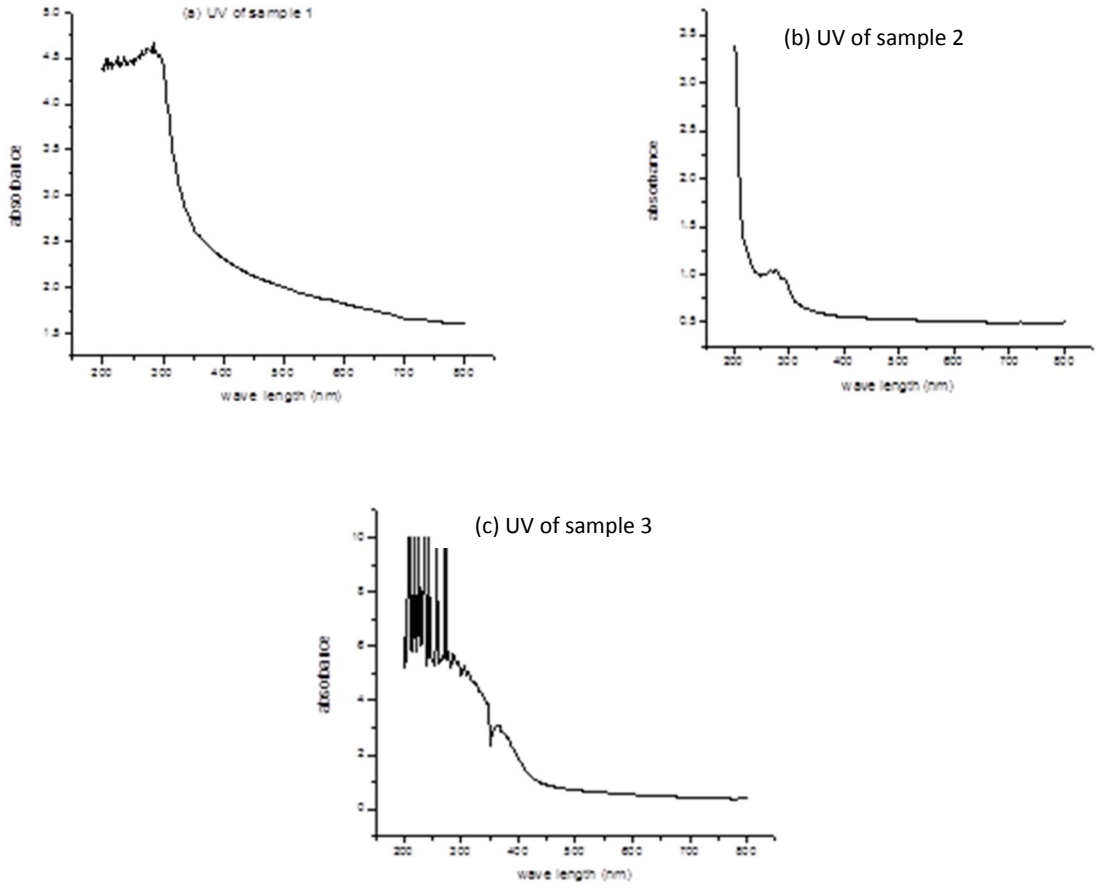

Fig. 4: UV-Visible spectrum of different samples.

UV-Visible spectrum indicating formation of Copper nanoparticles from pomegranate pellet ranged from wavelength 268 to 294nm was indicated by Nguyen *et al.* (2011) and Pavani *et al.* (2013) whom explained a red shift was observed in SPR from 268 to 294nm. This may be due to collision between smaller nanoparticles, which leads to particle growth.

Caroling *et al.* (2015) Found that The CuNp were formed at 80^0C this is explained as at room temperature and 60^0C the formation of CuNp were formed after 1 day and 2 hours respectively and above 80^0C under boiling condition the solution becomes charred with no particle formation. The result showed that EG acted as capping agent as shift position from 326-294 this goes in accordance with Thi *et al.* (2011) who studied the Effect of EG to prevent agglomeration and oxidation process and showed. The stabilization is commonly achieved by using surfactants, which prevents the aggregation binding to the nanoparticle surface, and indicated that EG is low in cost and non-toxic, the shape of Nano material depends strongly on EG and works as size controller and polymeric capping agent (Thi *et al.,* 2011). During particle synthesis, Cu ions can coordinately bond with carbon

and oxygen present in PEG, so that the synthesized CuNp is covered by an adsorbed layer of EG **(Jing *et al.*, 2011).**

UV–Visible spectrophotometer, The present work, reveals that the formation of Copper nanoparticles from Pomegranate pellet ascorbic concentration in the extract has effect on the UV-Visible absorbance spectroscopy of the synthesized CuNp: The result showed a single peak at around 294nm, but the formation of Copper nanoparticles from pomegranate supernatant which resulted in Cu whose dispersion did not show a Plasmon peak at around 570nm, but displayed a broadened peak at a short wavelength. The results indicating the presence of very small separated CuNp less than 4nm, in this concern, our observation agrees with Lisiecki and Pelin, (1993) and Lisiecki and Pelin, (1996).

Scanning electron microscope (SEM) and EDAX

Results in Photo 2, Illustrate SEM image for the formation of copper nanoparticles from pomegranate pellet. The experimental result of SEM of Copper nanoparticles from Pomegranate pellet showed that the prepared nanoparticle has a shape like flakes. A similar phenomenon was reported by Rong *et al.* (2013) and Caroling *et al.* (2015). However, copper nanoparticles from Pomegranate supernatant showed no SEM for it according to its colloidal phase.

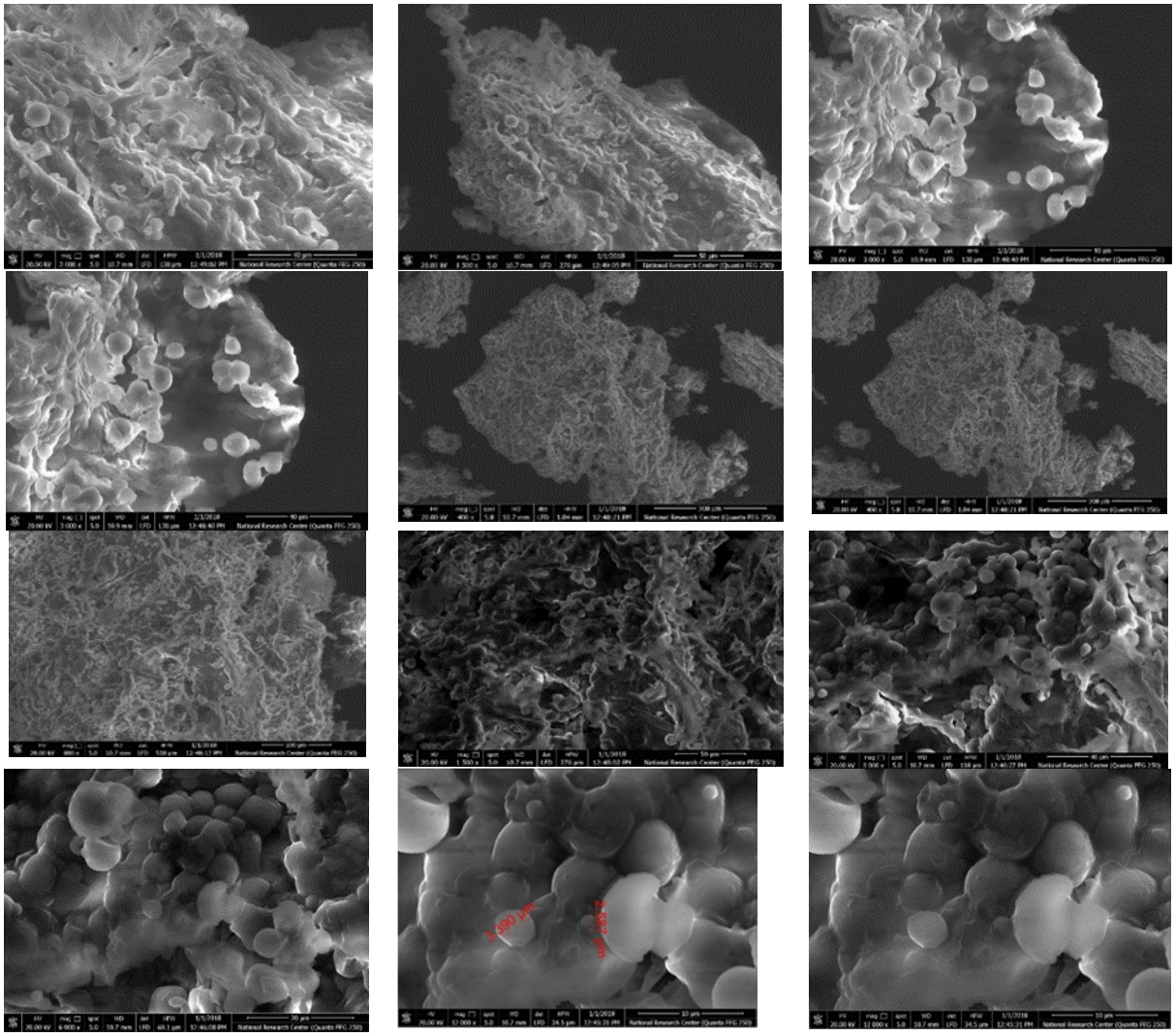

Photo 2: SEM of Copper nanoparticles from pomegranate pellet**.**

TEM

Results in Photo 3 (a) and 3 (b), Illustrate TEM image for the formation of Copper nanoparticles from Pomegranate pellet and supernatant, respectively TEM analysis revealed that the Copper nanoparticles from Pomegranate pellet were spherical. Although and on the other hand, the Copper nanoparticles from Pomegranate supernatant are predominantly spherical. The overall morphology of the copper nanoparticles produced by reduction of cu^{2+} ions with 2Mm CUSO4 is composed of almost uniform nanoparticles but their size is less than 4nm and the capping ability of pomegranate nanoparticles was observed. Similar phenomenon was reported by Caroling *et al.* (2015) and Jing *et al.* (2011).

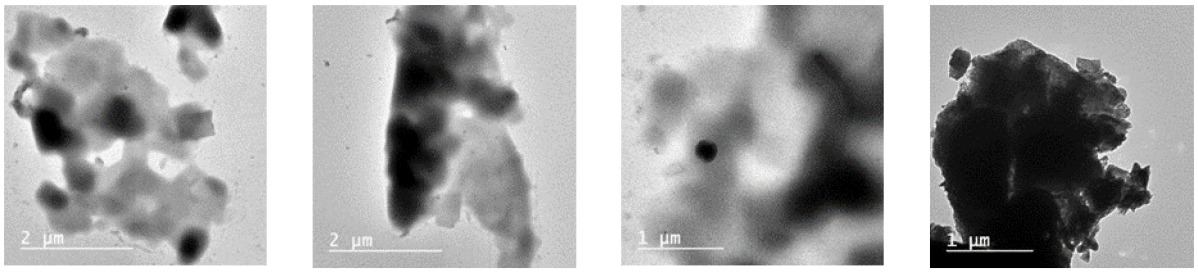

Photo 3 (a): TEM of Copper nanoparticles from Pomegranate pellet.

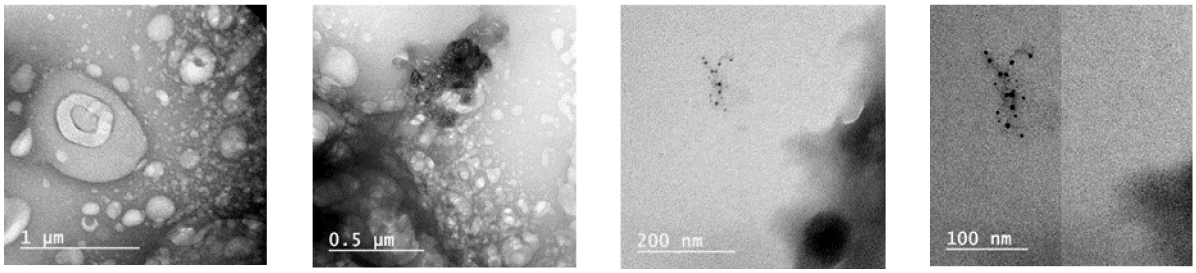

Photo 3 (b): TEM of Copper nanoparticles from Pomegranate supernatant.

Antimicrobial activity of Pomegranate lyophilized juice, Copper nanoparticles from Pomegranate pellet and supernatant

Result in figure 6, Illustrate Antibacterial effect of three samples of pomegranate lyophilized juice (sample 1, a), copper nanoparticles from pomegranate pellet (2, b) and supernatant (3, c) concentrations against S. aureus and B. cereus. Initial concentration solid 0.1g sample /9mL Phosphate Buffer Solution (PBS) and liquid 1mL sample /9mL (PBS). Then by sequencing 1=1%= 100µL from initial/9mL (PBS), 2=2%= 200µL from initial/9mL (PBS), 3=3%= 300µL from initial/9mL (PBS), 4=4%= 400µL from initial/9mL (PBS) and 10=10%= 1mL from initial/9mL (PBS) of three samples 1, 2 and 3 in a, b and c figures, respectively.

Result in figure 7: Illustrate Antibacterial effect of three samples of pomegranate lyophilized juice (sample 1, a), copper nanoparticles from pomegranate pellet (2, b) and supernatant (3, c) concentrations against *Yersenia enterocolitica and E. coli.* Initial concentration solid 0.1g sample /9mL Phosphate Buffer Solution (PBS) and liquid 1mL sample /9mL (PBS). Then by sequencing 1=1%= 100µL from initial/9mL (PBS), 2=2%= 200µL from initial/9mL (PBS), 3=3%= 300µL from initial/9mL (PBS), 4=4%= 400µL from initial/9mL (PBS) and 10=10%= 1mL from initial/9mL (PBS) of three samples 1, 2 and 3 in a, b and c figures, respectively.

Result in figure 8: Illustrate Antifungal effect of three samples of pomegranate-lyophilized juice (sample 1, a), copper nanoparticles from pomegranate pellet (sample 2, b) and supernatant (sample 3, c) concentrations against *Aspergillus flavus.* Initial concentration solid 0.1g sample /9mL Phosphate Buffer Solution (PBS) and liquid 1mL sample /9mL (PBS). Then by sequencing 1=1%= 100µL from initial/9mL (PBS), 2=2%= 200µL from initial/9mL (PBS), 3=3%= 300µL from initial/9mL (PBS), 4=4%= 400µL from initial/9mL (PBS) and 10=10%= 1mL from initial/9mL (PBS) of three samples 1, 2 and 3 in a, b and c figures, respectively.

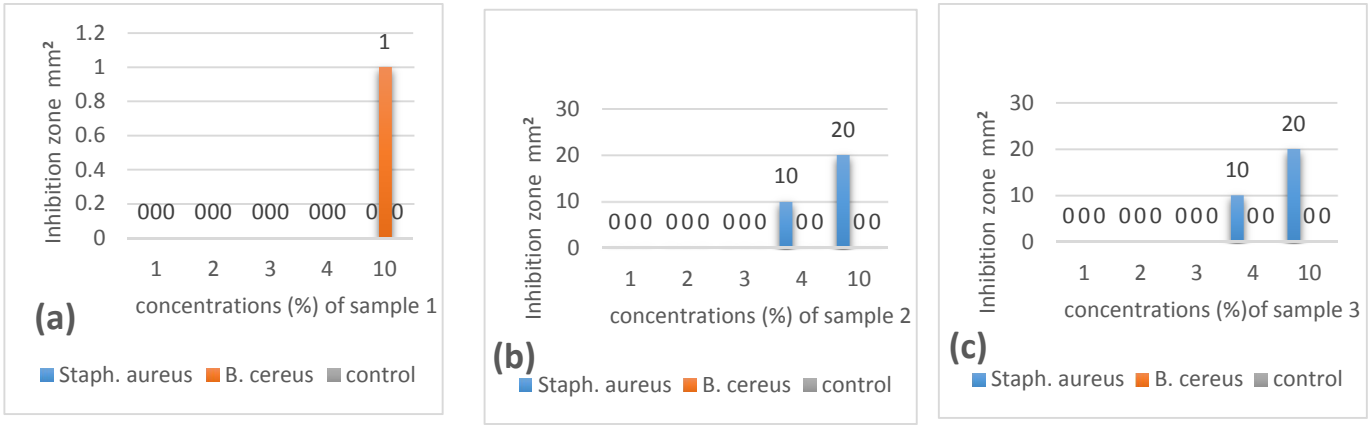

Fig. 6: Antibacterial effect of three samples of pomegranate-lyophilized juice (sample 1, a), copper nanoparticles from pomegranate pellet (sample 2, b) and supernatant (sample 3, c) in different concentrations against *S. aureus and B. cereus*.

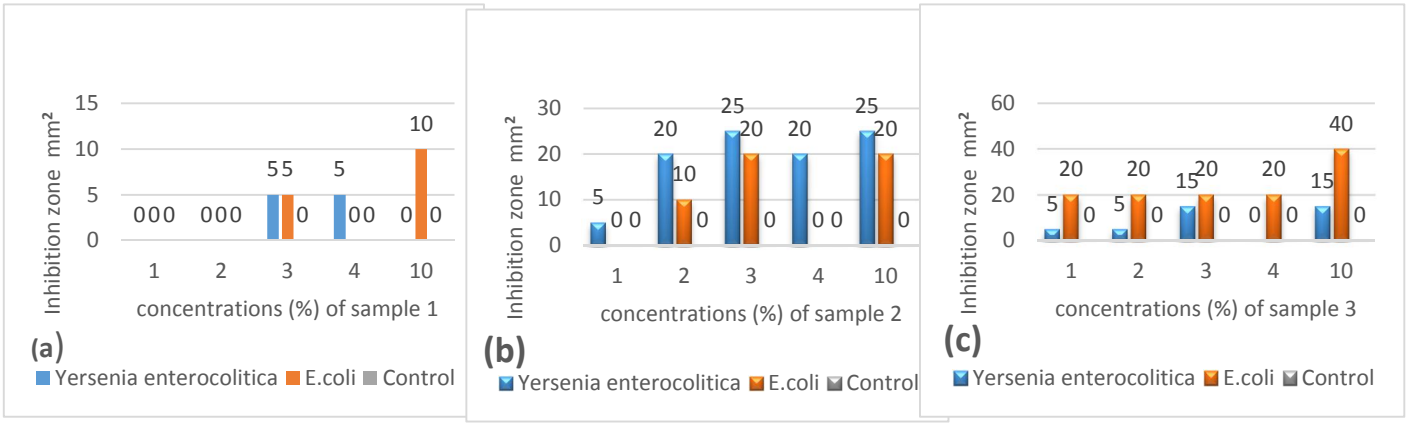

Fig. 7: Antibacterial effect of three samples of pomegranate-lyophilized juice (sample 1, a), copper nanoparticles from Pomegranate pellet (sample 2, b) and supernatant (sample 3, c) in different concentrations against *Yersenia enterocolitica* and *E. coli*.

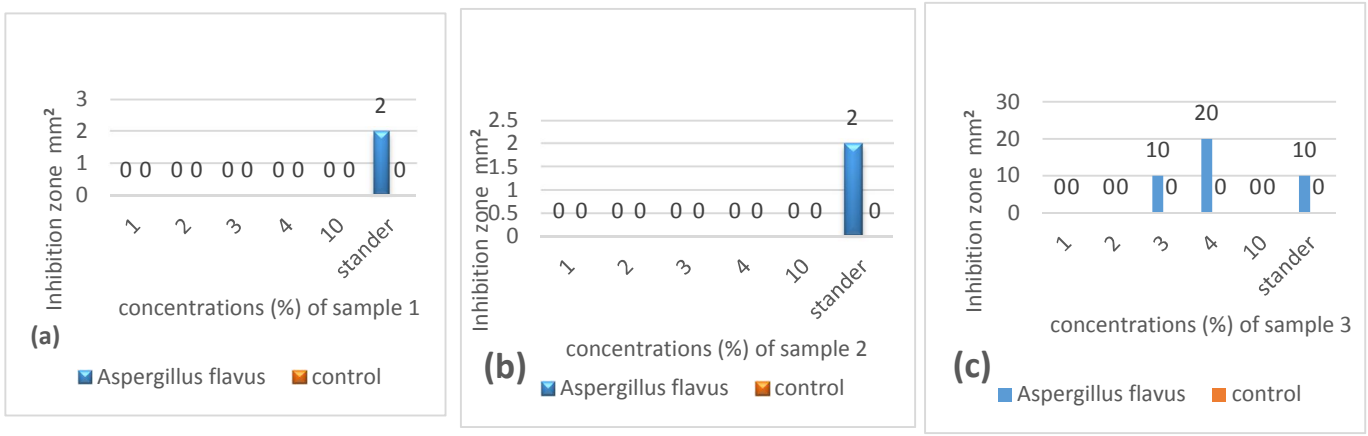

Fig. 8: Antifungal effect of three samples of pomegranate lyophilized juice (sample 1, a), copper nanoparticles from pomegranate pellet (sample 2, b) and supernatant (sample 3, c) in different concentrations against Aspergillus flavus.

Generally, the obtained result indicated that *S. aureus* and *E.coli are* more susceptible to Copper nanoparticles from Pomegranate pellet and Copper nanoparticles from Pomegranate supernatant than *B. cereus* and *Yersenia enterocolitica* to concentration 10%. While *Aspergillus flavus* was more resistant. Similar results were reported by Sankaranarayanan *et al.* (2016), Dibrov *et al. (*2002) and Bankalgi *et al.* (2016) who studied the effects of different investigated samples separately against bacteria which differ against fungi, also *Aspergillus flavus* compared to standard antibiotics and antifungal activity agent.

Anticancer effect of Pomegranate lyophilized juice, Copper nanoparticles from Pomegranate pellet and supernatant against cell line MCF.

Result in table 1 and Figure 9, Illustrate the cytotoxic effect of the tested samples against human breast adenocarcinoma cell line (MCF-7). The sample (1, a), illustrates cytotoxic effect of Pomegranate lyophilized juice concentration. The sample (2, b), illustrates cytotoxic effect of Copper nanoparticles from Pomegranate pellet concentrations. The sample (3, c), illustrates cytotoxic effect of Copper nanoparticles from Pomegranate supernatant concentrations.

Anticancer effect of Pomegranate lyophilized juice, Copper nanoparticles from Pomegranate pellet and supernatant against cell line HepG.

Result in Table2 and Figure 10, illustrates the cytotoxic effect of the tested samples against Human hepatocarcinoma cell line (HepG2). The sample (1, a), illustrates cytotoxic effect of Pomegranate lyophilized juice concentration. The sample (2, b), illustrates cytotoxic effect of Copper nanoparticles from Pomegranate pellet concentrations. The sample (3, c), illustrates cytotoxic effect of Copper nanoparticles from Pomegranate supernatant concentrations.

Anticancer effect of Pomegranate lyophilized juice, Copper nanoparticles from Pomegranate pellet and supernatant against cell line Caco-2.

Result in table 3 and Figure 11, illustrates the cytotoxic effect of the tested samples against human colorectal adenocarcinoma cells (Caco-2). The sample (1, a), illustrates cytotoxic effect of Pomegranate lyophilized juice concentration. The sample (2, b), illustrates cytotoxic effect of Copper nanoparticles from Pomegranate pellet concentrations. The sample (3, c), illustrates cytotoxic effect of Copper nanoparticles from Pomegranate supernatant concentrations.

Anticancer properties:

The experimental result of Pomegranate lyophilized juice showed the highest efficiency at IC50=81.6 µg/ml, which reduces the proliferation of colon cancer in vivo when experimented on human colorectal adenocarcinoma cells (Caco-2) cells. Similar phenomenon was reported by Herber, 2008 and Gonzalez-Sarrias *et al.,* 2009).

Table 1 sample 1	The cytotoxic effect of the Pomegranate lyophilized juice against (MCF-7).	
Conc. µg/ml	MCF/ sample 1	stdev
100	73.6	11.78553
50	76.4	2.036869
25	92.4	1.214316
12.5	94.3	1.017979
IC50	**185.3**	

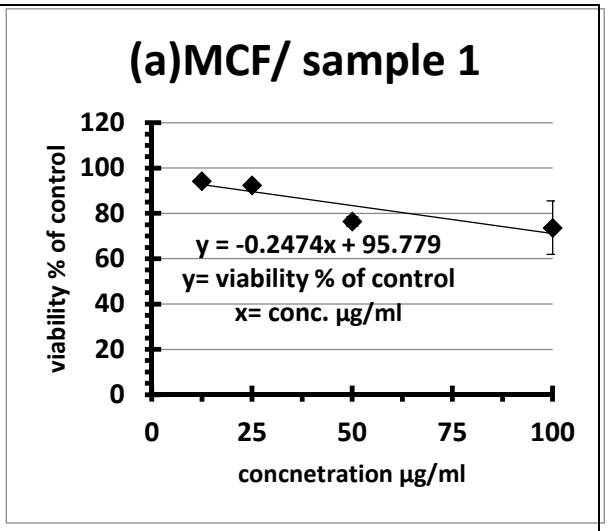

Table sample**2**	The cytotoxic effect of the Copper nanoparticles pellet against (MCF-7).	
Conc. µg/ml	MCF/ sample 2	stdev
100	70.3	2.011178
50	97.5	6.042146
25	103.3	1.554988
12.5	106.2	1.876466
IC50	**153.2**	

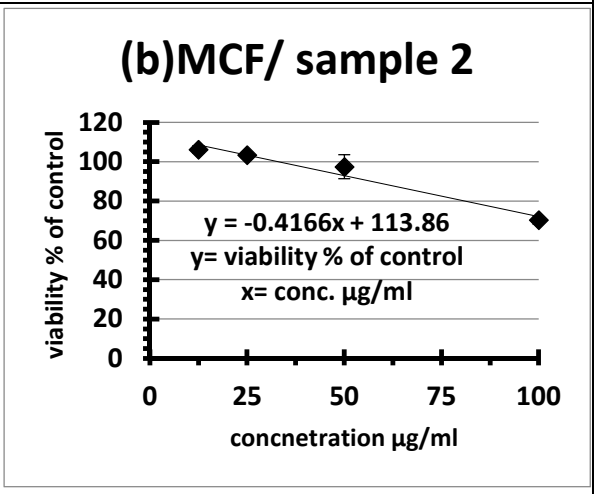

Table sample3	The cytotoxic effect of the Copper nanoparticles supernatant against (MCF-7).	
Conc. µg/ml	MCF/ sample 3	stdev
100	80.8	8.654803
50	104.1	0.581376
25	106.8	4.453976
12.5	110.0	4.338652
IC50	**198**	

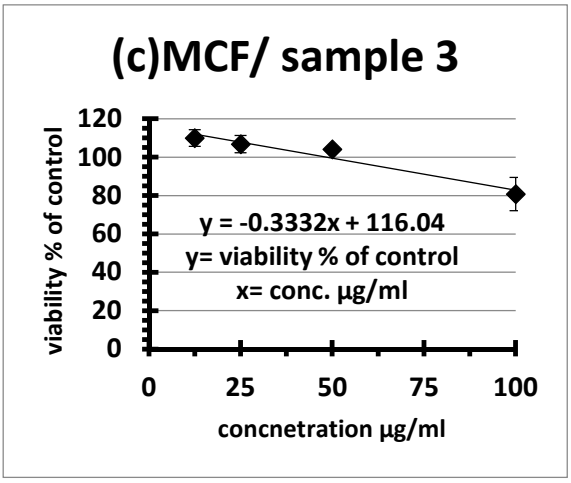

Table 1: Anticancer effect of three samples of Pomegranate lyophilized juice (sample 1), Copper nanoparticles from Pomegranate pellet (sample 2) and supernatant (sample 3) against cell line MCF.

Fig. 9: Anticancer effect of three samples of Pomegranate lyophilized juice (sample 1, a), Copper nanoparticles from Pomegranate pellet (sample 2, b) and supernatant (sample 3, c) against cell line MCF.

Table 2 sample1	The cytotoxic effect of the lyophilized juice concentration against (HepG2)	
Conc. µg/ml	Hep G2/ sample 1	stdev
100	90.68	5.696806
50	96.56	0.941356
25	100.44	0.853968
12.5	103.10	2.595351
IC50	**391**	

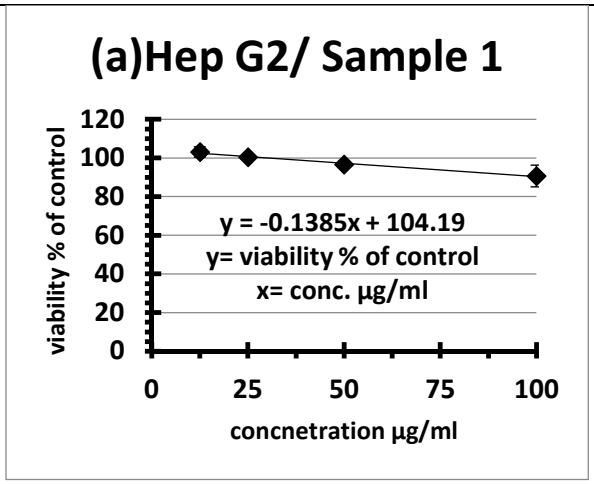

Table sample2	The cytotoxic effect of the Copper nanoparticles from pellet against (HepG2)	
Conc. µg/ml	Hep G2/ sample 2	stdev
100	80.01	6.113907
50	96.22	2.022759
25	100.15	0.475943
12.5	100.80	1.763862
IC50	**227**	

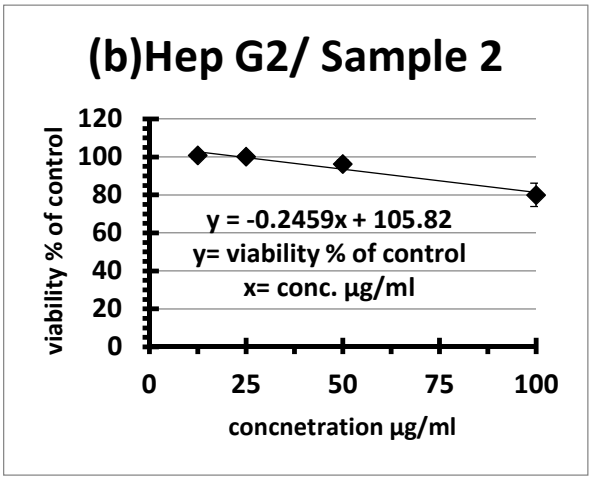

Table sample3	The cytotoxic effect of the Copper nanoparticles supernatant against (HepG2).	
Conc. µg/ml	Hep G2/ sample 3	stdev
100	92.81	12.48332
50	102.44	4.433865
25	103.28	7.711217
12.5	103.44	4.548697
IC50	**449**	

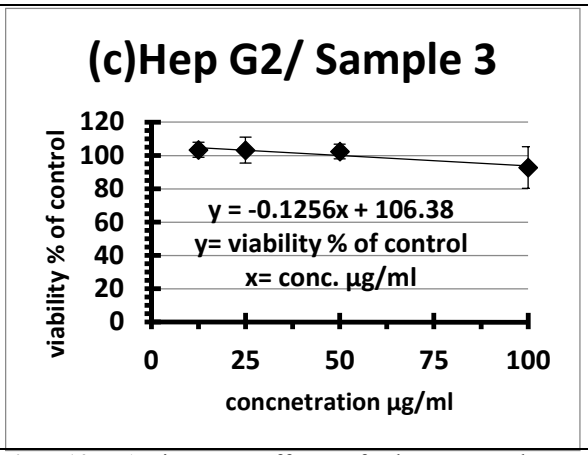

Table 2: Anticancer effect of three samples of Pomegranate lyophilized juice (sample 1), Copper nanoparticles from Pomegranate pellet (sample 2) and supernatant (sample 3) against cell line HepG2.

Fig. 10: Anticancer effect of three samples of Pomegranate lyophilized juice (sample 1, a), Copper nanoparticles from Pomegranate pellet (sample 2, b) and supernatant (sample 3, c) against cell line HepG2.

Table 3 sample1	The cytotoxic effect of the lyophilized juice against (Caco-2).	
Conc. µg/ml	Caco/Sample 1	stdev
100	44.37	5.682208
50	55.25	4.21576
25	84.08	6.902896
12.5	77.80	5.327827
IC50	**81.6**	

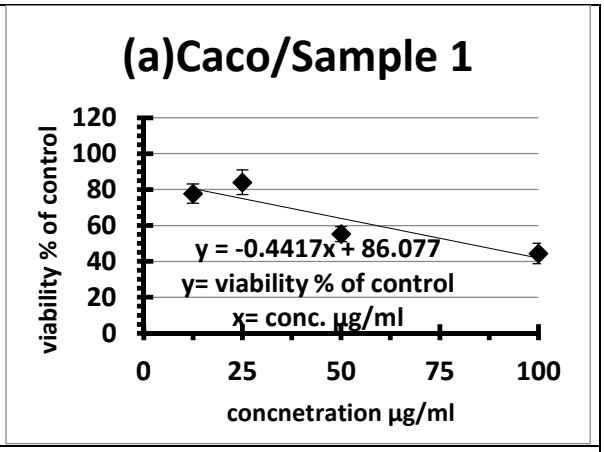

Table sample2	The cytotoxic effect of the Copper nanoparticles pellet against (Caco-2).	
Conc. µg/ml	Caco/Sample 2	stdev
100	92.14	1.927237
50	96.77	10.50348
25	106.36	9.085588
12.5	115.77	6.787317
IC50	**260.6**	

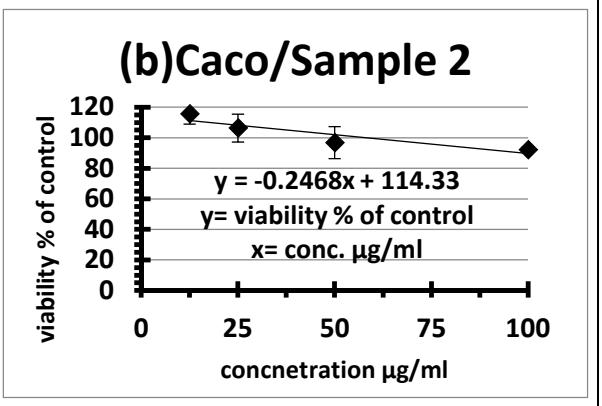

Table sample3	The cytotoxic effect of the Copper nanoparticles supernatant against (Caco-2).	
Conc. µg/ml	Caco/Sample 3	stdev
100	97.26	7.151982
50	109.85	2.487359
25	99.22	12.12892
12.5	113.60	5.643694
IC50	**483.9**	

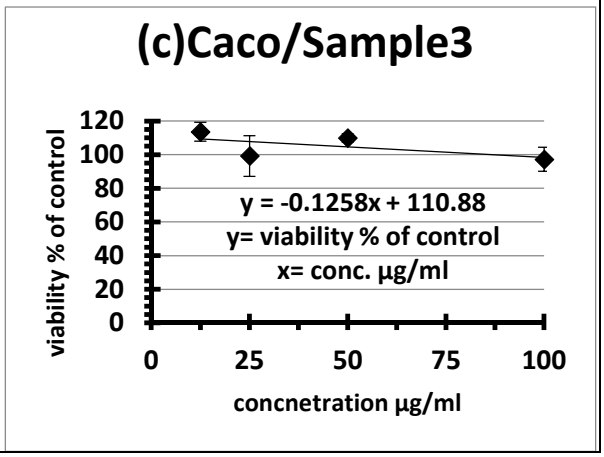

Table 3: Anticancer effect of three samples of Pomegranate lyophilized juice (sample 1), Copper nanoparticles from Pomegranate pellet (sample 2) and supernatant (sample 3) against cell line Caco-2.

Fig. 9: Anticancer effect of three samples of Pomegranate lyophilized juice (sample 1, a), Copper nanoparticles from Pomegranate pellet (sample 2, b) and supernatant (sample 3, c) against cell line Caco-2.

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