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Biosynthesis of silver nanoparticles using *Hibiscus sabdariffa* and their biological application

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Abstract
Silver has a good history of use in medicine and people healthcare. The biosynthesis of silver nanoparticles (AgNPs) was prepared by the ethanolic extract of Hibiscus sabdariffa**.** The UV, FTIR, and SEM were used for the detection of AgNPs. We have compared the antibacterial activity between the ethanolic extract and nano extract. The antibacterial activity was high in nano extract. DPPH assay was estimated the antioxidant action of nano extract and the effects was showed 82.22 % in 75µg/ml concentration. MTT assay was used for detecting the cytotoxicity of nano extract against Colon cancer, the outcomes showed the moral level of cytotoxic action with increasing concentration.

Keywords: Hibiscus sabdariffa, Silver nanoparticles, Antibacterial activity

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INTRODUCTION

Hibiscus sabdariffa was used as ornamental plants, it has three hundred species in tropical and subtropical areas. *Hibiscus sabdariffa* has medicinal properties (Qi, et al. 2005). *Hibiscus sabdariffa* is usually called roselle or red sorrel (Duke, 1983). Even still pervious soil is the greatest, *Hibiscus* can acclimate to a diversity of soil in a more humid climate and warmer. (Duke, 1983, Robert, 2005) roselle is a medical plant fellow to the family of Malvaceae (Mohagheghi, et al. 2011). *Hibiscus* can be found in all hot countries Malaysia, Saudi Arabia, India, Philippines, Vietnam, Thailand, Indonesia, Sudan, Mexico, and Egypt. (Rao, 1996., Chewonarin, Kinouch,i Kataoka 1999). Mexico, Sudan, Egypt, China, and Thailand are the main producers of Roselle blossoms. Other hibiscus kinds are planted aimed at the fibers they harvest. (Naturland, 2004)

Roselle calyx is largely used that is of many forms: dark red green and red. The calyxes are described by their combination anthocyanin Cyanidin3-Sambubioside3-Sambubioside and Delphinidin are the main anthocyanins. Roselle has contained minerals, organic acids, amino acids, carotene, sugar, and vitamin C, in its leaves seeds, and calyx in different stages determined by the geographical area and variety. (Singh, Khan, & Hailemariam, 2017). Roselle is contained several compounds that have been characterized and isolated from containing anthocyanidins, triterpenoids, flavonoids, alkaloids, and steroids (Mat Isa, et al. 1985).

Roselle the nontoxic medical plant having essential complexes called phytochemicals is healthy known for weakness and its medical things and food. (Abu-et al. 1997). The plant has applications in different medicinal problems such as cardiovascular problems, inflammatory diseases, and cancer has been good investigated by many researchers in many sites. (Singh, Khan, & Hailemariam, 2017; Yerkinbayeva, et al, 2015).

Now, many parts of plant-like bark, fruit, seed, leaf, and stem extracts have been prepared for the creation of nanoparticles (Bar, et al. 2009). Silver nanoparticles have been prepared by plant extract to their potent antifungal, antimicrobial, and anticancer activity(Kokura, et al. 2010). This investigation was aimed to prepared *Hibiscus* extract then create silver nanoparticles by the extract and examine the nano extract with anticancer, antioxidant, and antibacterial activities.

MATERIAL AND METHOD

Hibiscus sabdriffol

The *Hibiscus* calyxes were composed from the popular market of Baghdad, after that calyxes were washed with distilled water, and dried with air drying.

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***Hibiscus sabdriffol* extract:** Soxhlet apparatus method was used for extraction. Fifty gms of *Hibiscus* calyxes were extracted with ethanol 250 ml (70%) for seven-hour. The extract was dried and kept.

Strains of bacteria

The laboratory of microbiology was supplied the work with strains of bacteria these: *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aerugenosa*.

Silver Nanoparticles

Ten milliliters of extract were mixed by 90 mL of 0.01 mmol/mL aqueous silver nitrate, After that the mixture presentation to the light of the sun for 1 h. The color was changed from yellow to brown.(Bar, et al. 2009).

Characterization of nanoparticles

UV, FTIR, and SEM were used to check the production of nanoparticles. UV was checked with the TechcompUV2300 spectrophotometer. The organic molecules were appeared by the FTIR examination. The shape and size of the nanoparticles were shown by SEM.

Maintenance of cell cultures

The Iraq biotech Cell Bank Unit was obtained to get a colon cancer cell line. The cell line was kept in 1640-RPMI improved by penicillin 100 units/mL, streptomycin 100 µg/mL, and Fetal bovine 10%. EDTA-Trypsin was used for passage the cells and reseeded at 50% confluence double in a week, then incubated at 37 °C.(Mueller, Kassack, & Wiese, 2004)

MTT assay

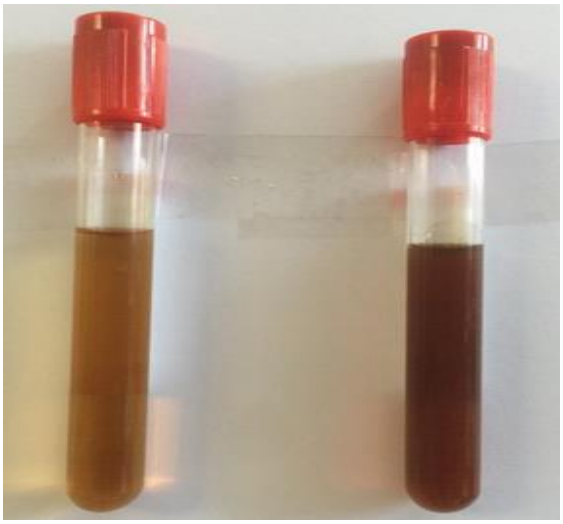
The cytotoxic effect was examined by MTT assay. 96-well plate was used to conduct the cell viability. The colon cell line was seeded at 1 × 10^4cells/well. The joining monolayer was completed after 24 hrs. Then, Tested material was added to the cells. After 72 hrs, the cell activity was examined by taking out the medium, addition 28 µL of 2 mg/mL solution of MTT Then, the cells were incubated for 60 min. at 37 °C. The MTT solution was removed, The crystals residual in the well was solubilized with DMSO 130 µL of with shaking and incubation at 37 °C to 15 min (Hayon, et al. 2003). The microplate reader at 492 nm was used to examine the absorbency. The test was done in triplicate. The next equation was used to calculate the ratio of cytotoxicity:-

$$\text{The cytotoxicity rate} = \text{A-B/A} * 100$$

A :optical density of control B :optical density of test,

Antioxidant activity

DPPH (1,1-Diphenyl-2-picryl-hydrazyl) 2.3mg was thawed with 3.3 ml of ethanol in the test tube. The test tube was covered with a foil of aluminum to keep from light. Ascorbic acid was used (20µg/ml) as control positive. (Keser, et al. 2012)



A B

Fig. 1. Creation of AgNPs by *Hibiscus sabdriffol* A-extract with $AgNo_3$ B- the color was altered by sunlight

The altered concentration of *Hibiscus sabdriffol* (75,50,25,µg/ml) were tested to determine the antioxidant activity. Ethanol (490µl) was added to Every concentration (10µl), then 500µl of DPPH solution was added to complete the amount to 1 ml. after that incubation 15 min in the room. The absorbency at 517 nm was determined by the following formula. (Keser, et al. 2012)

$$\text{Antioxidant activity \%} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control OD control}} \times 100$$

OD: optical density

***Hibiscus sabdriffol* activity against bacteria**

The *Hibiscus sabdriffol* activity against bacteria was examined with *E.coli, P. aeruginosa,* and *Staph. aureus.* The bacterial cultures were grown overnight taking CFU 10^5ml. The disc diffusion method was determined to test the antibacterial activity. The test was examined by using Muller Hinton agar plates. Sterile Hi-media cotton swab was used to extend the inoculum on agar plates. The plates were incubated at $37c^0$overnight and the inhibition zone diameter (mm) was determined after 24 hrs. All samples were tested in triplicate. Controls included solvent without plant extract.(Pepeljnjak, KosalecKalođera, & BLAŽEVIĆ, N. 2005)

RESULT AND DISCUSSION

UV analysis

The UV-visible spectroscopy was used to checking the formation of nanoparticles. The ethanolic extract of *Hibiscus sabdriffol* was yellow color next, silver nitrate solution was added and presentation to the light of sun became brown (**Fig. 1**). The surface of the silver plasmon resonance band at 410 nm (**Fig. 2**).

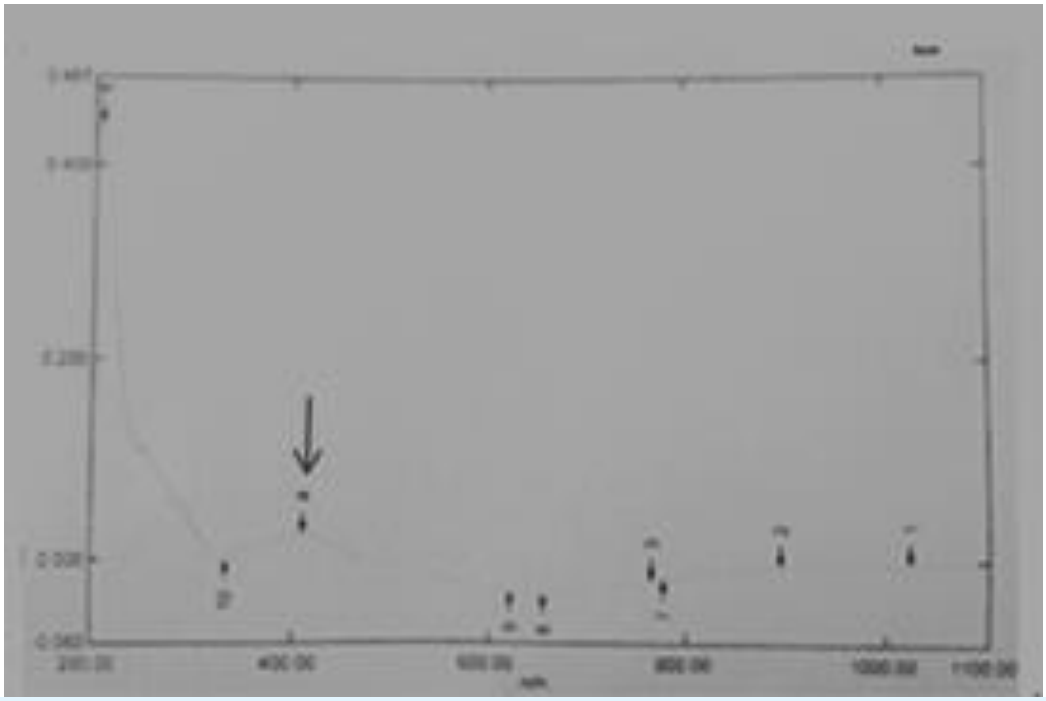


Fig. 2. UV-Vis absorption spectra of silver nanoparticle suspension synthesize by Hibiscus sabdriffo

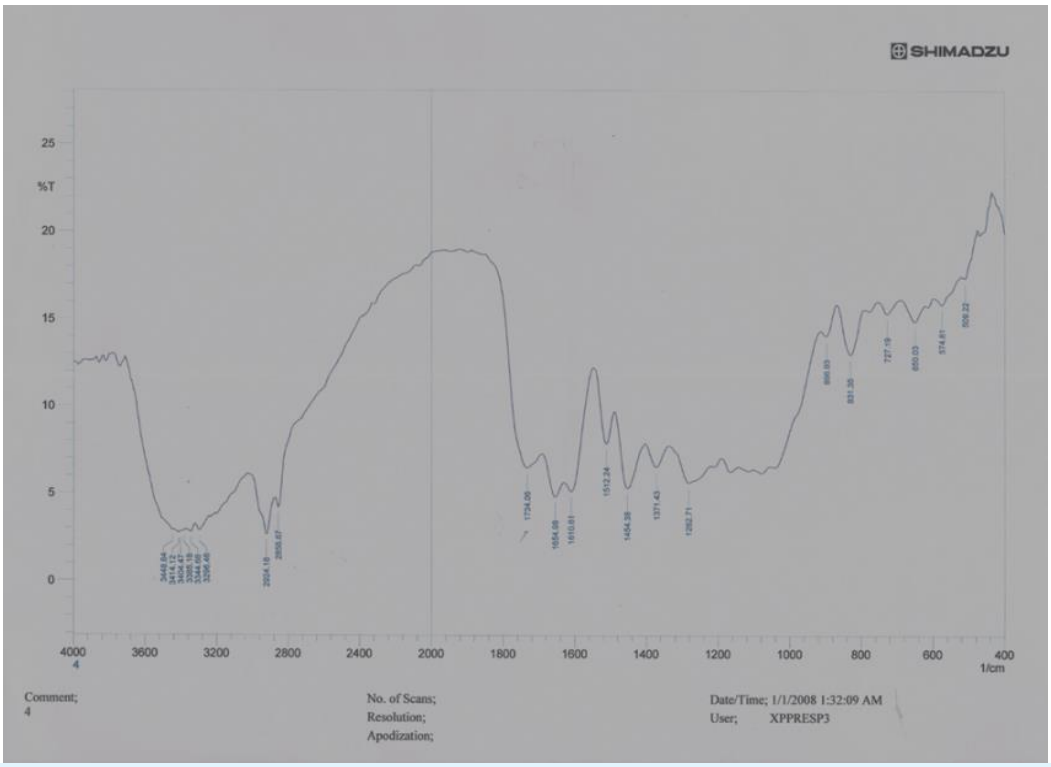


Fig. 3. FTIR for ethanol extract

FTIR: fourier transform infrared spectroscopy

Advantageous groups of the extract were determined by FTIR. The ethanolic extract showed the bands 3448.84 cm-1 due to phenolic, 2924.18 cm-1 due to C-H, 1734.06 cm-1 due to C=O 1654.98, 1610.61 due to C=C,1516.10, 1454.38,1342.50 cm-1 due to C-H,1147.68,1033.88, 1232.55 due to C-O **Fig. 3**, whereas the same result was presented with nano extract but absence the bands at 1734.06,1654.98,1512.24 due to the silver ions were redacted **Fig. 4** (Berthomieu, & Hienerwadel, 2009, Cisse, et al. 2009).

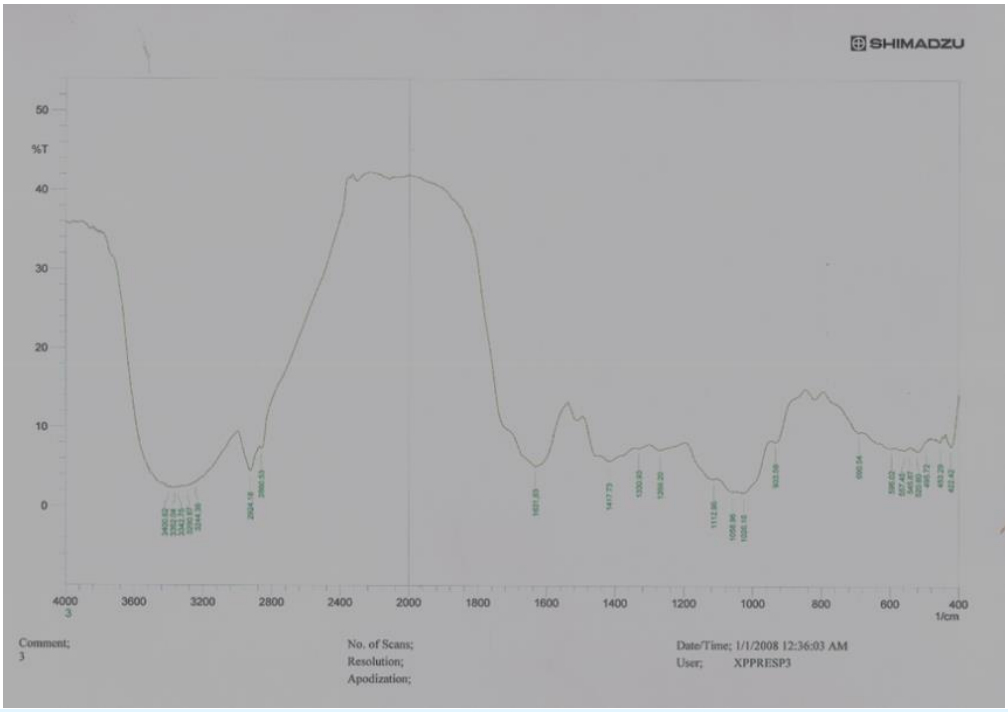


Fig. 4. FTIR for nano extract

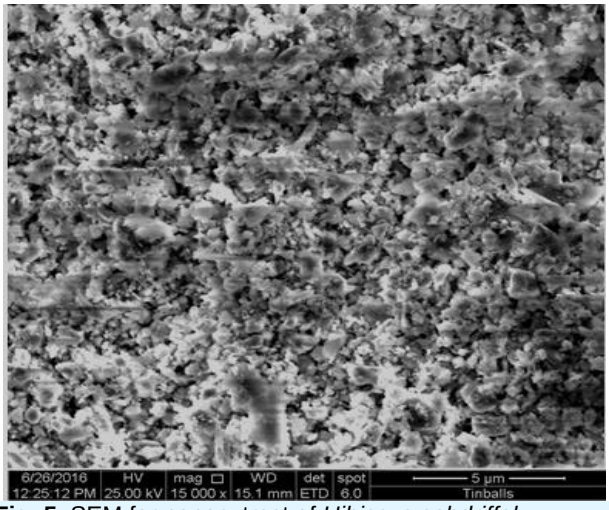


Fig. 5. SEM for nanoextract of *Hibiscus sabdriffol*

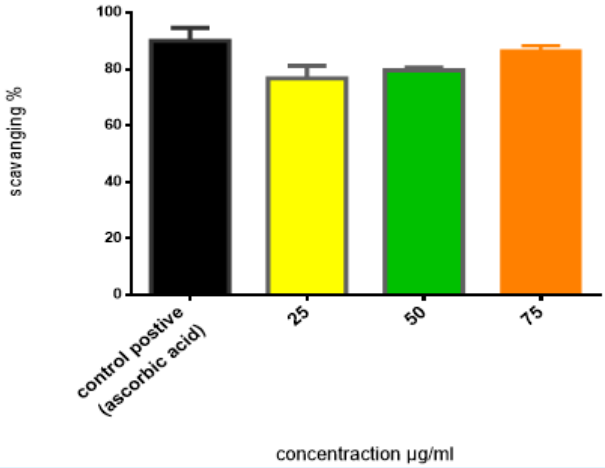


Fig. 6. DPPH scavenging activity of Hibiscus sabdriffol

SEM: scanning electron microscopy.

The SEM explanation the shape of nanoparticles, **Fig. 5** displays sphere-shaped nanoparticles.

The activity of *Hibiscus sabdriffol* extract against bacteria

Table 1 shows the nano extract of *Hibiscus sabdriffol* was presented top effect with *P. aeruginosa* by inhibition zone (24mm), *S.aureus* (28mm) and *E.coli* (23mm), While, This result be different with ethanolic extract, the inhibition zone reached to (15mm) with *P. aeruginosa*, *S.aureus* (17 mm) and *E.coli* (14mm) **Fig. 7**.

The activity of *H.sabdariffa* extract against bacteria was credited from phytochemical materials like Alkaloids, Flavonoids, phenolics, and tannins which affect bacteria(Nkumah, 2015, Zahraa, et al. 2020). protocatechuic acid (PCA) and *Hibiscus* anthocyanins (HAs) were found in *H.sabdariffa* have properties of therapeutic against cancer diseases and bacteria, *H.sabdariffa* (PCA) prevented the development of

Table 1. Antibacterial activity of *Hibiscus sabdriffol* extract Concentration mg mL-1

Microorganism	ethanoic extract				Silvernano extract			
	C	1	2	3	C	1	2	3
P. aeruginosa	-	10mm	13mm	15mm	-	19mm	20mm	24mm
S. aureus	-	13mm	14mm	17mm	-	19mm	20mm	28mm
E.coli	-	10mm	12mm	14mm	-	15mm	20mm	23mm

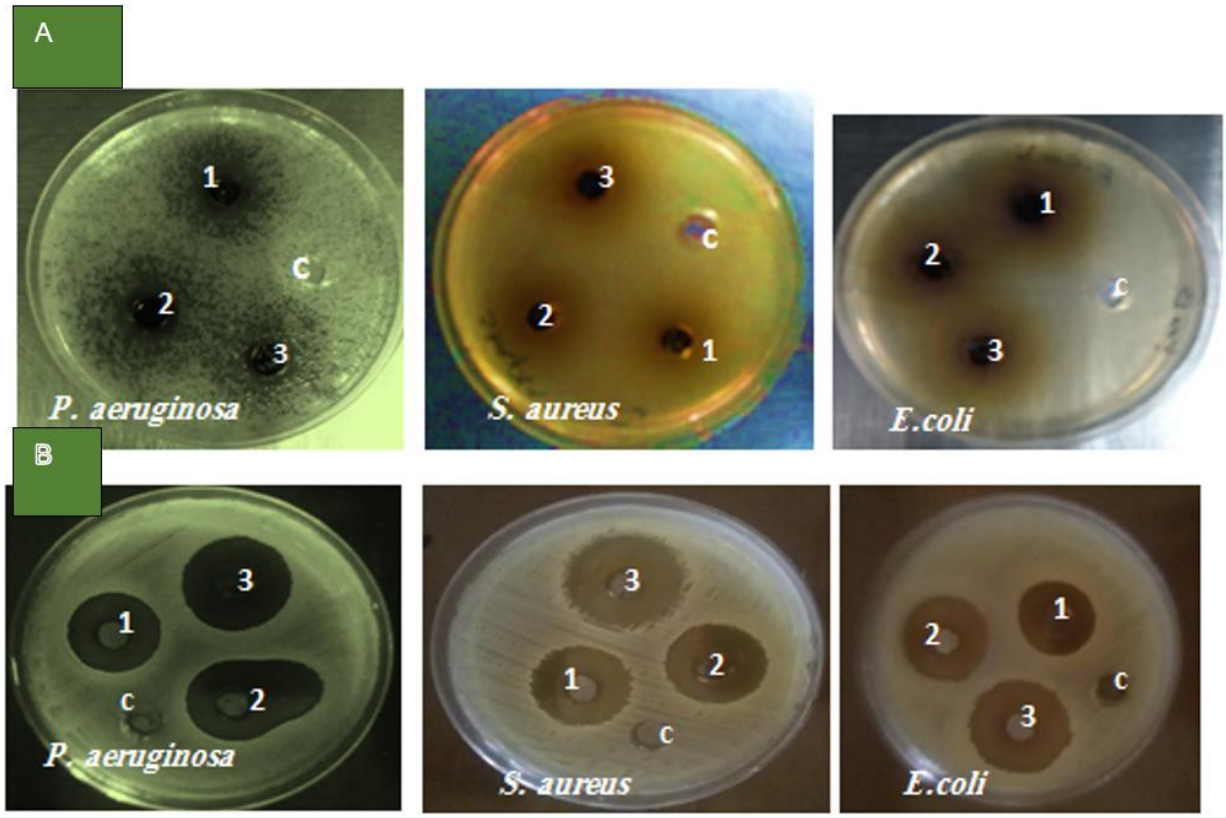


Fig. 7. Inhibition zone effects of *Hibiscus* A- ethanolic extract and B- anoextract.20,40 and 80mg per ml, concentration respectively.C-control

methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Klebsiella pneumonia,* and *Acinetobacter baumannii*. PCA has greater antibacterial action against microbes (Fullerton, et al. 2011, Liu, Tsao, & Yin, 2005.) The table (Qi, et al. 2005) was exhibited differently in antibacterial action related to the cell wall structure of gram negative and gram positive bacteria. The nanosilver mechanism was indistinguishable, but There is numerous readings commend that, the phosphorus and sulfur were affected by Ag+, thus in DNA Ag^+ will relate with phosphorus moieties and causes mutation of DNA, The sulfur-proteins were found in the cell membrane of bacteria and react with Ag+ that's lead to the destruction of bacteria. (Sahayaraj, & Rajesh, 2011, Holt, & Bard, 2005).

Hibiscus nano extract and Antioxidant activity

The DPPH was exhibited relational to the rise of concentration. The free radicals were given 82.22, 79.56, 77.94 by the concentration of 75,50 and 25 µg/ml one-to-one liken with the ascorbic acid (positive control) figure (Chewonarin, Kinouch,i Kataoka 1999). The *Hibiscus sabdriffol* extract was content high rate of specific phytochemical components as presented by FTIR, for example, tannin, flavonoids, phenols, and anthocyanins were aimed at antioxidant compounds (Cisse, et al. 2009). AgNPs are acting as donors of electron re-joining through free radicals to alteration them to products more stable which can conclude radical chain reaction. Also, AgNPs related fine with the activity of radical scavenging (Bar, et al. 2009, Aqil, Ahmad, & Mehmood, 2006).

***Hibiscus* nano extract and anticancer activity:**

Figs. 8 and **9** was exhibited the role of *H.sabdariffa* nano extract against colon cancer. The cancer cell apoptosis was stimulated by the *Hibiscus* anthocyanins (HAs), mainly in leukemia and gastric cancer, while *Hibiscus* protocatechuic acid (PCA) was established to destroy the cancer action of many materials in the rat tissues, Although the mechanism is not understood, it has been theorized that the high levels of ROS reactive of oxygen species will stimulate the mechanisms of cells stress and the production of ROS was sensitized cancer cell to apoptosis(Ovadje, et al. 2016). The mechanism of PCA anticancer actions was developed because of the ability to stimulate anticancer activities by GI arrest, DNA fragmentation and apoptosis (Philion, et al. 2017).

CONCLUSION

Hibiscus sabdriffol extract can be used in a simple way to create AgNps. The FTIR, UV, and SEM were exhibited the formation of AgNps. *Hibiscus sabdriffol* nanoextract displayed a high level in antibacterial, antioxidant and anticancer action, There for *Hibiscus sabdriffol* nanoextract can be used successfully in the application of biology.

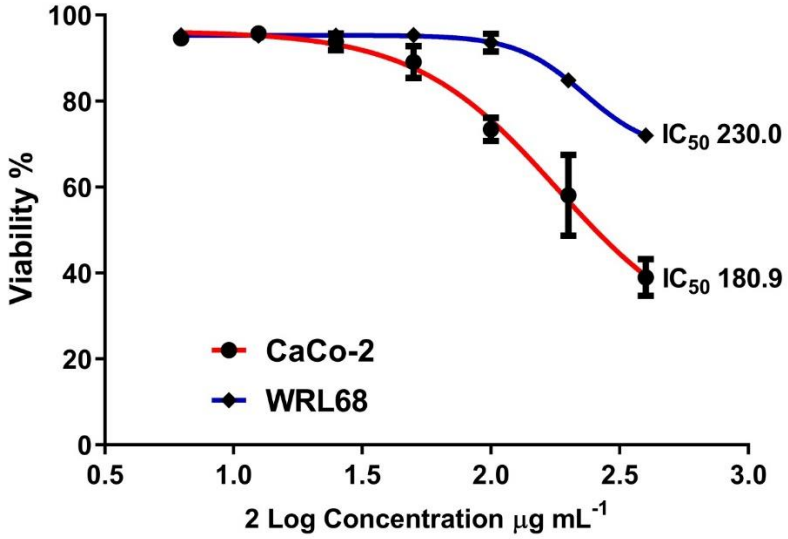


Fig. 8. The role of *Hibiscus sabdriffol* against colon cancer

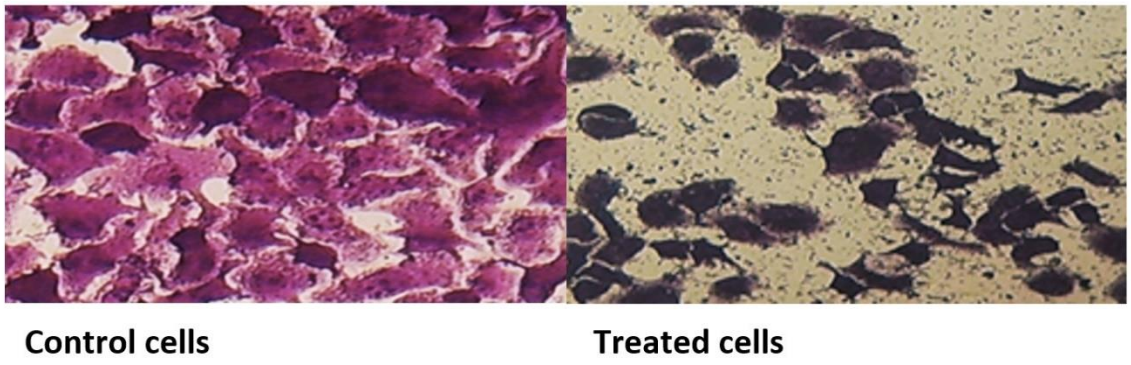


Fig. 9. The shape of cells after treatment with Hibiscus sabdriffol

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