Preparation and Characterization of Silver Nanoparticle by *Cordia myxa* Extract and their Study Anticancer, Antioxidant, Antibacterial Activity

Butheina A. Hasoon[1], Afnan I. Abdulwahab[2], Raghad Khwater Maeh[1], Khalida F. Al-azawi[2]

[1] Scholar Researcher, Biotechnology Division, [2]Scholar Researcher, Applied Chemistry Division, Applied Science Department, University of Technology, Baghdad, Iraq

Abstract

The ethanolic extract leaves of the *Cordia myxa* plant were used to prepare the biosynthesis of silver nanoparticles (Ag-NPs). The detection of Ag-NPs was done using UV, FTIR, and SEM. We evaluated the anti-bacterial property of ethanolic and nano extract against the growth of two types of pathogenic microbes: *Gram –ve* bacteria (*E. coli*) and *Gram +ve* bacteria (*S. aureus*). The antibacterial activity of the nano extract was high. In vitro, extracts demonstrated strong antioxidant activity against DPPH at a concentration of 0.8mg/ml. The MTT test was utilized to detect the cytotoxicity of nano extract against lung tumor, and the results indicated that nano extract treatment significantly inhibited the growth of cells (A594 cell line) in a concentration dependent manner. The results indicate, nano extract is a particularly valuable source of antiproliferative and cytotoxic agents.

Keywords: *Ethanolic- extract, Nano- extract, Cordia myxa leves, antibacterial, antioxidant, anticancer activity.*

Introduction

Nanotechnology has gotten a lot of widespread attention in recent years because of its many beneficial properties, such aseptically, electrical, electronic, chemical stability, and catalytic activity [1,2]. Nanoparticles' unique properties are commonly used in biomedical devices, cosmetics, medicine, and environmental applications [2,3]. The benefits and functions of nano-particles have being determined by their shape and size. As a result, precise control on the size and shape of the nano-particles is needed for improved antibacterial and catalytic action, which can be accomplished using various production methods, stabilizers, and reducing agents [3,4,5]. Though there are many physical and chemical methods to synthesis, chemicals are more costly and pose a threat to the environment. As a result, a healthier option is needed, that can be achieved through green -synthesis. Green -synthesis is environmentally friendly, expense-effective, and allows for single-step nanoparticle production [6]. Silver ions are reduced and stabilized by a mixture of tannins, terpenoids, phenolics, amino acids and proteins, contained in potted plant extracts that are widely available in the world. However, Biosynthesized nanoparticles are now thought to be more beneficial. Due to their biocompatibility, they are often cost-effective. According to a report Kumar and colleagues [7]. The medicinal properties of Cordia myxa plant are well known. The plant's leaves and bark have long been used to treat a variety of ailments, including dyspepsia, diarrhoea, fever, gonorrhoea, leprosy, and burning sensations. Leaves are often utilized as an astringent, demulcent, diuretic, and ulcer for cough in helminthic diseases [6]. The antibacterial activity of biosynthesized Ag-NPs, as well as photocatalytic detoxification of

Corresponding author:
Butheina A. Hasoon
buthenia.h.hasoon@uotechnology.edu.iq

colors like methylene blue (MB) and Congo red, are being investigated further (CR). The current study directs on the bio-synthesis of Ag-NPs from ethanolic extract of *Cordia myxa* leaves plant and their studies biomedical applications.

Materials and Methods

Extract Preparation

The extraction was done by soxhelet, and solvent 75% ethanol, then the solvent was totally removed by using a rotary evaporator to get a semi-solid form, then transferred to oven for obtain the crud extract, and stored at 40℃ until used[8].

Eco-friendly synthesis of Ag-NPs

Ten ml of leaf extract was mixed to 90 mL of 1 mM silver nitrate (AgNO3) solution, which was held at room temperature. The synthesized Ag-NPs were detected by altering the color to dark-brown [9].

Characterization of synthesized NPs

UV-Visible Spectrophotometer, FTIR&SEM

UV-Vis absorption spectra was employed for the optical test of Ag NPs and collected at various preparation conditions within the spectral range (350-650 nm) for NPs, solution were measured by UV-Vis double beam ectrophotometersSP-3000 plus (CE OPTMA TOKYO). All spectra were measured at room-temperature. The shape and size of Ag- nanoparticles was passed out using FTIR, scanning electron microscopic (SEM) wear applied in Department of Applied Science, University of technology, Iraq.

Phytochemical Screening of extract:

The extract was exposed to initial phytochemical screening for the determination of several phytoconstituents according to [10].

Antioxidant activity: DPPH assay:

Antioxidant activity of *Cordia myxa* extract & nono- extract wear calculated using DPPH assay with minor adjustments according to [8,3]. Extract was applied to investigate the scavenging activity. Every sample wear mix up with DPPH solution and then completes the quantity to one particular mL using ethanol. Absorbance was calculated at 517 nm. Scavenging activity computed corresponding to the equation formula:

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

OD= optical density

Preparation of bacterial isolates:

Microbial isolates were taken from isolated specimens. The microbes were seeds overnight at 37 ℃ on NB to preparation the suspensions cell. The microbial suspensions cell was homogenized and attuned to 0.5 McFarland standards (5×10^5 CFU/mL) by spectrophotometry.

Antimicrobial assays:

Isolates were obtained from the Biotechnology Branch/Applied Science Department at the University of Technology. Agar diffusion method was used to detect antimicrobial activity of plant. After the microbes were diffused with a spreader on MH agar, plant extracts were melted in 10% DMSO, and wells that were 6mm in diameter were filled with concentrations of (20,40,60,80,

and 100) mg mL−1, with 10% DMSO as a negative control. After incubation in 37ºC for 24h, the inhibition zone was measured around each well and compared with the control and conducted in in triplicate [4,11,12].

Detection of the Toxicity: Maintenance of Cell Cultures

Cell line of Lung cancer A549 cell line, from the unit of Cell Bank of Iraq biotech ,Saved RPMI-1640 Completed by (100) units/mL of both penicillin, streptomycin antibiotic's and (10%) from fetal bovine . Cells were passaged by Trypsin-EDT Are-seeded at (50%) confluence for 14 days week at 37 °C [8].

Cytotoxicity Assays

The MTT test was used to assess cytotoxic effects. Cell lines were cultured in a well of (1 104 cells). The monolayer was reached after 24 hours, and the cells were treated with the tested drug. After 72 h, the viability of the cells was determined by extracting the medium and incubating the cells for 1.5 h. at 37 °C with 28 µL of 2 mg/mL MTT solution. After that the MTT stain was eliminated and solubilized the rock crystal that staying in the wells by the add-on (130 µl) of DMSO followed by keep at 37 °C for 15 min [8 and 15]. The absorbency was determined on a micro plate reader at 492 nm. The percentage was determination as the following calculation:-

Inhibition rate = (A-B/A)*100

A =optical density of control, B =optical density of test.

Results and Discussion

The UV-visible spectroscopy of Cordia myxa was used to checking the formation of nanoparticles. The surface of the silver plasmon resonance band at 420 nm (Fig. 1).

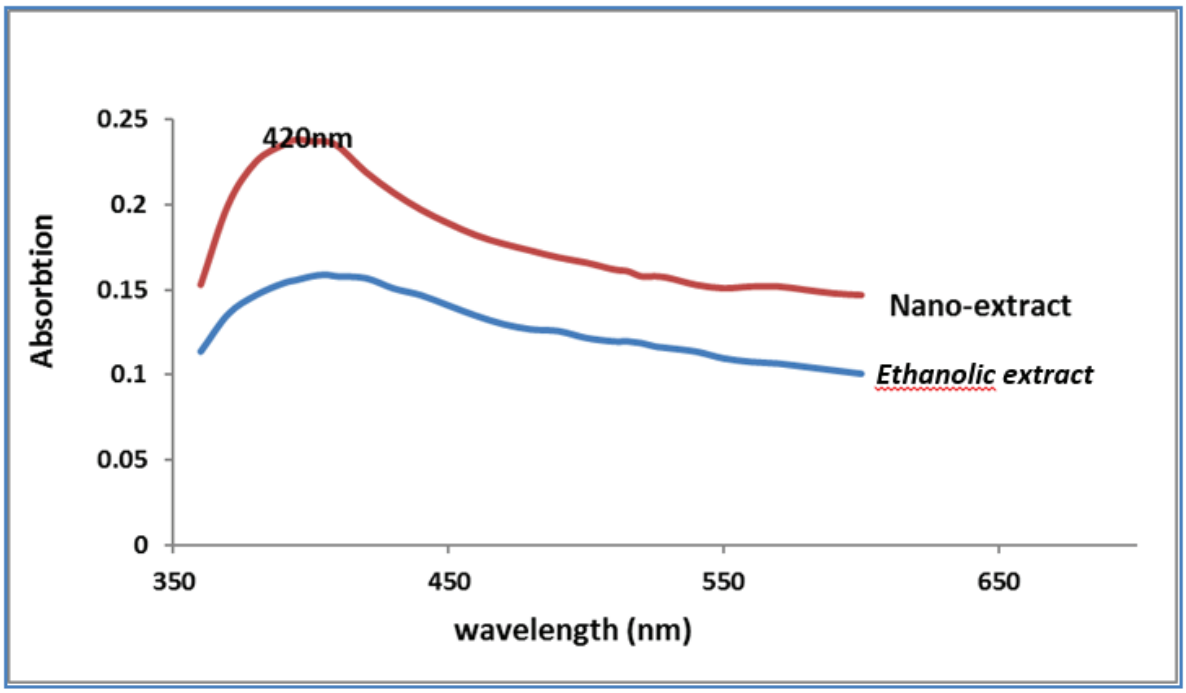

Figure.1. UV-Vis of ethanolic extract *and* of Ag *nanoparticle by Cordia myxa plant.*

SEM assay

SEM technique occurred employed to visualize the shape and size of leaves extracted nanoparticles. Dried powder of the leaves extracted nanoparticles was placed on carbon-coated copper grid. The SEM characterizations of the synthesized NPs was shown in figure2 (B), Images of SEM showed comparatively spherical shape NPs formed with diameter variety (70-178) nm. The nanoparticles were examined under various magnifications power. While ethanolic extract by Cordia myxa plant extract shown in figure 2A.

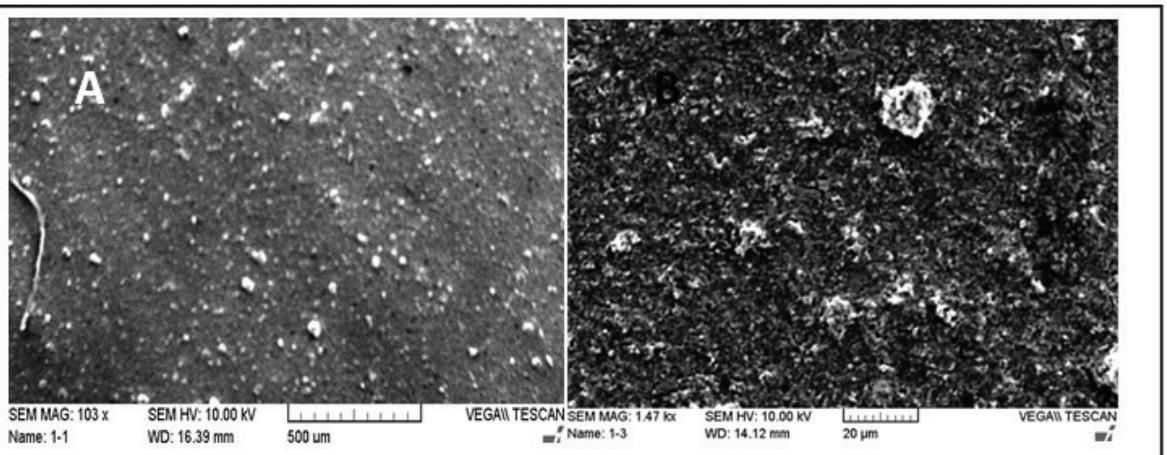

Figure 2. (A): SEM image of synthesized Ethanolic extract (B) :SEM image of synthesized nano extract.

FTIR assay

Beneficial groups of the extract were determined by FTIR. The ethanolic extract showed the bands 3448.84 cm-1 due to phenolic, 2934.18 cm^{-1} due to C=H, 1634.06 cm^{-1} due to C=O , 1655.98, 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 appeared in Fig. 4. 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. 3 B.

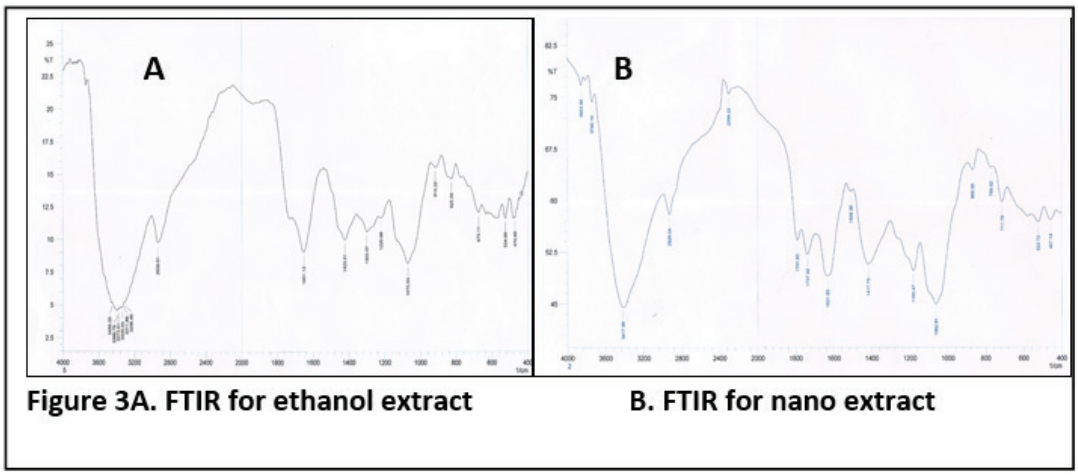

Figure 3A. FTIR for ethanol extract B. FTIR for nano extract

Preliminary Phytochemical Screening of plant extract:

Cordia myxa leaves extract: Preliminary Phytochemical Screening Based on the preliminary screening tannins, Flavonoids Ferric chloride test, Phenol, Alkaloids, Volatile oil, Saponins, were present in Ethanolic extract of Cordia myxa . Greenish (steroids), yellow to colorless (flavonoids), and dark green color suggest the presence of tannins and phenolics, respectively. The existence of volatile oil by yellow precipitate, yellow or reddish brown alkanoids, and yellow precipitate flavonoids is indicated by the formation of precipitate, such as yellow precipitate, while the presence of saponins is indicated by the persistence of frothing (Table 1).

Table 1 : Phytochemical Screening of *Cordia myxa* leaves plant.

NO.	Constituent	Presence(+) absent(-)
1	Tannins	+
2	Saponins	+
3	Phenol	+
4	Alkaloids Wagner's test	+
5	Flavonoids Ferric chloride test	+
6	Volatile oil	+
7	Glucosides	-

Antioxidant activity of *Cordia myxa leaves* plant

The DPPH was exhibited relational to the rise of concentration. The free radicals were given 55.40, 64.50, 75.54, 85.55 by the concentration of 0.2,0.4,0,6 and 0.8 µg/ml in table 2.

Table 2. DPPH radical scavenging of *Cordia myxa leaves.*

DPPH Radical Scavenging Activity % of ethanolic extract	**Ascorbic acid *(positive control)***	**Concentration (mg/ml)**	**DPPH Radical Scavenging Activity % of Nano-extract**	**Concentration (mg/ml)**	$P \leq$
43.20	93	0.2	55.40	0.2	0.001
54.44		0.4	64.50	0.4	0.001
65.50		0.6	75.54	0.6	0.001
3.517		0.8	85.55*	0.8	0.001

All the species showed a high antioxidant activity and DPPH radical action, which occurred probably due to the presence of high content of tannins, flavonoids, xanthones and benzophenones [10] and these constituents have been suggested to act as antioxidants [9]. Increased phenolic elements, such as flavonoids, phenolic acids, and phenolic diterpenes, increased DPPH radical scavenging activity. Many hydroxyl groups, including the O-dihydroxy group, are present in these phenolic components, which have a strong radical scavenging and antioxidant impact [10]. Phenols, Flavonoids and Alkaloids isolated from leaves of plant , decreased the cancer cells, induction apoptosis and use as anti-oxidant. Ag-NPs 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, Ag-NPs related fine with the activity of radical scavenging [16].

Evaluation of Antimicrobial activity:

Figure 4.B shows the nano extract of Cordia myxa was presented top effect with E.coli by inhibition zone (15.33mm), S. aureus (14.mm) ,While, This result be same with ethanolic extract, the inhibition zone reached to (12.5mm) with E.coli and S.aureus to(11.50 mm) in figure4 A. The activity of Cordia myxa extract against bacteria was credited from phytochemical materials like Alkaloids, Flavonoids, phenolics, and tannins which affect bacteria[10]. In Figure (4A,B) was exhibited differently in antibacterial action related to the cell wall structure of gram negative and gram-positive bacteria[17]. The nano-silver 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 [4].

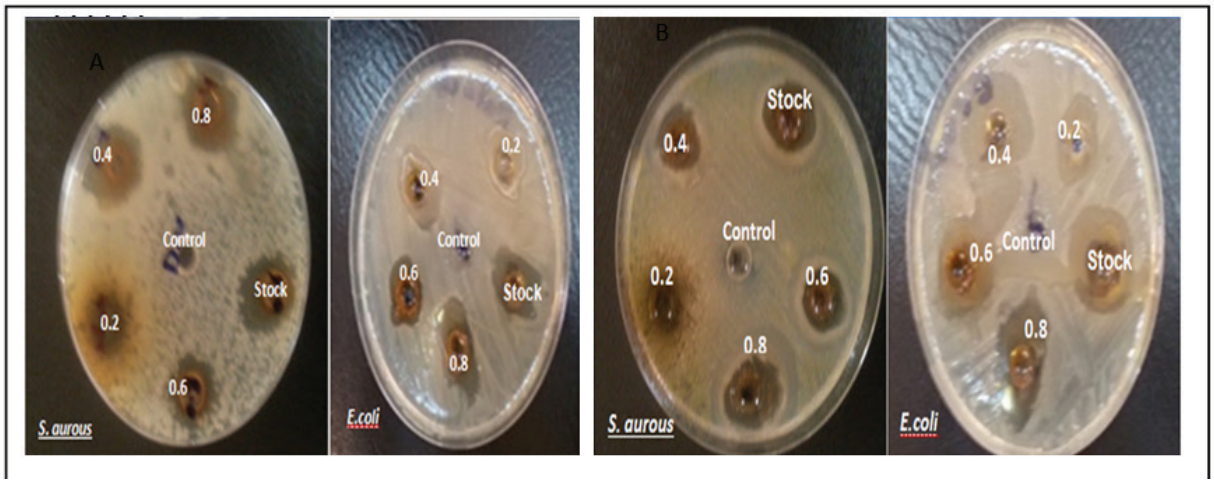

Figure 4. (A) Ethanolic extract and (B)nano extract include antibacterial activity against S. aureus and E.coli at different concentrations. 0.2, 0.4, 0.6, 0.8 and 1(stock) µgmL-1, Control.

Anticancer activity of *Cordia myxa* nano extract.

Cordia myxa nano extract were tested for their cytotoxicity against cancer lung cells. The ability of the Cordia myxa nano extract to prevent the proliferation of cancer cells was used to measure their antitumor activity. The results of this study revealed that against the human lung (A549) cancer cell lines had a highly important cytotoxic activity. The findings indicate that Cordia myxa nano extract have the ability to inhibit cell line formation, and that this effect is dose dependent. Figures 5 A&B was exhibited the role of Cordia myxa nano extract against lung cancer. 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[18]. The

sensitivity of A549 cell line to Cordia myxa nano extract appeared to be dose dependent, resulting in a significant decrease in viable cells viability that reached to a maximum inhibition with 2.6 mg/ml on concentration of Cordia myxa nano extract. The sensitivity of this cell line to 2.5/ml of Cordia myxa nano extract in comparison to untreated cell line as control. Inhibitor ratio of A549 cell line by Cordia myxa nano extract different concentrations. The IC_{50} for A549 was 98.48 µg/ml and that differ significantly ($P< 0.0001$) from the IC_{50} for WRL68 which was 326.6 µg/ml.

Conclusions

The size and shape of *Cordia myxa* nano extract were determined using SEM, UV, and FTIR. *Cordia myxa* nano extract has antibacterial activity against (G+ve) and (G-ve) bacteria, the ability of *Cordia myxa* nano extract to destroy cell lines A549 human (lung cells) was determined to be greater than 75%, and addition antioxidant.

Conflict of Interest: None

Funding: self

Ethical Clearance: Not required

References

1. Jawad KH, Hasoon BA, Hussein NN. Biological application of titanium dioxide nanoparticles prepared through laser ablation in liquid. Drug Invention Today. 2019 Oct 15;12(10).
2. Vilas V, Philip D, Mathew J. Catalytically and biologically active silver nanoparticles synthesized using essential oil. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014 Nov 11;132:743-50.
3. Jwad KH, Saleh TH, Abd-Alhamza B. Preparation of Aluminum Oxide Nanoparticles by Laser Ablation and a Study of Their Applications as Antibacterial and Wounds Healing Agent. Nano Biomed. Eng. 2019;11(3):313-9.
4. Jabir MS, Nayef UM, Jawad KH, Taqi ZJ, Ahmed NR. Porous silicon nanoparticles prepared via an improved method: a developing strategy for a successful antimicrobial agent against Escherichia coli and Staphylococcus aureus. InIOP Conference Series: Materials Science and Engineering 2018 Dec 1 (Vol. 454, No. 1, p. 012077). IOP Publishing.
5. Thakkar KN, Mhatre SS, Parikh RY. Biological synthesis of metallic nanoparticles. Nanomedicine: nanotechnology, biology and medicine. 2010 Apr 1;6(2):257-62.
6. Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. International journal of molecular sciences. 2016 Sep;17(9):1534.
7. Kumar VA, Uchida T, Mizuki T, Nakajima Y, Katsube Y, Hanajiri T, Maekawa T. Synthesis of nanoparticles composed of silver and silver chloride for a plasmonic photocatalyst using an extract from a weed Solidago altissima (goldenrod). Advances in Natural Sciences: Nanoscience and Nanotechnology. 2016 Jan 8;7(1):015002.
8. Abdulwahab AI, Hasoon BA, Maeah RK, Al-azawi KF. Preparation of Mentha Crispata Extract and Detection of its Biological Application, 12(1):1151-1155
9. Maeah RK, Hasoon BA, Abd-Alwahab AI, Al-Azawi KF, Hameedi WB. Biosynthesis of silver nanoparticles using Hibiscus sabdariffa and their biological application. EurAsian Journal of BioSciences. 2020 Sep 22;14(2):3377-83.
10. Maeah RK, Hasoon BA, Abd-Alwahab AI, Al-Azawi KF, Hameedi WB. Biosynthesis of silver nanoparticles using Hibiscus sabdariffa and their biological application. EurAsian Journal of BioSciences. 2020 Sep 22;14(2):3377-83.
11. Jwad KH, Saleh TH, Abd-Alhamza B. Preparation of Aluminum Oxide Nanoparticles by Laser Ablation and a Study of Their Applications as Antibacterial and Wounds Healing Agent. Nano Biomed. Eng. 2019;11(3):313-9.
12. Fadhil FA, Hasoon BA, Hussein NN, Khashan KS. Preparation and characterization of CuO NPs via laser ablation under electric field and study their antibacterial activity. InAIP Conference Proceedings 2018 Dec 6 (Vol. 2045, No. 1, p. 020002). AIP Publishing LLC.
13. Suganya RS, Priya K, Roxy BM. Phytochemical screening and antibacterial activity from Nerium oleander and evaluate their plant mediated nanoparticle synthesis. Int Res J Pharm. 2012;3:285-

8.

14. ALzubedy BA, ALhamdany SO, ALkazaly RK, ALzubedy MA, AbdulKareem Z, Al-Sadie HA, Sadoon AH. Biological effect of Lawsonia inermis plant. Al-Mustansiriyah Journal of Science. 2017 Mar 1;27(4).
15. Al-Shammari AM, Salman MI, Saihood YD, Yaseen NY, Raed K, Shaker HK, Ahmed A, Khalid A, Duiach A. In vitro synergistic enhancement of Newcastle Disease Virus to 5-fluorouracil cytotoxicity against tumor cells. Biomedicines. 2016 Mar;4(1):3.
16. Aqil F, Ahmad I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turkish journal of Biology. 2006 Nov 14;30(3):177-83.
17. Qi Y, Chin KL, Malekian F, Berhane M, Gager J. Biological characteristics, nutritional and medicinal value of roselle, Hibiscus sabdariffa. Circular-urban forestry natural resources and environment. 2005 Mar;604:1-2.
18. Ovadje P, Ammar S, Guerrero JA, Arnason JT, Pandey S. Dandelion root extract affects colorectal cancer proliferation and survival through the activation of multiple death signalling pathways. Oncotarget. 2016 Nov 8;7(45):73080.