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EVALUATION FOR ANTIBACTERIAL AND ANTIPROLIFERATIVE ACTIVITY AGAINST CANCER CELL LINES OF BIOSYNTHESIZED SILVER NANOPARTICLES FROM *VITEX AGNUS-CASTUS* AND *UNCARIA TOMENTOSA* EXTRACTS

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ABSTRACT

The present study aims to investigate the biosynthesis of silver nanoparticles using the aqueous extract of the *Vitex agnus-castus* (leaves and flowers) and *Uncaria tomentosa* (branches with claws) and investigate their antimicrobial and anticancer activities. In order to synthesise the silver nanoparticles, aqueous extract of the plant was prepared and introduced into a 1mM silver nitrate solution. The synthesised Ag NPs were characterised using various instrumental techniques, involved Scanning electron microscope (SEM), Ultraviolet-visible spectroscopy, Fourier transformed infrared spectroscopy (FTIR). Surface Plasmon Resonance (SPR) for AgNPs was noted at 390 nm using *V. agnus-castus* and *U. tomentosa* extracts. The synthesized AgNPs of *V. agnus-castus* and *U. tomentosa* were found with average diameter of 40.85- 49.41nm, 30.69- 54.53nm respectively. The antibacterial result (inhibition zone) against the experimental pathogens was found to be ranged between 12 to 24 mm and from 10 to 20 mm respectively. Toxicity effect of AgNPs against cancer cells was 75.46 of 50 µg/ml and 76.67 of 50 µg/ml respectively. In this research, silver nanoparticles of the plant extracts studied have antimicrobial against some bacterial strains and anticancer against breast cancer cells.

Keywords: *Silver Nitrate, Green Synthesis, Anticancer, Pathogenic Bacteria, Plant Extracts*.

1. INTRODUCTION

Nanomaterials have received much attention because of their unique chemical and physical that distinguish them from bulk-phase materials [1]. Nanoparticles (NPs) have different physical and chemical properties compared by the larger particles and the properties of NPs depend on their size and Morphology [2]. Novel implementation of nanomaterials and

nanoparticles are increasing quickly on different faces because it is totally new or enhanced characterized included (morphology, size and distribution) which quickly getting renovation in a lot of applications such as antibacterial, drug-gene delivery, health care, mechanics, cosmetics, biomedical, environment, food and feed health, electronics, optics, chemical industries, single electron transistors, catalysis, energy science, space industries, photo-electrochemical applications, light emitters and nonlinear optical devices [3,4,5,6]. Great growing in these developing technologies had opening up novel fundamentals, which involved creation of nanoscale materials afterwards in utilization or investigation their obscure optoelectronic and physicochemical characteristics [7].The metallic nanoparticles among the nanoparticles used for all the above targets, that regarded as highly hopeful as they having notable antibacterial characteristics because big surface area in comparison with volume ratio, that is researchers concern because the developing microbial resistance toward antibiotics, metal ions, as well as emerging resistant strains [8]. Amid the different kinds of metallic nanoparticles is silver nanoparticles (AgNPs) had been proved to be potent effective toward viruses and bacteria [9]. Furthermore, microbes are improbable to develop resistance to Ag, due to the metal targeting a wide range of sites in the organisms [10]. Silver nanoparticles from all-noble metal nanoparticles, are an arch product in the scope of nanotechnology that obtained infinite concerns related to their special features like catalytic, chemical stability, good conductivity, in addition anti-viral, antibacterial, antifungal as well as anti-inflammatory activities that are integrated as a composition of fibers, electronic components, cosmetic products, cryogenic superconducting materials, and food industry [11,12]. There is the various procedure in synthesis of AgNPs, but chemical and physical synthesis have numerous cons. Which is mostly based on the usage of highly reactive and toxic reagents, that is dangerous to the humans and environment and. Besides, these methods are much costly to be of industrial importance [13]. So, stable AgNPs synthesis by the green process with managed shape and size is so advantageous approach. Consequently, many novel techniques had recently been developed using either plant extracts (tubers, seeds, leaves) [14-16] or microbes (fungi and bacteria) [17, 18] to synthesize of AgNPs. Aromatic and Medicinal plants were utilized to a huge degree in medicine toward drug-resistant bacteria, that regarded among the most crucial reasons for the failing treatment of infectious diseases. The main sources of new medicines are medicinal plants and represent a replacement choice than routine drugs [19]. Medicinal plants are regarded a new resource for production agents which might be as replacement for antibiotics in treating of resistant bacteria [20]. Due to their low cost, safety and effects on a wide numeral of medicinal plants, microbes, might having the capability in treating resistance of bacteria [21]. The antimicrobial impacts of extracted aromatic oils by big numeral of plants had been reviewed and evaluated [22, 23] the mechanisms which allow the natural ingredients for species and herbs against resist microbes had been investigated [24]. The result revealed these mechanisms markedly varied based on essential oil composition [25, 26]. It usually utilized in the remedy of puerperal diseases, such digestive tonics, remedy of wounds, ulcers, fevers, other ailments and common debility. There is wisdom of utilizing plants as

herbal drugs in the local community, which is cooling, mucilaginous, sweet, anti-inflammatory diuretic, utilized in treatment carminative, digestive, spasmodic affections, tonic and puerperal affections, vitiated conditions of pita, spermatorrhea, dysmenorrhea, amenorrhea, common debility as well as inflammation. Leaves decoction was given in situation of gonorrhea, but root considered as anti-bilious. Total plant of *P. murex* was utilized as a treatment in curative headache, cold, cough, stomachache, intestinal infections, diarrhea and dysentery [27]. High recovery of *E.coli* OI57:H7 isolates from sewage waste water indicate the needs of active treatment. Isolation of the bacterium from tap water is alarming which requiring improvement of water quality. Isolation of the pathogen from river water is ordinary because the majority area of study people live in villages work in upbreed of cattles and buffaloes which is resorvoir (source) of the dispersed of the bacterium from feacesthat take the way to the surface water [28]. In the study of [29] they observed a high incidence of antibiotic resistance among the isolation of *E. coli*. Still yet, no recording about green synthesis of NPs from the extract of *V. agnus-castus* (common names: Chasteberry, Abraham's balm, Chastetree, Chaste Tree, Lilac chastetree, Monk's Pepper, Texas lilac) and *U. tomentosa* (common name: Cat's Claw), Current study examine the synthesis of AgNPs by these plants extract and determine related properties.

2. EXPERIMENTAL

2.1 Microorganisms

Microorganisms were obtained from some hospitals of Basrah. These microorganisms were from two resources, the first one was collected between Sep/2019 to Dec/2019 from patients of some hospitals in Basrah as shown in table (1). The second resource of isolates were collected from Al-Fayhaa hospital sewage. The isolates were identified using Vitek® 2 system and their sensitivity informations listed in table (2).

Table 1: Some characteristics of members of pathogenic bacterial species from patients of different hospitals in Basrah

Name of hospital	Select organisms	Sex of patient	Type of sample	Resistance to antibiotic	MIC
General Basrah Hospital	*Proteus mirabilis*	Male	Ear swab	Imipenem	4
General Basrah Hospital	*Psuedomonas aeruginosa*	Male	Suputum	Imipenem Meropenem	1 >=16
Al-Fayhaa General Hospital	*Proteus mirabilis*	Female	Tissue swab	Imipenem Meropenem	>=16 1

Al-Fayhaa General Hospital	*Proteus mirabilis*	Male	Wound swab	Imipenem Meropenem	8 1
Al-Fayhaa General Hospital	*Klebsiella pneumonia* ssp. *ozaenae*	Male	Wound swab	Imipenem Meropenem	>=16 >=16
Al-Fayhaa General Hospital	*Acinetobacter baumannii* complex	Female	Wound swab	Imipenem Meropenem	>=16 >=16
Al-Fayhaa General Hospital	*Psuedomonas aeruginosa*	Female	Wound swab	Imipenem Meropenem	>=16 4
Ibn Ghazwan Maternity and Pediatrics Hospital	*Acinetobacter baumannii*	Female	Wound swab	Imipenem Meropenem	>=16 >=16
Al-Fayhaa General Hospital	*Acinetobacter baumannii*	Female	Suputum	Imipenem Meropenem	>=16 >=16
Al-Fayhaa General Hospital	*Klebsiella pneumoniae* ssp. *Pneumonia*	Female	Urine	Imipenem Meropenem	>=16 >=16
General Basrah Hospital	*Klebsiella pneumoniae* ssp. *Pneumonia*	Female	Wound swab	Ertapenem Meropenem	4 8
General Basrah Hospital	*Klebsiella pneumoniae* ssp. *pneumonia*	Male	Ear swab	Ertapenem Meropenem	4 4
Al-Fayhaa General Hospital	*Acinetobacter baumannii* Complex	Female	Blood	Imipenem Meropenem	>=16 >=16
Al-Fayhaa General Hospital	*Acinetobacter baumannii*	Male	Suputum	Imipenem Meropenem	>=16 >=16
Al-Fayhaa General Hospital	*Psuedomonas aeruginosa*	Male	Blood	Imipenem Meropenem	>=16 >=16
Al-Fayhaa General Hospital	*Acinetobacter baumannii*	Female	Suputum	Imipenem Meropenem	>=16 >=16
Ibn Ghazwan Maternity and Pediatrics Hospital	*E.coli*	Male	Stool	Imipenem Meropenem	0.5 >=16

Table 2: Some characteristics of members of ecological bacterial species from sewage of Al-Fayhaa hospital in Basrah with Sensitivity informations

Organism	Antibiotic	Susceptibility	MIC
Enterobacter cloacae ssp. *dissolvens*	Imipenem	Sensitive	2
	Meropenem	Resistant	>=16
Raoultella ornithinolytica	Imipenem	Sensitive	<=0.25
	Meropenem	Sensitive	<=0.25
Acinetobacter baumannii	Imipenem	Sensitive	<=0.25
	Meropenem	Sensitive	<=0.25
Escherichia coli	Imipenem	Sensitive	2
	Meropenem	Sensitive	0.5
Enterobacter aerogenes	Imipenem	Sensitive	2
	Meropenem	Sensitive	0.5

2.2 Gas Chromatography-Mass spectrometry (GC-Ms) for plant powders

GC-MC is widely used in the medical industries for analytical research and development, quality control, quality assurance, production, and pilot plant divisions for active pharmaceutical ingredients (API), bulk drugs and formulations. It is an integral part of research related to medicinal chemistry (synthesis and characterisation of compounds), pharmaceutical analysis (stability testing, impurity identification), pharmaceutical process control, and pharmaceutical biotechnology. In this study the powder of seeds was dried. 20g of powder was soaked in methanol, then put in oven temperature 300°C with slow fan for 1 min then 60°C for 10 min then 10°C/min to 280°C for 3, the run time was 35 min. The samples were examined by (GC-MS) [30].

2.3 Preparation of Plant Extract

Two plants include of *Vitex agnus-castus* (It is one of the plants named by the scientist Carolus Linnaeus, Swedish botanist) and *Uncaria tomentosa* (Carl Ludwig Willdenow [1765-1812] was a German botanist and Josef August Schultes [1773-1831] was a Austrian biologist who classified this plant) were used to make the aqueous extract. For preparing extracts 5gm of thoroughly washed and finely crushed plants was taken and mixed with 100ml deionized water put in 500 ml of Erlenmeyer flask, the mixture was boiling for 10 min, after that filtered by using Whatman No. 1 filter paper (pore sized 25 µm) [31, 32].

2.4 Synthesis of Silver Nanoparticles (Ag NPs)

A 5mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. This aqueous solution was prepared by dissolving 0.39 g of silver nitrate in a liter of deionized distilled water. 10 mL of the plant extract was added to 90 mL of a 5mM aqueous solution of silver nitrate for reduction to Ag+ ions and kept

at room temperature for 4 h until changing the color from watery to dark brown. [31].

2.5 Characterization of Silver Nanoparticles

2.5.1 Ultra Violate Visible Spectroscopy (UV-Vis)

This method utilizing light in the visible and adjacent (near-UV and near-infrared (NIR)) spectrum. Visible range absorption directly impacts the sensed color of the chemicals included. The electromagnetic range, molecules subject electronic transitions in this region [32]. The spectrum was reported through 300-800 nm, the wavelength identical to maximum absorption was detected at room temperature.

2.5.2 Fourier Transform Infrared Spectroscopy (FTIR)

Centrifugation residual solution (30ml) at 10000 rpm for 10 min, resulting suspension was redispersed in 2ml sterile D.W for removing all free biomass compound or deposits which is not the capping ligand of the NPs. Repeating of the centrifuging process for 3 times. Then, freeze dried for the purified suspension was done to gain dried powder [31]. FTIR spectra were recorder at room temperature and analyzing of the dried NPs via FT-IR-4200 JASCO/ Japan**.**

2.5.3 Scanning Electron Microscopy (SEM)

SEM analysis is a process which scans a sample using an electron beam to give a magnified image for analysis. Preparing of thin films for the sample on a carbon coated copper grid through dropping a too little quantity of the sample on the grid, the film on the SEM grid were permit to dry via placing it below a mercury lamp for 5 min. [31].

2.6 Antibacterial Activity of Biogenic Silver Nanoparticles

Evaluation of antibacterial activity for AgNPs that synthesized from *Vitex agnus-castus* and *Uncaria tomentosa*. The synthesised particles were tested for their antibacterial activity by well diffusion method against pathogenic organisms. Sub culturing of pure culture of organism on nutrient agar and streaked regularly by sterile cotton swab. Gel puncture was used for making wells (sized 6mm) on Muller-Hinton agar plates. 50 µl (5 milli mol) of Ag NPs solution and plant extracts (negative control) and $AgNO_3$ (Positive control) were added in wells for all plates. After incubation at 37 °C for 18 h, the different levels of the inhibition zone were measured [33].

2.7 Anticancer Activity of Biogenic Silver Nanoparticles

Cell line: Breast Cancer cell line mcf7, Control: Cell culture medium

Human Breast Cancer Cell Line MCF-7 & human mammary cell line HBL100 were obtained from the Iraq Biotechnology Cell Bank unit in Basra and maintained in RPMI-1640 supplemented with 10% fetal bovine, 100 µg/ml penicillin and 100 µg/ml streptomycin. Cells were passaged with trypsin-EDTA re-seeded at 50% confluence two times a week and incubated at 37 °C [34]. For detecting the cytotoxic impacts, the MTT cell viability

assay was performed on 96-well plates. Seeding of cell lines MCF7& HBL100 at $1*10^4$ cells/well. Next 24h or a confluent monolayer was ompleted, cell lines were treated with the tested compounds (A: Ag NPs *of V. agnus-castus* and B: AgNPs of *U. tomentosa*) at a different concentrations (50 and 75 µg/ml), the concentration of extracts were 50 µg/ml, the concentration of $AgNO_3$ was 50 µg/ml. Measuring of cell viability after 72 hrs, elimination the medium, 28 µL of 2 mg/mL solution from MTT was dded (incubation the cells for 2 h at 37 °C). After elimination the MTT solution, the crystals residual in the wells were solubilized by adding 100 µL of Dimethyl Sulphoxide (DMSO), subsequent incubated and shacked for 15 min at 37 °C [35]. The absorbency was detected on a microplate reader at wavelength 620 nm; the assay was done in triplicate. Calculation of inhibition rate for cell growth (the percentage of cytotoxicity) as the following equation:

Proliferation or diffusion rate (PR) = B/A * 100 where A is the average optical density of untreated wells, B is the optical density of treated wells and inhibition rate (IR) = 100- PR [36].

2.10 Statistical analysis

The data were analyzed using the Statistical Package for Social Science (SPSS-11) and Analysis of Variance (ANOVA) and Revised Least Significant Differences (RLSD).

3. Result and discussion

3.1 Gas Chromatography-Mass spectrometry (GC-Ms) for Plant Powders

The active principles in *V. agnus-castus* with their retention time (RT), concentration (%), name of compounds, molecular formula, and molecular weight (MW), are presented in Table (3). The active principles in *U. tomentosa* with their retention time (RT), concentration (%), name of compounds, molecular formula, and molecular weight (MW), are presented in Table (4). The major components present in *V. agnus-castus* were Hydrazine, methyl (4.90 %), and in *U. tomentosa* were (R)-(-)-2-Amino-1-propanol (70.5%) identified as low levels. These phytochemicals are responsible for various pharmacological actions like antimicrobial, anti-inflammation, Anti-cancer, etc. (Table 5).

Table 3: phytocomponents identified in the V. agnus-castus

S. No.	RT	Area (%)	Name of compound	Molecular formula	M.W (g/mol)
1	1.85	4.90	Hydrazine, methyl-	N_2H_4	32.05
2	1.85	3.26	2- Hydrazinoethanol	$C_2H_8N_2O$	76.1
3	1.85	1.39	Ethanol, 2-nitro-	$C_2H_5NO_3$	91.0660

Table 4: phytocomponents identified in the U. tomentosa

S. No.	RT	Area (%)	Name of compound	Molecular formula	MW(g/mol)
1	1.28	35.0	Carbamic acid, monoammonium salt	CH_3NO_2	61.04
2	1.28	2.32	Nitrous oxide	N_2O	44.013
3	1.28	2.05	Phenol, 4-[2-(methylamino)ethyl]-	$C_9H_{14}BrNO$	232.12
4	1.28	1.48	(2-Aziridinylethyl)amine	$C_4H_{10}N_2$	86.14
7	1.37	3.46	Acetic acid, hydroxy-	C_2H_4O	76.0514
8	1.37	2.79	Acetic acid, ethoxyhydroxy-, ethyl ester	$C_6H_{12}O_4$	148.16
9	2	70.5	(R)-(-)-2-Amino-1-propanol	C_3H_9NO	75.11
10	2	16.6	(2-Aziridinylethyl)amine	$C_4H_{10}N_2$	86.14
11	2	4.53	Hydroxyurea	$CH_4N_2O_2$	76.055
12	2	1.65	Benzeneethanamine, 2-fluoro-β,3-dihydroxy-N-methyl-	$C_9H_{12}FNO_2$	185.2
13	2	1.39	Benzeneethanamine, 3-fluoro-β,5-dihydroxy-N-methyl-	$C_9H_{12}FNO_2$	185.2
14	2	1.28	Dextroamphetamine	C_9NH_{13}	135.2062
15	2	1.08	Benzeneethanamine, 2-fluoro-β,5-dihydroxy-N-methyl-	$C_9H_{12}FNO_2$	185.2
16	2.75	23.7	N-Hydroxymethyl-2-phenylacetamide	$C_9H_{11}NO_2$	165.19
17	2.75	12.9	Benzeneethanol, α-methyl-	$C_9H_{12}O$	136.1910
18	2.75	10.2	Benzeneacetamide	C_8H_9NO	135.1632
19	2.75	6.56	Hydrazinecarboxylic acid, phenylmethyl ester	$C_8H_{10}N_2O_2$	166.1772
20	2.75	4.37	Benzenesulfonamide, N,N,4-trimethyl-	$C_9H_{13}NO_2S$	199.270
21	2.75	3.08	Benzeneethanamine, 3,4-benzyloxy-2,5-difluoro-β-hydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219.18
22	2.75	2.60	Benzene, [(methoxymethoxy)methyl]-	$C_9H_{12}O_2$	152.19

Table 5: Active of phyto-components identified in plants by GC-MC

Plant extract	RT	Area (%)	Name of compound	Activity
Vitex agnus-castus	1.85	4.90	Hydrazine	Antimycobacterial activity, antidepressant activity
Uncaria tomentosa	1.28	35.0	Carbamic acid	Antibacterial activity
	2	70.5	(R)-(-)-2-Amino-1-propanol	Catalytic activity

3.2 Synthesis of Silver Nanoparticles (Ag NPs)

Recently using of Green synthesis of Ag NPs has been preformed via algae, bacteria, fungi and plants due to it is a simple, unexpensive, and eco-friendly technique. This method can be an appropriate replacement to chemical and physical technique. It is familiar that AgNPs had yellowish brown color in of agitation aqueous solution because ofsurface plasmon vibration in AgNPs [37]. The bioactive compounds of the extract had been accountable for the reduction and forming of AgNPs [38]. The utilization of different plant materials in biosynthesis of NPs is regarded a green technology due to never included hazard chemicals [39]. In current work the plant extracts were mixed in the aqueous solution of the Ag+ complex, till changing the color to deep brown because reduction of Ag ion; indicating the formating of AgNPs. Figures (1, 2). This result agreement with result of study Keshari et al [40] which concluded the forming of NPs began afterword extract mixing with AgNPs solution. Visible color altered (pale brown to dark brown) of the solution, also spectral analysis certain the forming of AgNPs. Dark brown color of solution indicated the reduction of $AgNO_3$ into the AgNPs. Moreover, AgNPs had potent antibacterial activity toward chosen bacteria as presence of capping agents and low sized. In future AgNPs might be utilized as antibiotics for many reasons unexpensive, non-toxic and environmental-friendly also greatlyeffectiveness toward bacteria. The current work proven plant extracts in and the Ag^0 to Ag^+ reducing aqueous Agquick forming environmental-friendly AgNPs and justly well-defined dimensions sized less than 100 nm. However, the exactly mechanism for forming of the NPs in this green synthesis was obscure, perhaps reduced cofactors or biosynthetic products had a basic role in the reduction of particular salts to NPs. Thus, these ecofriendly NPs probably used as a perfect source toward MDR bacteria. Study of Logeswari et al [33] noticed the change of the solution color from yellow to bright yellow, till dark brown after 24 and 48 h of the reaction, denoted the forming of AgNPs as Ag^+ were reduced to AgNPs during addition to extract of natural plant as *Ocimum tenuiflorum*, *Solanum tricobatum*, *Syzygium cumini*, *Citrus sinensis and Centella asiatica*.

Figure 1: synthesis of AgNPs after 1 hours **(C is AgNPs using *V. agnus-castus*, D is AgNPs using *U. tomentosa*)**

Figure 2: synthesis of AgNPs after 4 hours **(C is AgNPs using *V. agnus-castus*, D is AgNPs using *U. tomentosa*)**

3.3 Characterization of Silver Nanoparticles

3.3.1 UV-visible Absorption Spectrum of AgNPs

It is commonly known that UV-Vis spectroscopy can be utilized for examination shape and size-controlled NPs in aqueous suspensions [41]. Figure (3) reveals the UV-Vis spectra recoded in the reaction medium after 4 h by the UV spectrophotometer at the absorbance band (λ 350-900 nm) the color changes were measured absorption was scanned at 400 nm. The result show that the absorbance of visible light ranges (0.20 to 0.45) for the two plant extracts. This study recorded less than result of Thirunavoukkarasu *et al* [42] which the leaf extract of *Desmodium gangeticum* UV-visible spectra reported reaction vessels at various times of reaction, and strong peak centered at ca. 450 nm. Figure (4).

Forming of AgNPs in Keshari *et al* study [40] was assured via UV-Vis spectrophotometer (λ, 300-700 nm). Reporting the absorbance band at 442 nm because localized surface plasmon resonance (LSPR), proven the forming of AgNPs. But, lack of LSPR suggested the forming of tiny AgNPs or the Ag cluster that implicates a low number of atoms [43]. Study of Sulaiman *et al* [44] about using of leaves extract of *Eucalyptus chapmaniana* for green synthesis, cytotoxic effects and antimicrobial and cytotoxic impacts of AgNPs revealed that UV-Vis spectral analysis appeared Ag LSPR at 413 nm which agreement with present study. Study of Logeswari *et al* [33] showed the UV-vis spectra maximum absorbance at 420 nm identical to the LSPR of AgNPs. The observance occurs confirmed reducing of the Ag^{+} extracellularly. It was recorded before that absorbance at nearly 430 nm for Ag was a property for Nobel metal particles [45]. This result agreement with study of Khalil et al [10], the UV-Vis spectra of 3 formed AgNPs by constant AgNOM) with various 3 -concentration (1 × 10concentration of extract from olive after 24h at room temperature. Alteration of solution color from pale yellow to yellowish brown then deep brown based on the concentration of extract which confirm forming of AgNPs the reason of alteration in color related to surface Plasmon vibration excitation in AgNPs, at 440-458nm seeing the Surface Plasmon Resonance (SPR) of AgNPs. Study of Kumar *et al* [46] used the extract of *Boerhaavia diffusa* as a reducing-agents, Maximum absorption of prepared colloidal solution of Ag using UV-Vis spectroscopy was at 418 nm. UV-Vis spectroscopy result of study

Dhand *et al* [47] revealed the maximum adsorption at 459 nm that symbolize the characteristic SPR of NPs utilizing seed extract of *Coffea arabica*. UV-Vis spectrum of Dadashpour *et al* study [48] showed the maximum absorption of the biosynthesized AgNPs using *Matricaria chamomilla* extract was at 430 nm. In study Saxena *et al* [32] about utilizing extract of onion as biological methods in synthesis of AgNPs, the Surface Plasmon band in solution of AgNPs remainsclose to 413nm during reaction duration. In Umoren *et al* study [49], the absorption spectrum of UV-vis for solution revealed SPR derived from AgNPs using red apple fruit extract at around 409-448 nm. The aqueous extract for *Salvia officinalis* (common sage) utilized in biosynthesis of NPs give maximum absorbance peak on the curve at 430 nm [50]. All of previous studies above agreement with the present study.

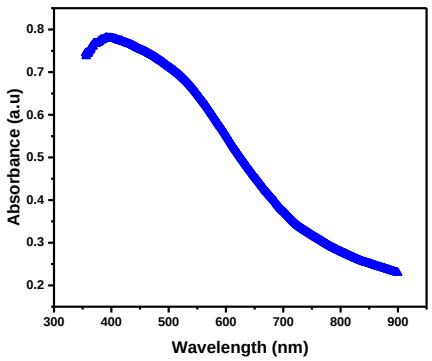

Figure 3: UV–Vis for Ag NPs of V. agnus-castus

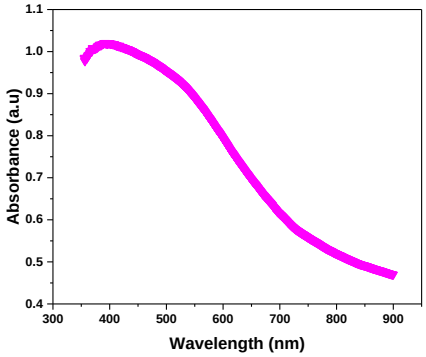

Figure 4: UV–Vis for Ag NPs of U. tomentosa

3.3.2 Fourier Transform Infrared Spectroscopy (FTIR)

Usage FTIR measurements in order to investigate the Ag NPs associated molecules. Centrifugation 100 ml of Ag NPs solution at 5000 rpm for 20 min, then washing the pellet 3 times by 10 ml of de-ionized water to eliminate of the free proteins/enzymes which not capping the Ag NPs. Drying and ground the samples with potassium bromide pellets then analyzed by a JASCO FT/IR-4200 (Japan) in the diffuse reflectance mode operating (resolution 4cm^{-1}). The FTIR spectrum analysis of *V. agnus-castus* and *U. tomentosa* are showed in figures (5, 6)**.**

The FTIR spectrum of *V.agnus-castus* showed peak at 3351 cm^{-1} refers to normal polymeric, OH stretch. The peak at 2928 cm^{-1} refers to methylene C-H stretch. The peak at 1737 cm^{-1} refers to ester. The peak at 1649 cm^{-1} refers to amide. The peak at 1427 cm^{-1} refers to carbonate ion. The peak at 1246 cm^{-1} refers to aromatic ethers, aryl-O stretch. The peak at 1060 cm^{-1} refers to primary amine, CN stretch. The peak at 611 cm^{-1} refers to di sulfides S-S stretch. The FT-IR spectrum of *U. tomentosa* showed peak at 3362 cm^{-1} refers to normal polymeric, OH stretch. The peak at 2928 cm^{-1} refers to methylene C-H stretch. The peak at 1649 cm^{-1} refers to amide. The peak at 1408 cm^{-1} refers to Vinyl C-H in-plane bend. The peak at 1249 cm^{-1} refers to aromatic ethers, aryl-O stretch. The peak at 1046 cm^{-1} refers to primary amine, CN stretch. The peak at 668 cm^{-1} refers to di sulfides C-S stretch. The result of FT-IR spectrum of both *V. agnus-castus* and *U. tomentosa* appear the compounds is contain of functional

groups of alkene compounds, hydroxyl compounds, ether compounds, primary amines, carbonyl compounds, and thiols compounds. Presence of band at 2358, 2361 cm-1 of both *V. agnus-castus* and *U. tomentosa* respectively is unknown and they are in the same position in wavenumber spectrum marked with a red star inside the figures. FTIR results of Keshari *et al* [40] study confirmed the various functional group present on the surface of bioactive compounds. This functional responsible for the capping of silver nanoparticles and stable in nanosize.

The result of this study agreement with study of Thirunavoukkarasu *et al* [42] which shows the FTIR spectra of aqueous silver nanoparticles prepared from the *Desmodium gangeticum* leaf extract. Peak at 3470 cm^{-1} and 1640 cm^{-1} corresponds to N-H stretching and bending vibrations, respectively in amines from proteins of plants. While stretching vibration of O-H bonds at 3280 cm^{-1} (alcohol and phenols) and C-H bonds at 2920 cm^{-1} arise from plant metabolites, stretching vibration of O-H at 2400cm^{-1} peak and C=O at 1680 cm^{-1} peak, arise from carboxylic acid. Two peaks at 1600 cm^{-1} and 1500 cm^{-1} corresponds to C=C stretching vibrations from aromatic rings, all from plant metabolites. Three peaks, vis., 1010 cm^{-1}, 1190 cm^{-1} and 1080 cm^{-1} correspond to C-O stretching from alcohol, carboxylic acid, ester and ether; all owing to functional groups of proteins and metabolites covering the silver nanoparticles. Peak at 800 cm^{-1} is attributed to aromatic groups. Result of [51] study about green synthesis of silver nanoparticles using *Hibiscus rosa sinensis* showed that from FTIR spectra the Ag nanoparticles are bound to carboxylate ion groups. FTIR results of Dhand *et al* [47] recorded a downward shift of absorption bands between (800-1500 cm^{-1}) indicting the formation of silver nanoparticles using *Coffea arabica* seed extract.

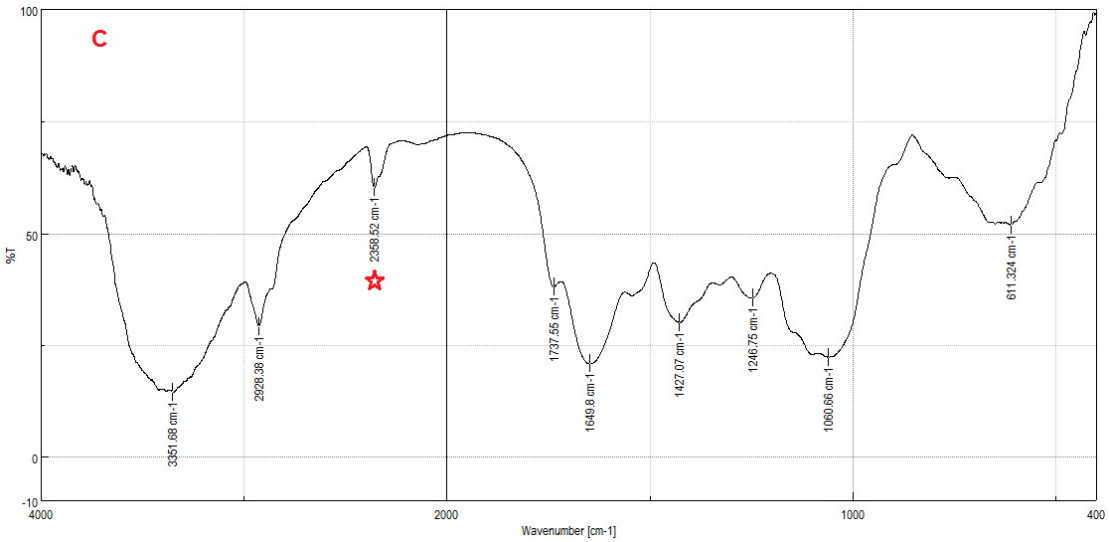

Figure 5: FTIR analysis of V. agnus-castus

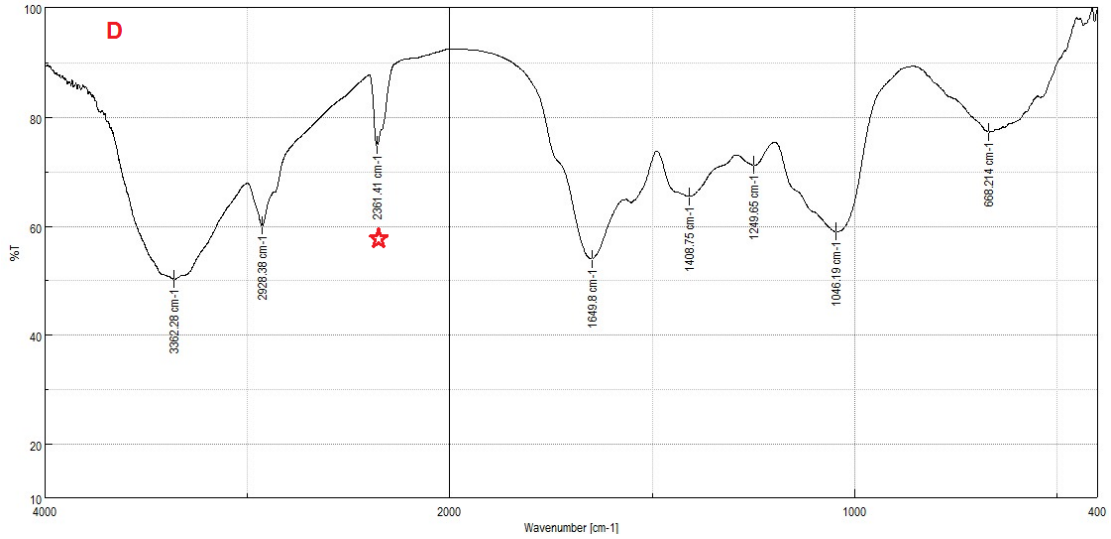

Figure 6: FTIR analysis of U.tomentosa

3.3.3 Scanning Electron Microscopy (SEM)

Synthesized AgNPs suspension was vaporized on clean glass slide in order to characterization of SEM, then left and covered for entirely drying at room temperature. Analysis of SEM by ESEM (Model: Supra 55; Zeiss, Germany). Figures (7, 8) reveals SEM of AgNPs of plant extracts which they were commonly spherical shaped, sized from 40.85 - 49.41 nm for AgNPs of *V. agnus-castus* in diameter and the diameter was from 30.69 - 54.53 nm for AgNPs of *U. tomentosa*.

The result study agreement with study of Thirunavoukkarasu *et al* [42] which the experimental results revealed about 18-39nm the diameter in the solution of prepared NPs. And agreement with the diameter ranged between 40-52 nm of metallic Ag NPs from leaf extract of *Caesalpinia coriaria* were the result by Jeeva *et al* [52]. Study of Vijayakumar *et al* [53] showed the average diameter of AgNPs from *Artemisia nilagirica* was 70-90 nm determined by SEM. This result showed SEM bigger than the resent study. Also the study of Kummar *et al* [54] showed bigger size and concluded that synthesized AgNPs using *Azadirachta idica* leaf extract having spherical shaped with average diameter 94 nm. Result of study Jagtap and Bapat [14] showed smaller size, the AgNPs using Artocarpus heterophyllus seed extract were commonly found irregular shaped sized 10.78 nm in average. Abalkhil et al [55] concluded that small-sized AgNPs synthesized by *Aloe vera* were more effective than those synthesized by *Portulaca oleracea* and *Cynodon dactylon*.

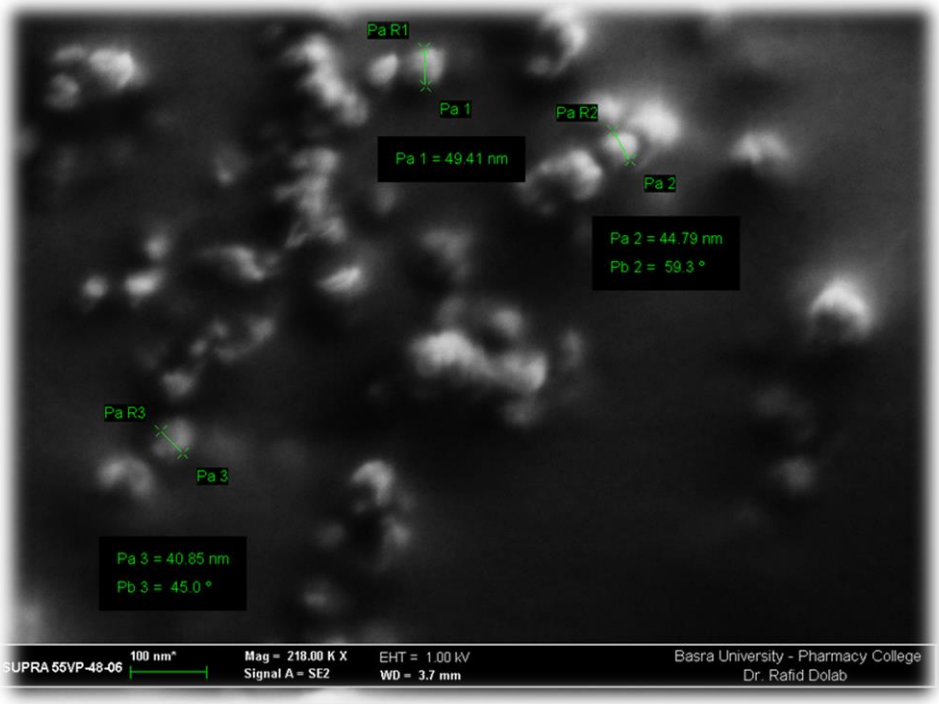

Figure 7: SEM of AgNPs of V. agnus-castus plant extract

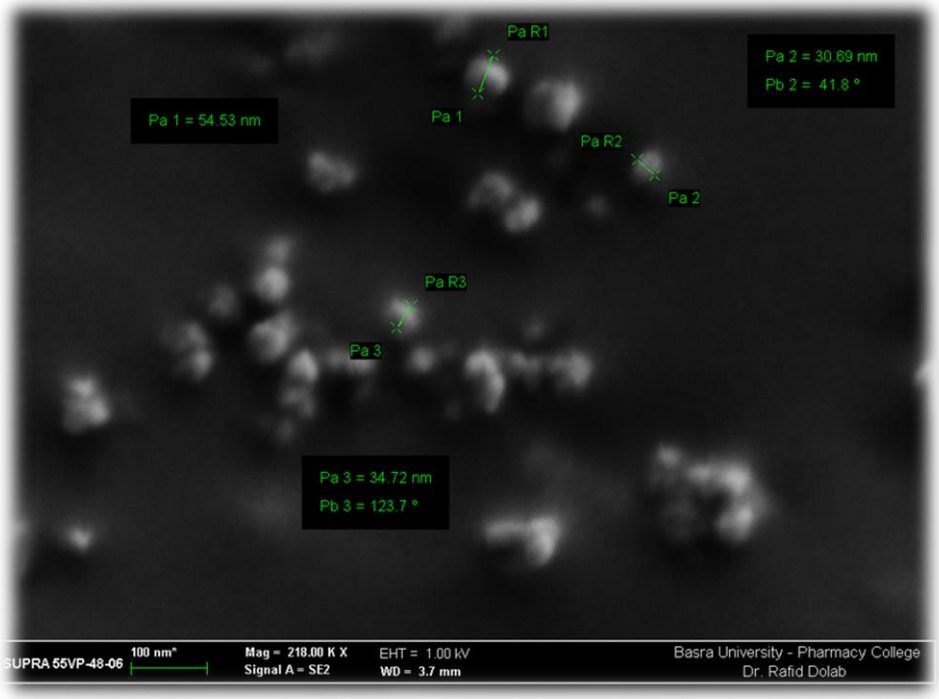

Figure 9: SEM of AgNPs of U. tomentosa plant extract

3.4 Antibacterial Activity of Biogenic Silver Nanoparticles

Antimicrobial activity of synthesizing AgNPs from extract of natural plants was examined toward different pathogenic organisms using well diffusion methods like *Proteus merabilis*, *E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The inhibition zones diameter (mm) is explained in table (6). The result showed that the silver nanoparticles synthesized by *V. agnus-castus* were found to have highest antimicrobial activity against *Proteus mirabilis* (24 mm) and the lesser antimicrobial activity toward *Klebsiella pneumoniae* ssp. *pneumoniae* (12 mm) table (6). The diameter of inhibition zones (mm) around each well full with nanoparticles solution and $AgNO_3$ is explained in table (7) of the second type of hospital sewage isolates. The result show that there was no effect of silver nanoparticles and silver nitrate on *Enterobacter cloacae* ssp. *dissolvens* and there was clear effect on *Acinetobacter baumannii*. The result study agreement with study of Logeswari et al [33], synthesis of AgNPs using extract of *Solanum tricobatum*, *Ocimum tenuiflorum* having highest antimicrobial activity toward *E. coli* with inhibition zone 30 mm but less activity of AgNPs from *Solanum tricobactum* was toward *P. aeruginosa* and *E. coli* (12 mm). Antibacterial sensitivity results certain the $AgNO_3$ and AgNPs had antibacterial activity. The maximum inhibition zone of AgNPs toward *Vibrio cholera*e was 41 mm while minimum inhibition zone toward *Enterococcus faecalis* 15 mm. Inhibition zone of silver nitrate (14 mm) and silver nanoparticles (23 mm) against *Escherichia coli* bacteria [40]. AgNPs revealed effective antimicrobial characters in comparison with other salts as related quite large surface area that gives best connection with microorganisms, NPs bind to the cell membrane and permeated within bacteria. Cell membrane of bacteria implicates Sulfer containing proteins so AgNPs interface with these proteins in the cell in addition with Phosphorus containing compounds as DNA. During AgNPs penetration the bacterial cell forming in the center of bacteria a low molecular weight region that bacteria agglomerates so, protect DNA from Ag^+ ions. NPs prefer to target the respiratory chain, cell division then causing cell death, which liberate Ag^+ ions in the bacterial cells, that increase related activity as bactericidal [56, 57].

Table 6: Zone of inhibition of Ag NPs and $AgNO_3$ (concentration 50 µg/ml) against pathogenic bacteria from Vitek® 2 system of hospitals

Type of Bacteria	Diameter of inhibition zone (mm)			Std. Dev.	Average
	D*	C*	$AgNO_3$		
Proteus mirabilis **(ear swab)**	16	16	15	0.58	15.67
Pseudomonas aeruginosa **(suputum)**	19	16	17	1.53	17.33
Proteus mirabilis **(tissue swab)**	17	19	20	1.53	18.67

Proteus mirabilis **(wound swab)**	20	24	20	2.31	21.33
Klebsiella pneumonia **ssp.** ***Ozaenae*** **(wound swab)**	20	17	20	1.73	19.00
Acinetobacter baumannii **complex (wound swab)**	17	18	19	1.00	18.00
Pseudomonas aeroginosa **(wound swab)**	15	14	18	2.08	15.67
Acinetobacter baumannii **(wound swab)**	20	16	20	2.31	18.67
Acinetobacter baumannii **(suputum)**	20	20	14	3.46	18.00
Klebsiella pneumonia **ssp.** ***Pneumonia*** **(urine)**	10	15	15	2.89	13.33
Klebsiella pneumonia **ssp.** ***Pneumonia*** **(wound swab)**	13	12	16	2.08	13.67
Klebsiella pneumonia **ssp.** ***Pneumonia*** **(ear swab)**	14	15	17	1.53	15.33
Acinetobacter baumannii **complex (blood)**	16	14	17	1.53	15.67
Acinetobacter baumannii **(suputum)**	16	16	18	1.15	16.67
Pseudomonas aeruginosa **(blood)**	15	18	16	1.53	16.33
Acinetobacter baumannii **(suputum)**	19	16	19	1.73	18.00
E. coli **(stool)**	17	17	15	1.15	16.33

* C is AgNPs using *V. agnus-castus* plant extract, * D is AgNPs using *U. tomentosa* plant extract

There are no significant differences under the probability level of P≤0.05

Table 7: Zone of inhibition of Ag NPs and $AgNO_3$ (concentration 50 µg/ml) against ecological bacteria from hospital sewage

Type of Bacteria	Diameter of inhibition zone (mm)			Std. Dev.	Average
	$AgNO_3$	D*	C*		
Enterobacter cloacae ssp. *dissolvens*	5	-	9	2.89	1.67
Raoultella ornithinolytica	7	7	7	0.00	7.00
Acinetobacter baumannii	19	19	19	0.00	19.00
Acinetobacter baumannii	15	15	15	0.00	15.00
Escherichia coli	15	14	14	0.58	14.33
Escherichia coli	15	16	16	0.58	15.67
Escherichia coli	15	14	15	0.58	14.67
Enterobacter cloacae ssp. *dissolvens*	-	-	-	0.00	0.00
Enterobacter cloacae ssp. *dissolvens*	7	14	13	3.79	11.33
Enterobacter cloacae ssp. *dissolvens*	-	-	-	0.00	0.00
Enterobacter cloacae complex	5	7	5	1.15	5.67
Enterobacter aerogenes	13	14	14	0.58	13.67
Enterobacter cloacae complex	14	12	13	1.00	13.00
Enterobacter cloacae complex	14	12	12	1.15	12.67
Escherichia coli	16	14	12	2.00	14.00
Enterobacter cloacae complex	-	-	-	0.00	0.00

* C is AgNPs using *V. agnus-castus* plant extract, * D is AgNPs using *U. tomentosa* plant extract

There are no significant differences under the probability level of P≤0.05

3.5 Anticancer Activity of Biogenic Silver Nanoparticles

The results of MCF7 (The cancer cells) of C: AgNPs of *V. agnus-castus* and D: AgNPs of *U. tomentosa* are shown in tables (8, 9). The results of HBL100 (The normal cells) of C: AgNPs of *V. agnus-castus* and D: AgNPs of *U. tomentosa* are shown in tables (10, 11).

The results show that the nanomaterials in this study have a low toxic effect towards normal cells and this

is what we observe in drugs used to treat cancers as they have many side effects. Result of study Kathiravan *et al* [58] showed the ability implantation in vitro-anticancer activity toward human breast cancer cell line by using AgNPs from leaf extract of *Melia dubia*. Using single pot technique in synthesis of AgNPs, which so quick, appropriate, less time consuming and eco-friendly as well as abilities for applying in several of existing implantation. AgNPs had been tested using various concentrations anti-breast cancer activity invitro using MCF-7 cell line, they found increasing the death of cancer cell when increasing concentrations of AgNPs solution. In study by Devi *et al* [59] showed the synthesized AgNPs by *Gelidiella* sp. extract were evaluated for cytotoxic activity on Hep-2 (Human laryngeal) cell lines. The cytotoxicity assay of Kelkawi *et al* [60] showed significance activity of AgNPs against cancer cells using *Mentha pulegium* on MCF-7 and Hela cancer cells. Green synthesis AgNPs from leaf extract of cannonball revealed cytotoxicity to human breast cancer cell line (MCF-7). Another study by Devaraj *et al* [61]. Using fruit extract from *Cleome viscosa* L for green synthesis of AgNPs. Revealed credible activity against cancer cells in on ovarian (PA1) and the lung (A549) cancer cell lines [62].

Table 8: Rate viability of mcf7 cell line treated with three replicate of concentrations (µg/ml) from C

Concentration of materials%	AgNPs (50)	AgNPs (75)	Extract (50)	Ag NO_3 (50)	Average	Std. Dev.
Mean of viability %	24.54	23.44	22.66	29.85	25.12	3.24
Mean of toxicity %	75.46	76.56	77.34	70.15	74.88	3.24

Table 9: Rate viability of mcf7 cell line treated with three replicate of concentrations (µg/ml) from D

Concentration of materials%	AgNPs (50)	AgNPs (75)	Extract (50)	$AgNO_3$ (50)	Average	Std. Dev.
Mean of viability %	23.33	33.72	21.56	29.85	27.12	5.67
Mean of toxicity %	76.67	66.28	78.44	70.15	72.89	5.67

Table 10: Rate viability of HBL100 cell line treated with three replicate of concentrations (µg/ml) from C

Concentration of materials%	AgNPs (50)	AgNPs (75)	Extract (50)	$AgNO_3$ (50)	Average	Std. Dev.
Mean of viability %	49.93	42.73	37.93	53.54	46.03	7.03
Mean of toxicity %	50.07	57.27	62.07	46.46	53.97	7.03

Table 11: Rate viability of HBL100 cell line treated with three replicate of concentrations (µg/ml) from D

Concentration of materials%	AgNPs (50)	AgNPs (75)	Extract (50)	$AgNO_3$ (50)	Average	Std. Dev.
Mean of viability %	44.41	39.37	38.89	53.54	44.05	6.80
Mean of toxicity %	55.59	60.63	61.11	46.46	55.95	6.80

4. CONCLUSION

Colloidal silver nanoparticles have been successfully prepared using botanicals Calendula and Cat's Claw which are environmentally friendly, economical, and more effective than physical and chemical approaches. The plant extract not only acts as a reducing agent, but also coats the produced nanoparticles, providing them with stability. The two plants used are a good reducing agent for preparing stable colloidal silver nanoparticles. Silver nanoparticles prepared using it have an intense absorption peak in the visible region with a peak at 450 nm. Moreover, its average diameter is nanometers from 40.85 - 49.41 nm for AgNPs of *V. agnus-castus* in diameter and the diameter was from 30.69 - 54.53 nm for AgNPs of *U. tomentosa*. On the other hand, antibacterial tests showed that these nanoparticles have antibacterial activity against the experimental pathogens ranged between 12 to 24 mm for AgNPs of *V. agnus-castus* and from 10 to 20 mm for AgNPs of *U. tomentosa*. Toxicity effect of AgNPs against cancer cells was 75.46 and 76.67 of 50 µg/ml respectively. In this study, this is the first time to use these plant extracts for antibacterial and anticancer. So, green synthesis of AgNPs via plant extracts (*V. agnus-castus* and *U. tomentosa*) can cause a huge effect in advent decades.

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الخلاصة

تهدف الدراسة الحالية إلى التخليق الحيوي لجسيمات الفضة النانوية باستخدام المستخلص المائي من نبات كف مريم *Vitex agnus-castus* (باستخدام الأوراق والزهور الجافة) وكذلك نبات مخالب القط *Uncaria tomentosa* (باستخدام الفروع ذات المخالب) والتحقيق في نشاطها ضد الميكروبي وضد الخلايا السرطانية، ومن أجل تصنيع الجسيمات النانوية الفضية ، تم تحضير المستخلص المائي للنبات ودمجه في محلول نترات الفضة 1 ملي مولاري. تم تمييز جسيمات الفضة النانوية Ag NPs المُصنَّعة باستخدام تقنيات مفيدة مختلفة ، تتضمن استخدام مجهر المسح الإلكتروني (SEM) ، والتحليل الطيفي المرئي فوق البنفسجي(Uv-Vis) ، والتحليل الطيفي للأشعة تحت الحمراء .(FTIR). لوحظ رنين البلازمون السطحي (SPR) لـ AgNPs عند 390 نانومتر باستخدام مستخلصات *V.agnus-castus* و *U. tomentosa* تم العثور على AgNPs المركب لـ *V. agnus-castus* و*U. tomentosa* بمتوسط قطر 40.85- 49.41 نانومتر ، 30.69- 54.53 نانومتر على التوالي. وجد أن النتيجة المضادة للبكتيريا (منطقة التثبيط) ضد مسببات الأمراض التجريبية تراوحت بين 12 إلى 24 ملم ومن 10 إلى 20 ملم على التوالي. كان تأثير السمية لـ AgNPs ضد الخلايا السرطانية 24.54 بتركيز 50 ميكروغرام / مل و 23.33 بتركيز 50 ميكروغرام / مل على التوالي.