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SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *COCOS NUCIFERA* L. MALE FLOWERS: AN INVESTIGATION INTO THEIR POTENT ANTIBACTERIAL AND ANTICANCER EFFICACY

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ABSTRACT. The current research focused on the production of silver nanoparticles using dichloro methane-mediated *Cocos nucifera* male flower extract and their minimum inhibitory concentration against ten different bacterial pathogens. *C. nucifera* male flowers were extracted by 100% DCM, and the phytochemicals present in this extract were studied. These plant phytochemicals act as reducing agents to reduce the silver nitrate ($AgNO_3$) to silver nanoparticles (AgNPs) which was confirmed by UV/Vis spectrophotometer, Fourier transform infra-red spectrum, X-ray diffraction analysis, and scanning electron microscopy. The 15 µL of silver nanoparticles was the minimum inhibitory concentration for *Klebsiella pneumonia* and *Bacillus subtilis*. The 20 µL of silver nanoparticles is the MIC for *Pseudomonas aeruginosa, Vibrio harveyi, and Plesiomonas shigelloides*. 30 µL of plant flower extract concentration highly inhibits the *E. coli*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Vibrio mimicus*, and *Vibrio alginolyticus* bacterial pathogens. *C. nucifera* male flowers extract derived silver nanoparticles are highly stable and have high potential activity against MCF-7 Cell line.

KEY WORDS: *Cocos nucifera*, Nanoparticles, Phytochemicals, Antimicrobials, Bacterial pathogens, MCF 7 Cell line

INTRODUCTION

Green chemistry has received high attention in all fields of science knowledge and technology expertise in the last few years. Green synthesis of nanoparticles prepared for their low toxicity, eco-friendly, and cost-effective. Green-based silver nanoparticles are used in many fields of science, including medicine [1-3]. Nano-sized particles are mainly synthesized through physical or chemical methods [4-8]. However, these types of nanoparticles are toxic to which one benefited. So, many researchers are searching for an alternative called the biosynthesis method. This biosynthesis method is a synthesis of nanoparticles with the help of bio-based materials extracted from plants, animals, microbes, and minerals [9-11]. This type of synthesized nanoparticles is eco-friendly and low-toxic compared to the chemical/physical method of synthesized nanoparticles.

The biologically synthesized silver nanoparticles perform as efficient microbial growth inhibitory agents against bacteria, fungi, viruses, etc [12]. The role of biological materials extracted from natural sources as a reducing agent in biosynthesis. The *Teucrium polium* leaf extract-based green synthesized silver nanoparticles inhibit the MNK45 human gastric cancer cell [13]. The synthesis of Ag-Nps from aqueous leaf extract of *Eichhornia crassipes* (Mart.) was used to study the cytotoxicity and genotoxicity through *the Allium cepa* assay [14]. Native rice starch-based silver nanoparticles are used as nano fertilizers for onions without any noticeable genotoxicity [15]. The *Allium ampeloprasum* aqueous extract used to synthesize the silver nanoparticles has good antioxidant, antibacterial, and cytotoxicity, as reported by Jalilian *et al*. [16]. In vivo, experimental inflammation was assessed by *Cornus sanguinea* L. fruits extract-based silver nanoparticles, and the results confirmed an increase in oxidative stress in COX-2 expressions of NFkB phosphorylation [17]. The marine red algae *Gelidium corneum*-mediated

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silver nanoparticles highly inhibit the biofilm formation [18]. The *Andrographis paniculata*-mediated eco-friendly silver nanoparticles induced mitochondrial-mediated apoptosis in parasites through oxidative stress by high ROS production. During this mechanism, the silver nanoparticles decline GST, GR, TRxR, and GSH levels [19]. The exact mechanism and toxicity of *Thymus vulgaris* leaf extract-based silver nanoparticles were studied through methicillin-resistant *Staphylococcus aureus* (MRSA) [20]. Green synthesized silver nanoparticles were used to degrade the inorganic dyes and industrial effluents [21-23]. The biological impacts of *C. nucifera* encompass antihelminthic, anti-inflammatory, antinociceptive, antioxidant, antifungal, antimicrobial, and antitumor properties [24].

The present research discussed the production of silver nanoparticles using Dichloro methane-mediated *C. nucifera* male flower extracts and their biological properties, such as minimum inhibitory concentration against ten different bacterial pathogens, antioxidants, and anticancer properties.

EXPERIMENTAL

Plant material collection and extraction

The male flowers of the coconut (*C. nucifera*) tree were purchased from Chezhiyan Organics, Tenkasi district, Tamil Nadu, India. The collected plant materials from the˜ twenty years old coconut trees from the Western Ghats, Tenkasi at the latitude and longitude range of 8°53'21.5"N and 77°19'13.1"E. The collected flowers were shade-dried at room temperature (36±1 ºC) for 10 days. The dried flowers were extracted using a solvent extraction method. Dichloro methane (100%) is used as a solvent for extraction. After extraction, excess of solvent was removed by water bath at 65 ºC.

Phytochemical study

The flower extract was analyzed their phytochemicals of carbohydrates, proteins, amino acids, alkaloids, amino acids, flavanoids, terpenoids, triterpenoids, tannins, saponin, aromatic acids, phenolic compounds, xanthoprotein, reducing sugar, phlobatinin [25].

Silver nanoparticles synthesis

The silver nanoparticles were synthesized using the green synthesis method. The 10 mL of DCM-based *C. nucifera* male flower extract was mixed with 90 mL of 1 mM silver nitrate solution at room temperature (36±1 ºC). The reaction mixture was stirred continuously to change its color [26].

Characterization silver nanoparticles

The synthesized *C. nucifera* male flower-based silver nanoparticles were characterized using ultraviolet (UV)/Vis spectrophotometer (Laboao-LU-T3C), FT-IR (Shimadzu FTIR-8400S), XRD (Rigaku), and scanning electron microscope (Jsm-6380LA).

Minimum inhibitory concentration

The minimum inhibitory concentration of plant extract and plant-based silver nanoparticles were analyzed against ten diverse gram positive and gram negative bacterial pathogens like *E. Coli*, *K. pneumonia*, *P. aeruginosa*, *B. subtilis*, *Vibrio harveyi*, *P. shigelloides*, *S. paratyphi*, *S. aureus*, *V. mimicus*, and *V. alginolyticus* through standard protocol [27].

In-vitro antioxidant studies

The synthesized Ag-NPs and *C. nucifera* male flower extract were analyzed in In-vitro antioxidant studies like the conditions of hydrogen donating ability, with the firm of radical DPPH (free radical scavenging activity), hydroxyl radical scavenging, nitric oxide scavenging, superoxide radicals scavenging activity, phospho molybdenum reduction assay, ferric reducing power (FRAP) assay, and chelation of ferrous ions (metal chelating activity) using standard protocols.

In vitro cytotoxicity and determination of cell viability by MTT assay

Determination of cytotoxic properties of *C. nucifera* male flower-based silver nanoparticles was analyzed through the selected cell line, MCF-7, using standard procedure. The MCF-7 cells were purchased from ATCC and maintained at Department of Biochemistry, College of Science, King Saud University. 96 microtitre plates were used for all experiments on cytotoxicity [17].

The percentage cytotoxicity was considered by the following calculation:

% of Growth Inhibition = 100 – (Mean OD of test group/Mean OD of the control group x 100)

RESULTS AND DISCUSSION

Plants are rich in phytochemicals like carbohydrates, phenolic compounds, sugars, alkaloids, aromatics, etc. These phytochemicals are significant in nanoparticle synthesis in the green synthesis method. Many researchers have reported the biosynthesis of silver nanoparticles with attractive biological and chemical properties [13, 22]. In this green route, the silver nitrate was reduced by plant phytochemicals to form silver nanoparticles [27]. The carbohydrate, terpenoids, phenolic compounds, and reducing sugars are the phytochemicals present in the dichloro methane-mediated *C. nucifera* male flower extract (Table 1).

Table 1. Phytochemical study of dichloro methane mediated *C. nucifera* male flowers extract.

S. No.	Test for	Coconut tree male flower extract
1	Carbohydrate	+
2	Protein	-
3	Amino acid	-
4	Alkaloids	-
5	Flavanoid	-
6	Terpenoids	+
7	Tannins	-
8	Saponins	-
9	Aromatic acids	-
10	Phenolic compounds	+
11	Xanthoprotein	-
12	Reducing sugar	+
13	Triterpenoids	-
14	Phlobatinins	-

These plant phytochemicals act as reducing agents to reduce the silver nitrate ($AgNO_3$) to silver nanoparticles (AgNPs). When the nitrate molecules (NO_3) are oxidized and Ag^+ ions are reduced by the plant-lowering agents, the color of the reaction mixture slowly changes to reddish pink, indicating silver nanoparticle synthesis (Figure 1).

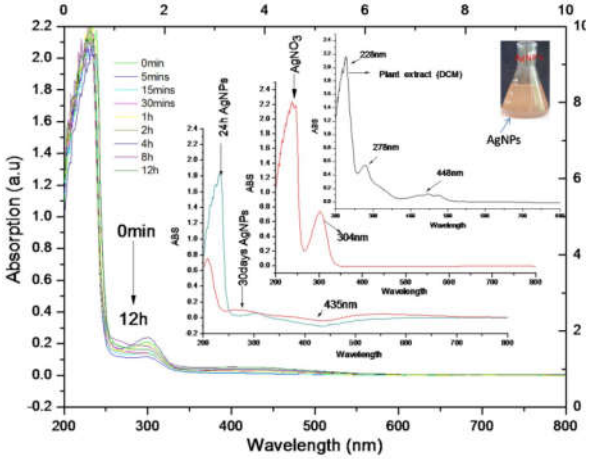

Figure 1. UV spectrum of the coconut tree male flower extract; extract mediated silver nanoparticles.

The UV spectrum of plant extract contains three major absorption peaks at 228, 278, and 448 nm. The absorption of silver nitrate has a single absorption peak located at 304 nm. The initial time of plant extract mixed silver nitrate reaction mixture has a single absorption peak at 304 nm. The reaction time was increased, and the range of absorption at 304 nm slowly decreased (0, 5, 15, 30 min, 1, 2, 4, 8, and 12 h) represented in Figure 1. The UV spectra of plant extract and silver nitrate solution mixture at 24 h had a single prominent peak at 435 nm to confirm silver nanoparticle synthesis (Figure 1). The same opinion was reported by Huang *et al*. [28]. The stability of 30 days of stored plant-based silver nanoparticles was analyzed by UV spectrum, having a single prominent absorption peak in 435 nm. This confirmed that this nanoparticle is highly stable for a long time (Figure 2a).

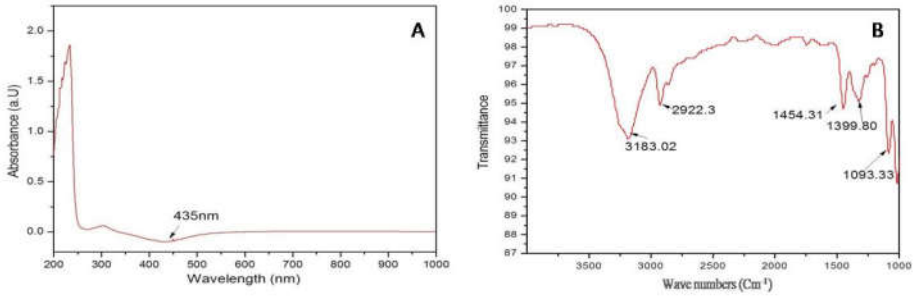

Figure 2. a) UV/Vis spectral characterization of green synthesized Ag-NPs. b) FT-IR characterization of green synthesized Ag-NPs.

Further, the synthesized nanoparticles were confirmed through FT-IR, SEM, and XRD characterization. Figure 2b depicts the FTIR analysis of Ag-NPs synthesized using plant extracts. The figure showed a strong band at 1093.33, 1399.80, 1454.31, 2922.30, and 3182.03 cm^{-1}. The FTIR bands 1454.31, 2922.30, and 3182.03 cm^{-1} confirmed the functionally active groups present in the synthesized silver nanoparticles [29]. Scanning electron microscope images to confirm that the silver nanoparticles are spherical (Figure 3).

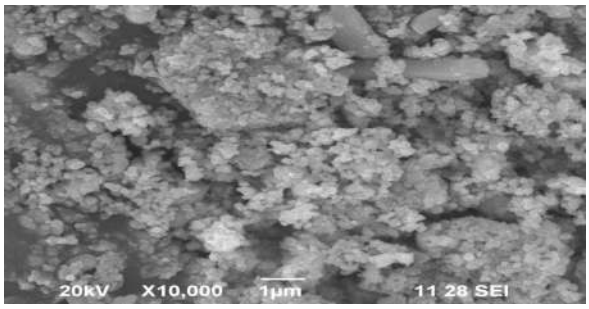

Figure 3. Scanning electron microscope images of synthesized Ag-NPs.

The XRD spectra of Ag-NPs synthesized using *C. nucifera* male flower extract are mentioned in Figure 4. Solid and sharp peaks were read at 38.52, 44.65, 64.57, and 77.60º for the spectrum ranging from 10 to 80º; the corresponding plane values were (111), (200), (220), and (311). The Scherrer equation used to calculate the size of the AgNPs, $D = k\lambda/\beta\cos\theta$.

The estimated sizes of the AgNPs from the XRD results are 48.4 nm to 65.5 nm. The secondary phase of Ag-NPs, e.g., Ag_3O_4, was from our spectral data, and its respective crystalline planes were measured. The peaks 38.52, 44.65, 64.57, and 77.60º were confirmed crystalline silver nanoparticles [30].

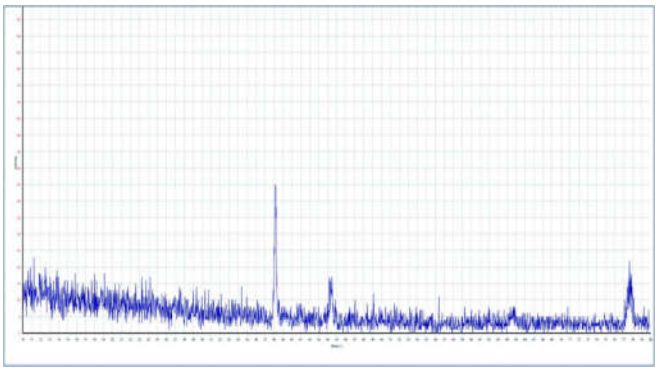

Figure 4. XRD characterization of synthesized Ag-NPs.

The minimum inhibitory concentration (MIC) of DCM-based *C. nucifera* male flower extract against different bacterial pathogens is shown in Figure 5a and Table 2. The 20 µL of plant flower extract is the MIC for *Escherichia coli*, *S. aureus, P. aeruginosa*, *B. subtilis*, *V. harveyi*, *P. shigelloides*, and *V. alginolyticus*. The 15 µL of plant extract was MIC for *Klebsiella pneumonia*. 35 µL of concentration of DCM-based *C. nucifera* male flower extract highly inhibits the *Salmonella paratyphi* bacterial pathogen. 25 µL of *C. nucifera* male flower extract concentration highly inhibits the *Vibrio mimicus* bacterial pathogen.

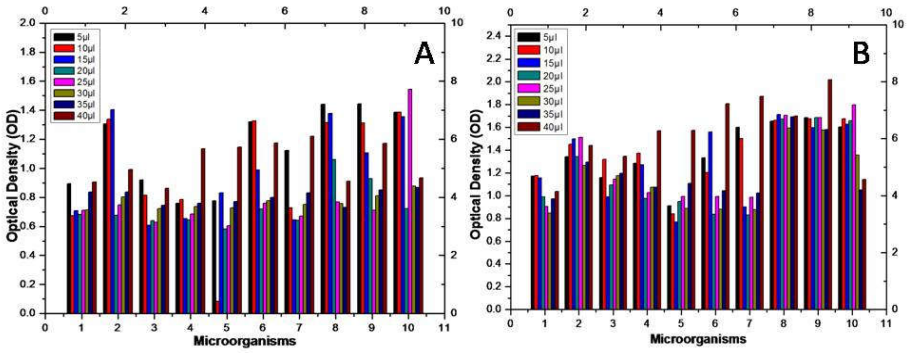

Figure 5. a) MIC of coconut tree male flower extract. b) MIC of synthesized silver nanoparticles. 1. *E. coli*, 2. *Staphylococcus aureus*, 3. *Klebsiella pneumonia*, 4. *Pseudomonas aeruginosa*, 5. *Bacillus subtilis*, 6. *Vibrio harveyi*, 7. *Plesiomonas shigelloides*, 8. *Salmonella paratyphi*, 9. *Vibrio mimicus*, and 10. *Vibrio alginolyticus*.

Table 2. Optical density values of coconut tree male flower extract MIC.

Microorganisms	The OD value of coconut tree male flower extract MIC							
	5 µl	10 µl	15 µl	20 µl	25 µl	30 µl	35 µl	40 µl
E. Coli	0.893	0.673	0.709	0.680*	0.710	0.712	0.835	0.905
Staphylococcus aureus	1.307	1.339	1.405	0.677*	0.748	0.803	0.835	0.992
Klebsiella pneumoniae	0.919	0.813	0.606*	0.638	0.628	0.723	0.746	0.864
Pseudomonas aeruginosa	0.759	0.786	0.653	0.645*	0.685	0.735	0.760	1.133
Bacillus subtilis	0.777	0.083	0.830	0.582*	0.605	0.727	0.769	1.146
Vibrio harveyi	1.321	1.326	0.988	0.718*	0.760	0.777	0.799	1.174
Plesiomonas shigelloides	1.124	0.726	0.645	0.642*	0.670	0.751	0.831	1.221
Salmonella paratyphi	1.440	1.314	1.377	1.059	0.767	0.757	0.731*	0.910
Vibrio mimicus	1.445	1.312	1.105	0.929	0.711*	0.811	0.851	1.172
Vibrio alginolyticus	1.387	1.388	1.355	0.722*	1.545	0.877	0.868	0.934
Control	0.657	0.434	0.556	0.627	0.633	0.657	0.729	1.000

The MIC of plant flower extract-based silver nanoparticles against different bacterial pathogens is shown in Figure 5b and Table 3. The 15 µL of silver nanoparticles was MIC for *Klebsiella pneumonia* and *Bacillus subtilis*. The 20 µL of silver nanoparticles is the MIC for *Pseudomonas aeruginosa*, *Vibrio harveyi*, and *Plesiomonas shigelloides*. 30 µL of plant flower extract concentration highly inhibits the *E. coli*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Vibrio mimicus*, and *Vibrio alginolyticus* bacterial pathogens.

The observed DPPH* scavenging radical activity of DCM-based *C. nucifera* male flower extract and selected plant extract-mediated nanoparticles is given in Figure 6a. The test silver nanoparticles showed higher DPPH* scavenging activity than the plant extracts. The *OH scavenging ability, Superoxide radical scavenging activity, and Nitric oxide–scavenging activity of isolated plant compounds and Ag-NPs were shown in Figures 6a, b, and c. The synthesized silver nanoparticles were more active than the *C. nucifera* male flower extract.

Table 3. Optical density values of coconut tree male flower extract mediated silver nanoparticles MIC.

Microorganisms	The OD value of Ag-NPs MIC							
	5 µl	10 µl	15 µl	20 µl	25 µl	30 µl	35 µl	40 µl
E. Coli	1.174	1.177	1.156	0.990	0.904	0.848*	0.971	1.038
Staphylococcus aureus	1.343	1.452	1.499	1.343	1.512	1.265*	1.294	1.440
Klebsiella pneumoniae	1.158	1.317	0.988*	1.093	1.145	1.175	1.198	1.346
Pseudomonas aeruginosa	1.284	1.372	1.271	0.977*	1.027	1.073	1.075	1.569
Bacillus subtilis	0.909	0.840	0.768*	0.949	0.993	0.887	1.109	1.572
Vibrio harveyi	1.331	1.202	1.560	0.836*	0.991	0.881	1.042	1.808
Plesiomonas shigelloides	1.599	1.503	0.900	0.828*	0.985	0.876	1.022	1.873
Salmonella paratyphi	1.654	1.661	1.712	1.673	1.707	1.593*	1.691	1.697
Vibrio mimicus	1.686	1.675	1.597	1.686	1.686	1.575*	1.579	2.017
Vibrio alginolyticus	1.605	1.675	1.627	1.659	1.798	1.354*	1.051	1.141
Control	0.767	0.604	0.875	0.603*	0.620	0.649	0.708	1.227

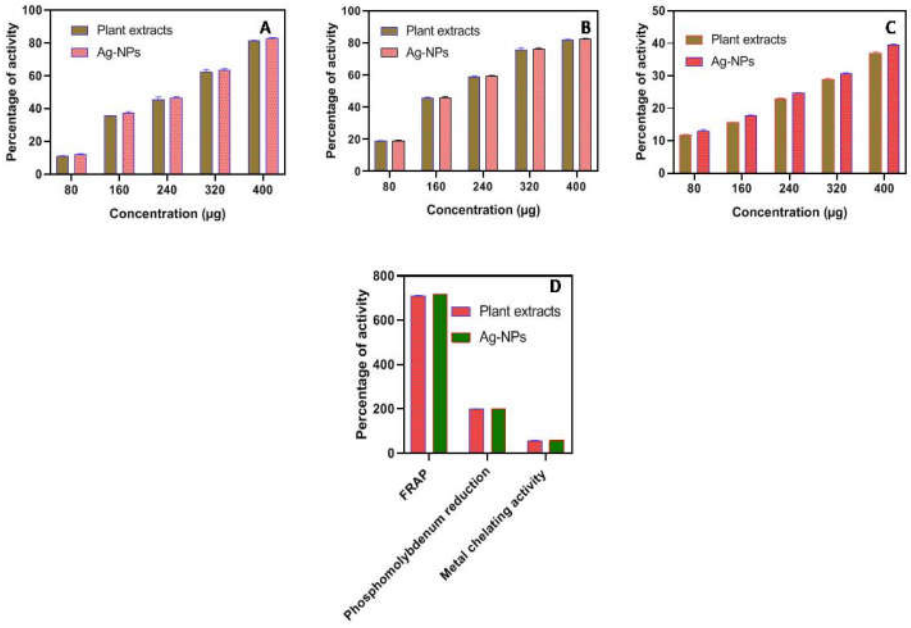

Figure 6. a) DPPH radical activity. b) Hydroxyl radical scavenging activity. c) Superoxide radical scavenging activity. d) FRAP, Phosphomolybdenum reduction and metal chelating property of DCM based *Cocas nucifera* male flower extract and Ag-NPs.

The *C. nucifera* male flower extract and plant extract mediated Ag-Nps were analyzed for FRAP, phosphohomolybdenum reduction, and metal chelating properties. The FRAP phoshomolybdenum reduction and metal chelating properties of *C. nucifera* male flower extract were followed by 709.41 ± 1.018, 198.86 ± 1.506, and 57.65 ± 0.390. The FRAP phoshomolybdenum reduction and metal chelating property of *C. nucifera* male flower extract

mediated silver nanoparticles were followed by 716.22 ± 0.610, 199.35 ±0.742, and 58.55 ±0.636 (Figure 6d).

The cytotoxic effects of isolated compounds from coconut tree male flower extracts and AgNPs were tested against the MCF-7 cell line through MTT assay (Figure 7a, b and c). Cells were treated with increasing concentrations of isolated compounds for 3 days. Figure 7 shows a dose-dependent increase in the proportion of death cells among *MCF-7* cells. More than 73% of cells died in response to 1000 µg/mL of silver nanoparticles, and at the same concentration, around 40% of MCF-7 cells died by coconut tree male flower extracts (Figure 7a). The silver nanoparticles induced cellular toxicity by generating high-level ROS, which can lead to MCF-7 cell death for free radical formation [31 - 33].

Figure 7. a) Anticancer studies of DCM based *cocas nucifera* male flower extract and Ag-NPs against MCF 7 cell line. b) Microscopic images of *cocas nucifera* male flower extract treated MCF 7 cell line. c) Ag-NPs treated MCF 7 cell line.

CONCLUSION

The synthesized silver nanoparticles through Dichloro methane mediated *C. nucifera* male flowers extract contains carbohydrates, terpenoids, phenolic compounds, and reducing sugars that act as reducing agents to reduce the silver nitrate ($AgNO_3$) to silver nanoparticles (AgNPs). The 20 µL of plant flower extract is the MIC for *Escherichia coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *Vibrio harveyi*, *Plesiomonas shigelloides*, and *Vibrio alginolyticus*. The 15 µL of plant extract was MIC for *Klebsiella pneumonia*. 35 µL of plant flower extract concentration highly inhibits the *Salmonella paratyphi* bacterial pathogen. 25 µL of plant flower extract concentration highly inhibits the *Vibrio mimicus* bacterial pathogen. The 15 µL of silver nanoparticles was MIC for *Klebsiella pneumonia* and *Bacillus subtilis*. The 20 µL of silver nanoparticles is the MIC for *Pseudomonas aeruginosa*, *Vibrio harveyi*, and *Plesiomonas shigelloides*. 30 µL of DCM extract of *C. nucifera* male flowers highly inhibits *E. coli*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Vibrio mimicus*, and *Vibrio alginolyticus* bacterial pathogens. As confirmed by the UV spectral study, the synthesized silver nanoparticles are highly stable for a long time. *C. nucifera* male flowers extract mediated silver nanoparticles are highly stable and have high potential activity against MCF-7 Cell lines.

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