

## Supplementary data

# Bacterial Responses to *Ephedra aphylla* Extract and Green-Synthesized Ag-TiO<sub>2</sub> and Ag-SeO<sub>2</sub> Core/Shell Nanocomposites: Unveiling Antimicrobial and Antioxidant Properties

## Section S1: Materials and Methods

### Reagents

Folin-Ciocalteu reagent (analytical grade, Fluka, Biochemical Inc., Bucharest, Romania), Gallic acid ( $\geq 98\%$ , Biomedical Inc., Orange City, FL, USA), 1,1-Diphenyl-2-picrylhydrazyl (DPPH $\cdot$ ) ( $\geq 97\%$ ), aluminum chloride (anhydrous,  $\geq 99\%$ ), sodium hydroxide (pellets,  $\geq 99\%$ ), sodium nitrite ( $\geq 99\%$ ), catechin hydrate ( $\geq 98\%$ ), vanillin ( $\geq 99\%$ ), hydrochloric acid (37%), and ascorbic acid ( $\geq 99\%$ ) were purchased from Sigma Aldrich (St. Louis, USA). Sodium Carbonate (analytical grade), and tannic acid ( $\geq 98\%$ ) (El-Nasr Pharmaceutical Chemicals, Cairo, Egypt). Silver nitrate (AgNO<sub>3</sub>) was purchased from PIOCHEM for laboratory chemicals; CAS Number: 7761-88-8; purity: 99.5%, titanium and selenium dioxides ( $\geq 99\%$ ) were purchased from Sigma Aldrich, St. Louis, USA.

### Phytochemical Analysis

#### Folin-Ciocalteu Assay

The total phenolic content in the samples was determined using the Folin-Ciocalteu assay according to [1]. Briefly, 100  $\mu$ L of the sample was transferred to a cuvette. Then, 5 mL of Folin-Ciocalteu reagent was added (diluted reagent (1 mL) with distilled water (9 mL)), and the mixture was mixed thoroughly. After 5 minutes of incubation, 4 mL of 7.5% sodium carbonate solution was added followed by further mixing. The final volume was adjusted by distilled water to 10 mL. The mixture was allowed to stand for 30 minutes at 40 °C in the dark. The absorbance of the blue-colored solution was then measured at 765 nm using a spectrophotometer. To quantify the phenolic content, a standard curve of gallic acid was prepared in the concentration range of 0-100 mg/L. The absorbance values of the standards were plotted against their corresponding concentrations, and the linear regression equation was obtained. The phenolic content of the samples was then calculated by interpolating the absorbance values of the samples into the standard curve equation and expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry sample (mg GAE/g DW).

### **Aluminum Chloride Colorimetric Assay**

The flavonoid content in the samples was determined using the aluminum chloride colorimetric assay [2]. Briefly, 100  $\mu$ L of the sample was transferred to a cuvette. Distilled water (4 mL) was added, followed by the addition of 0.3 mL of 5% sodium nitrite solution. The mixture was mixed thoroughly and allowed to stand for 5 minutes. Subsequently, 0.3 mL of 10% aluminum chloride solution was added, mixed well, and incubated for 6 minutes. Next, 2 mL of 1 M sodium hydroxide solution was added, and mixed thoroughly, and the mixture was allowed to stand for 15 minutes at room temperature. The final volume was adjusted by distilled water to 10 mL. Finally, the absorbance of the orange solution was measured at 510 nm using a spectrophotometer. To quantify the flavonoid content, a standard curve of quercetin was prepared in the concentration range of 0-100 mg/L. The absorbance values of the standards were plotted against their corresponding concentrations, and the linear regression equation was obtained. The flavonoid content of the samples was then calculated by interpolating the absorbance values of the samples into the standard curve equation and expressed as milligrams of quercetin equivalents (mg QE) per gram of dry sample (mg QE/g DW).

### **Vanillin-Hydrochloride Assay**

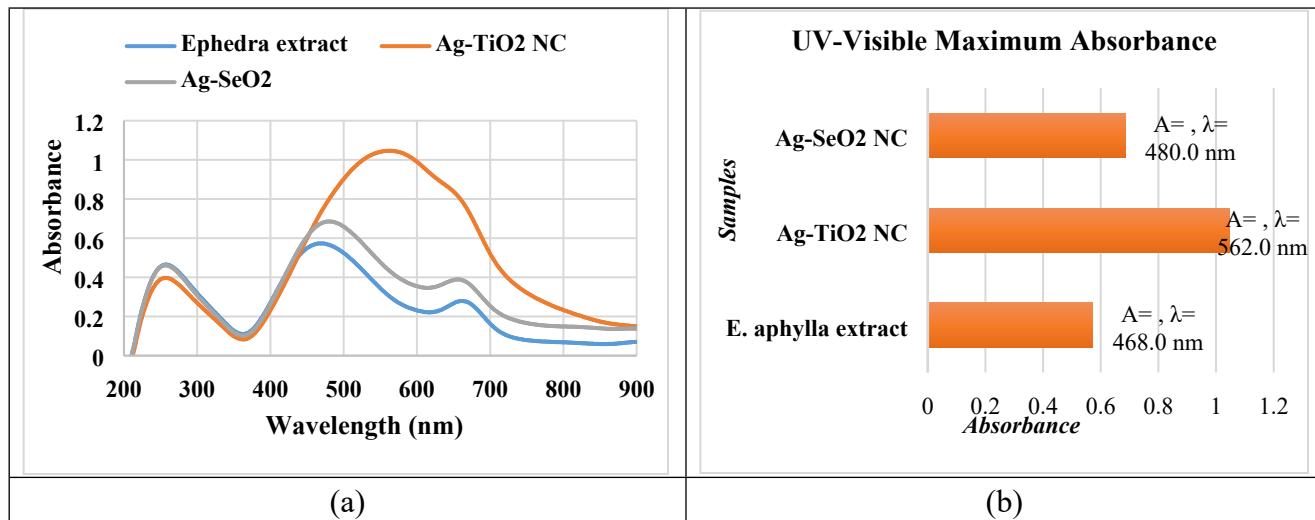
The tannin content in samples was determined using a modified vanillin-hydrochloride assay according to the reported work by Aberoumand [3]. Briefly, freshly prepared vanillin-hydrochloride reagent 5 mL (prepared by dissolving equivalent amounts of hydrochloric acid (30%) in methanol with vanillin solution in methanol (4%)) was added to the samples (1 mL) followed by incubation for 20 minutes. The absorbance of the orange solution was measured at 510 nm using a spectrophotometer. A standard curve of tannic acid was prepared and used to convert the absorbance values of the samples to tannic acid equivalents (TAE). The results were expressed as grams of TAE per 100 grams of dry extract.

### **Statistical analysis**

The results were articulated as means  $\pm$  standard deviation (SD). All analyses of *Ephedra aphylla* extract and the nanocomposites were implemented in triplicate. The mean data of different samples were analyzed by the Statistical Package for Social Sciences (SPSS, version 21). Statistical significance was determined using a one-way analysis of variance. Differences were deliberated statistically significant when the *p*-value was  $\leq 0.05$ .

## Section S2: Characterization of Nanocomposites

### UV-Visible Spectroscopy



**Figure S1.** UV-visible spectroscopy of *E. aphylla* extract and nanocomposites. (a) Presented the UV-visible scale diagram of the scanned samples at different wavelengths (nm). (b) Presented the maximum absorbance recorded by each sample at varied wavelengths.

### FTIR Spectroscopy

**Table S1** summarizes the key functional groups identified in the FTIR spectra of *E. aphylla* extract, Ag-TiO<sub>2</sub>, and Ag-SeO<sub>2</sub> NCs. The absence of certain peaks in the nanomaterial spectra, such as those associated with O-H and C=O stretching vibrations, indicates the participation of these functional groups in the biosynthesis process. The emergence of new peaks, such as those associated with metal-oxygen bonds, confirms the successful formation of the desired nanomaterials.

**Table S1:** FTIR Analysis of *E. aphylla* Extract and Greenly Synthesized Nanomaterials.

Wavenumber (cm <sup>-1</sup> )	<i>E. aphylla</i> Extract	Ag-TiO <sub>2</sub> NC	Ag-SeO <sub>2</sub> NC	Interpretation
3550-3540	Present	Absent	Absent	O-H stretching (alcohols, phenols)
2989, 2965, 2915, 2848	Present	Present	Present	C-H stretching (aliphatic)
1709	Present	Absent	Absent	C=O stretching (carbonyl groups)
1601	Present	Absent	Absent	C=C stretching (aromatic)
1469-1447, 1408, 1379	Present	Present	Present	C-H bending (aliphatic)
1254, 1226, 1078, 1044, 897, 868, 762, 718	Present	Absent	Absent	C-O stretching (alcohols, ethers, esters)

1105	Absent	Present	Present	Ti-O-Ti stretching ( $\text{TiO}_2$ )
1034, 822, 762, 709, 665, 490, 430, 378	Absent	Absent	Present	Se-O, Ag-O, and other metal-oxygen bonds ( $\text{Ag}-\text{SeO}_2$ )
414, 378	Absent	Present	Present	Metal-oxygen bonds (Ag-O, Ti-O)

### Zeta Potential Analysis

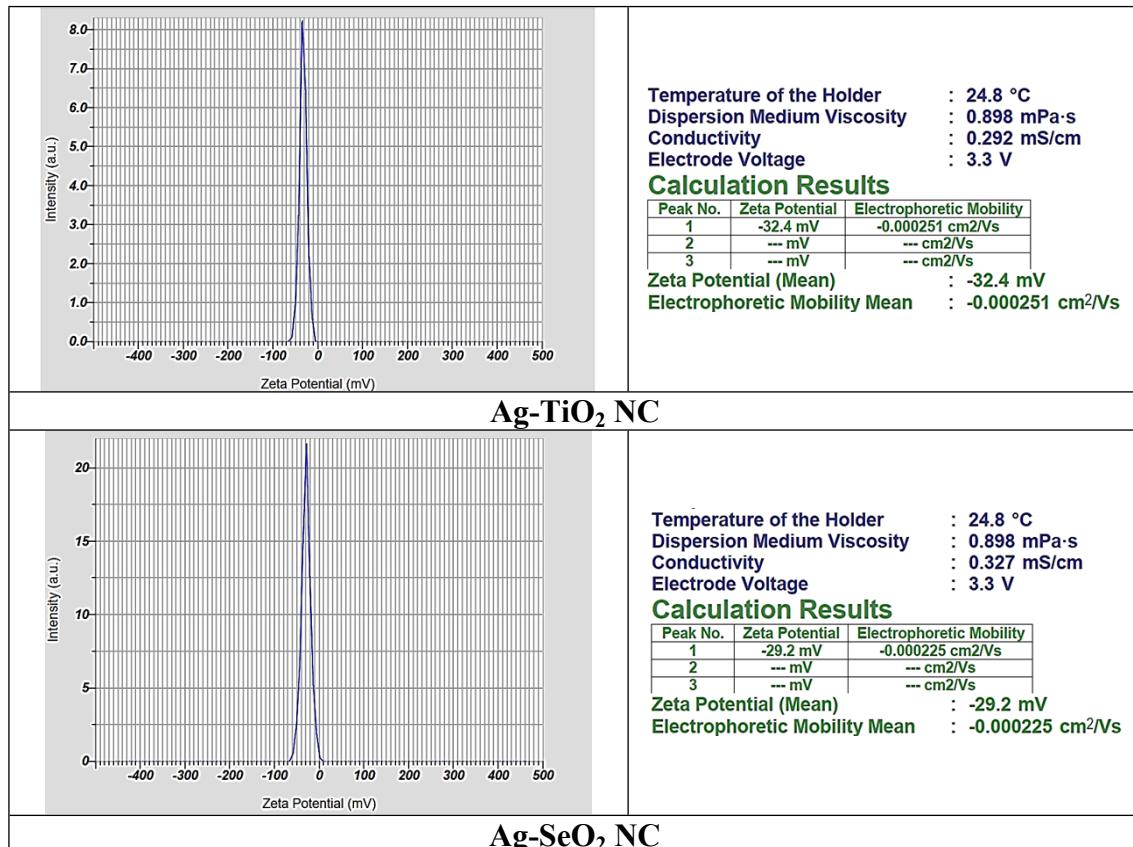


Figure S2. Zeta potential analysis of  $\text{Ag}-\text{TiO}_2$  and  $\text{Ag}-\text{SeO}_2$  NC.

### Dynamic Light Scattering (DLS)

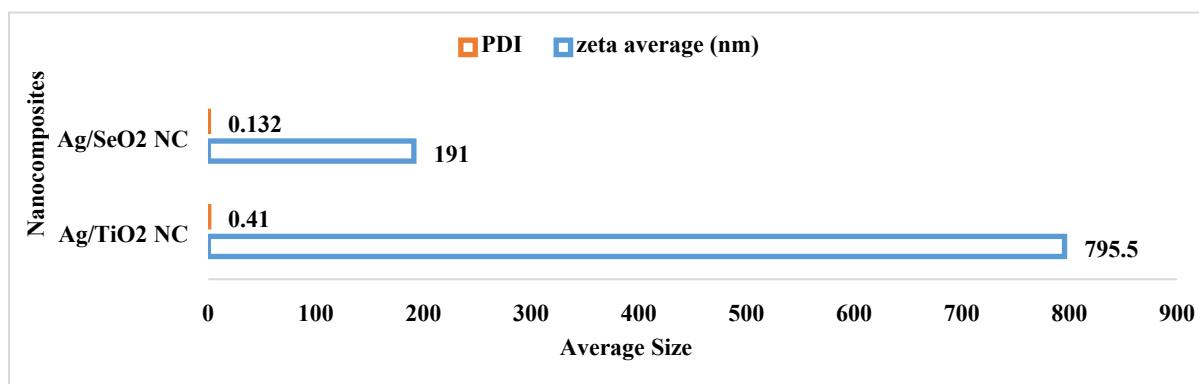
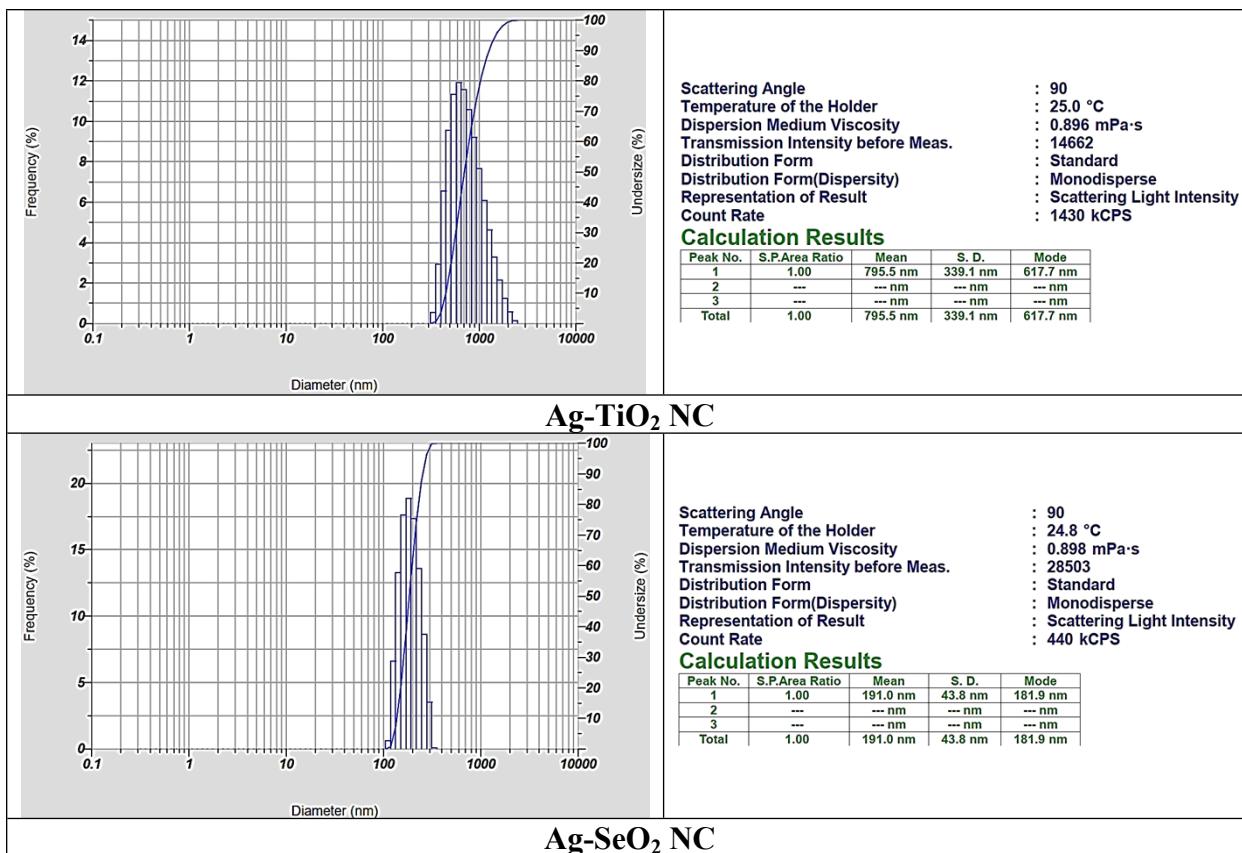


Figure S3. Dynamic Light Scattering (DLS) results of  $\text{Ag}-\text{TiO}_2$  and  $\text{Ag}-\text{SeO}_2$  NC.



**Figure S4.** DLS analysis of Ag-TiO<sub>2</sub> and Ag-SeO<sub>2</sub> NC.

### Energy-dispersive X-ray spectroscopy (EDX)

**Table S2.** The results of EDX analysis of Ag/TiO<sub>2</sub> and Ag/SeO<sub>2</sub> nanocomposites.

Ag-TiO <sub>2</sub> NC		
Element	Weight %	Atomic %
C	16.13	20.43
O	43.64	52.53
Ti	15.02	16.01
Ag	25.21	12.03
Ag-SeO <sub>2</sub> NC		
Element	Weight %	Atomic %
C	33.63	45.29
O	28.50	34.02
Se	12.63	11.33
Ag	25.24	14.36

## X-ray Diffraction (XRD)

### Scan Parameters of Ag-TiO<sub>2</sub> NC:

Operator	cwi
Raw Data Origin	XRD measurement (*.XRDML)
Scan Axis	Gonio
Start Position [°2Th.]	5.0100
End Position [°2Th.]	89.9900
Step Size [°2Th.]	0.0200
Scan Step Time [s]	0.3000
Scan Type	Continuous
Offset [°2Th.]	0.0000
Divergence Slit Type	Fixed
Divergence Slit Size [°]	0.4785
Specimen Length [mm]	10.00
Receiving Slit Size [mm]	0.1000
Measurement Temperature [°C]	25.00
Anode Material	Cu
K-Alpha1 [Å]	1.54060
K-Alpha2 [Å]	1.54443
K-Beta [Å]	1.39225
K-A2 / K-A1 Ratio	0.50000
Generator Settings	30 mA, 40 kV
Diffractometer Type	0000000000005545
Diffractometer Number	0
Goniometer Radius [mm]	240.00
Dist. Focus-Diverg. Slit [mm]	91.00
Incident Beam Monochromator	No
Spinning	No

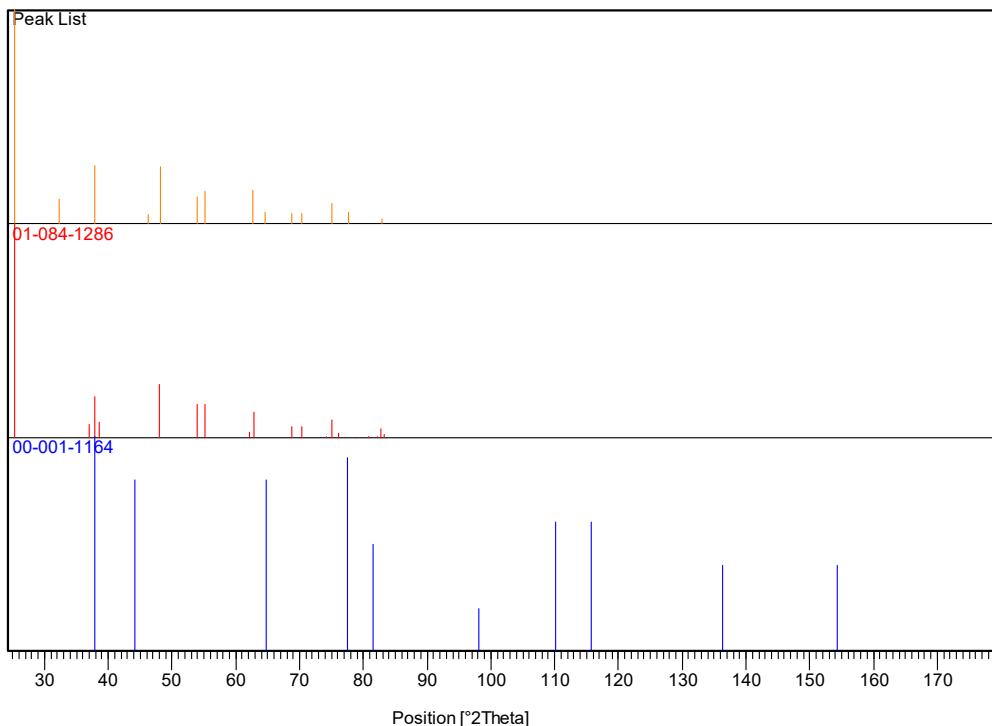
**Table S3.** Peak List of Ag-TiO<sub>2</sub> NC from XRD Analysis

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Tip width [°2Th.]	Matched by
25.3705	109.18	0.1378	3.51073	100.00	0.1653	01-084-1286
32.2905	12.45	0.1968	2.77242	11.40	0.2362	
37.9114	29.66	0.4723	2.37330	27.17	0.5668	01-084-1286; 00-001-1164
46.2985	4.65	0.2362	1.96103	4.26	0.2834	
48.0907	28.84	0.1968	1.89206	26.42	0.2362	01-084-1286
53.9631	13.96	0.3149	1.69921	12.79	0.3779	01-084-1286
55.1590	16.58	0.3149	1.66517	15.19	0.3779	01-084-1286
62.7276	17.05	0.1181	1.48123	15.62	0.1417	01-084-1286
64.5772	5.81	0.4723	1.44321	5.32	0.5668	00-001-1164
68.7912	5.25	0.3149	1.36472	4.80	0.3779	01-084-1286
70.4096	5.37	0.3149	1.33727	4.92	0.3779	01-084-1286
75.1112	10.45	0.3936	1.26480	9.58	0.4723	01-084-1286
77.6040	5.95	0.7872	1.23029	5.45	0.9446	00-001-1164

82.8935	2.60	1.1520	1.16373	2.38	1.3824	01-084-1286
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**Table S4.** Identified Patterns List of Ag-TiO<sub>2</sub> NC:

Visible	Ref. Code	Score	Compound Name	Displacement [°2Th.]	Scale Factor	Chemical Formula
*	01-084-1286	65	Anatase, syn	0.000	0.865	TiO <sub>2</sub>
*	00-001-1164	15	Silver	0.000	0.117	Ag



**Figure S5.** Plot of Identified Phases of Ag-TiO<sub>2</sub> NC.

**Scan Parameters of Ag-SeO<sub>2</sub> NC:**

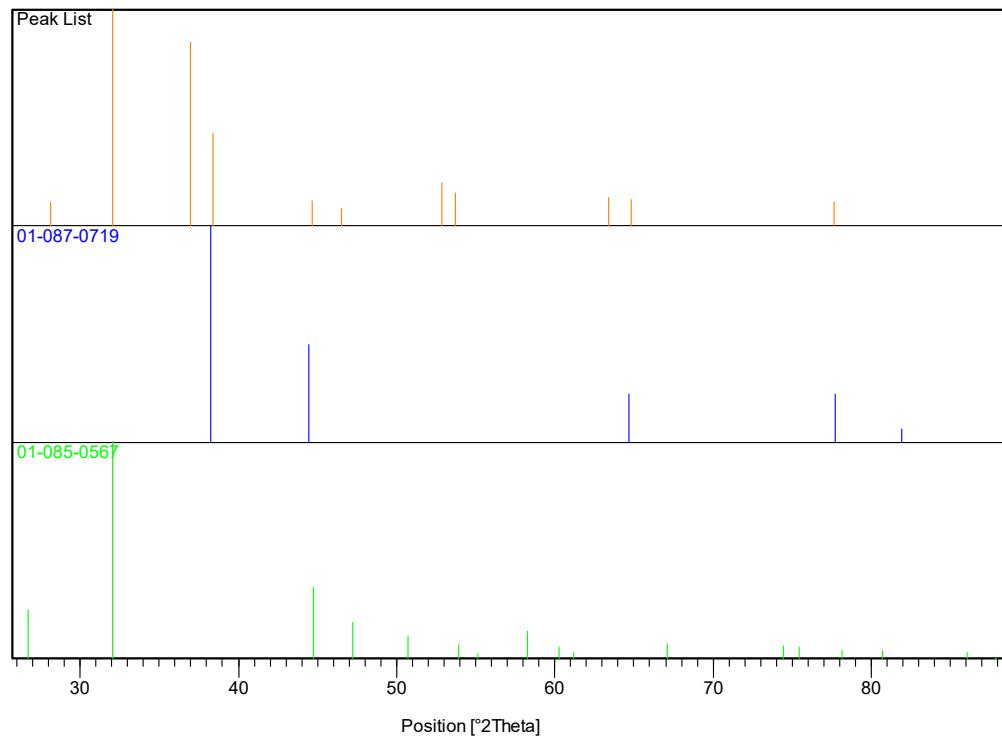
Operator	cwi
Raw Data Origin	XRD measurement (*.XRDML)
Scan Axis	Gonio
Start Position [°2Th.]	5.0100
End Position [°2Th.]	89.9900
Step Size [°2Th.]	0.0200
Scan Step Time [s]	0.3000
Scan Type	Continuous
Offset [°2Th.]	0.0000
Divergence Slit Type	Fixed
Divergence Slit Size [°]	0.4785
Specimen Length [mm]	10.00
Receiving Slit Size [mm]	0.1000
Measurement Temperature [°C]	25.00
Anode Material	Cu
K-Alpha1 [Å]	1.54060
K-Alpha2 [Å]	1.54443
K-Beta [Å]	1.39225
K-A2 / K-A1 Ratio	0.50000
Generator Settings	30 mA, 40 kV
Diffractometer Type	0000000000005545
Diffractometer Number	0
Goniometer Radius [mm]	240.00
Dist. Focus-Diverg. Slit [mm]	91.00
Incident Beam Monochromator	No
Spinning	No

**Table S5.** Peak List of Ag-SeO<sub>2</sub> NC from XRD Analysis:

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Tip width [°2Th.]	Matched by
28.1142	5.89	0.2362	3.17403	11.14	0.2834	
32.0635	52.91	0.1968	2.79153	100.00	0.2362	01-085-0567
36.9520	44.86	0.1574	2.43269	84.78	0.1889	
38.3697	22.56	0.3936	2.34600	42.63	0.4723	01-087-0719
44.6656	6.21	0.9446	2.02886	11.73	1.1336	01-087-0719; 01-085-0567
46.5360	4.14	0.3149	1.95158	7.82	0.3779	
52.8852	10.40	0.2362	1.73127	19.65	0.2834	
53.7036	7.97	0.4723	1.70681	15.07	0.5668	01-085-0567
63.4276	6.87	0.6298	1.46656	12.98	0.7557	
64.8311	6.31	0.4723	1.43817	11.94	0.5668	01-087-0719
77.6319	5.93	0.9600	1.22890	11.20	1.1520	01-087-0719; 01-085-0567

**Table S6.** Identified Patterns List of Ag-SeO<sub>2</sub> NC:

Visible	Ref. Code	Score	Compound Name	Displacement [°2Th.]	Scale Factor	Chemical Formula
*	01-087-0719	33	Silver 3C	0.000	0.591	Ag
*	01-085-0567	17	Selenium, syn	0.000	0.923	Se



**Figure S6.** Plot of Identified Phases of Ag-SeO<sub>2</sub> NC:

### Section S3: Phytochemical and Biological Profile

#### Phytochemical Analysis

**Table S7.** The results of the phytochemical analysis of the investigated extracted samples.

Samples	Phenolics Content <sup>[a]</sup>	Flavonoids Content <sup>[b]</sup>	Tannins Content <sup>[c]</sup>
<i>Ephedra aphylla</i> extract	193.28±1.73	76.55±1.48	123.8±1.77
Ag-TiO <sub>2</sub> NC	129.53±1.09	66.19±1.18	90.2±1.86
Ag-SeO <sub>2</sub> NC	82.889±1.51	17.89±1.89	28.83±1.31

<sup>[a]</sup> Phenolic Content “mg gallic acid/1 gm dry sample”

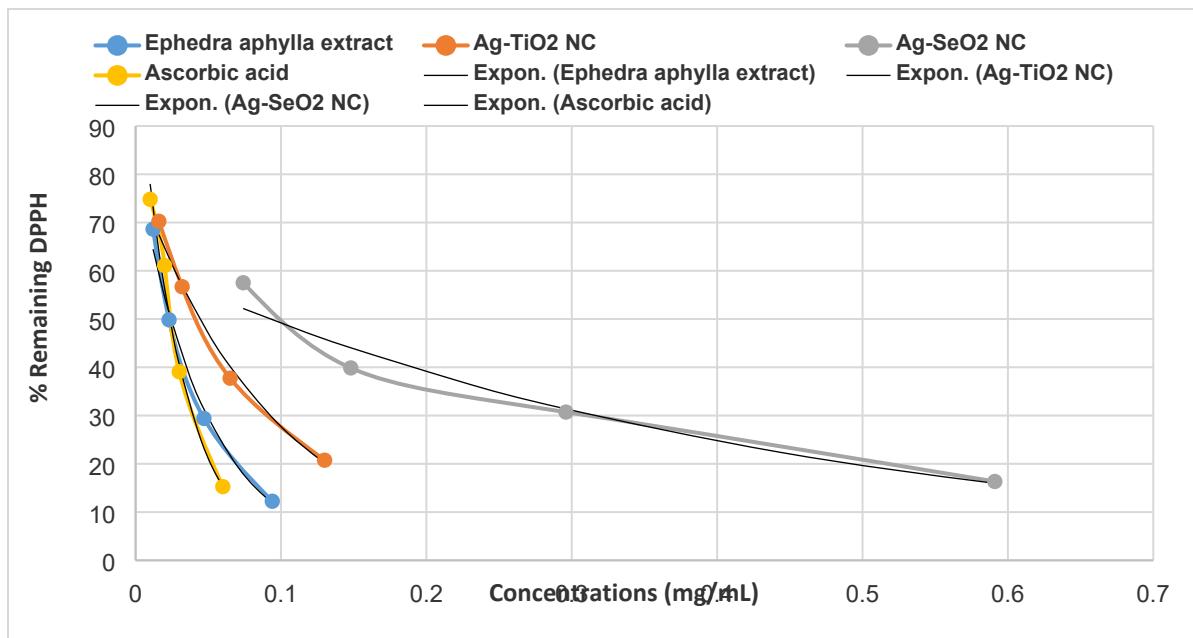
<sup>[b]</sup> Flavonoid Content “mg catechin acid/1 gm dry sample”

<sup>[c]</sup> Tannins Contents “mg tannic acid acid/1 gm dry sample”

#### Antioxidant Activity

**Table S8.** The antioxidant results of the tested samples.

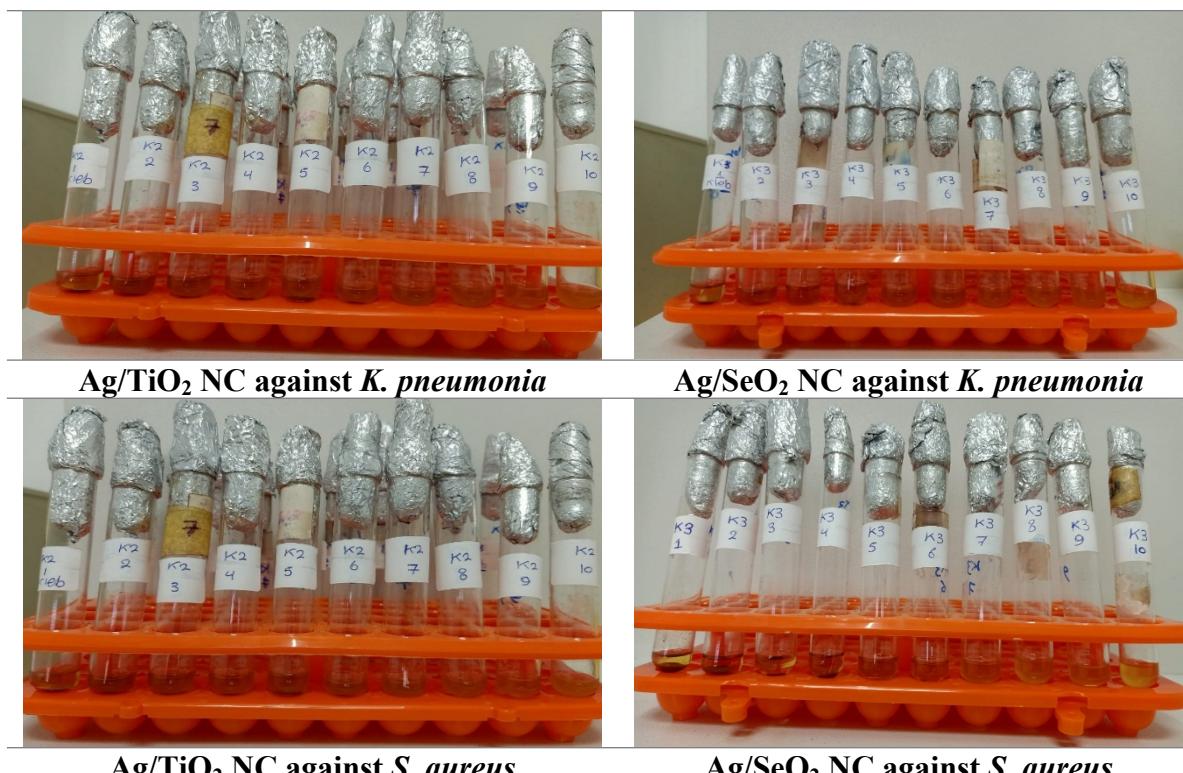
Samples	Concentrations (mg/mL)	% Remaining DPPH	% Scavenging Activity	IC <sub>50</sub> (mg/mL)
<i>Ephedra aphylla</i> extract	0.094	12.25	87.75	0.024
	0.047	29.41	70.59	
	0.023	49.84	50.16	
	0.012	68.63	31.37	
Ag-TiO <sub>2</sub> NC	0.13	20.75	79.25	0.044
	0.065	37.75	62.25	
	0.032	56.7	43.3	
	0.016	70.26	29.74	
Ag-SeO <sub>2</sub> NC	0.591	16.34	83.66	0.092
	0.296	30.72	69.28	
	0.148	39.87	60.13	
	0.074	57.52	42.48	
Ascorbic acid	0.06	15.27	84.73	0.022
	0.03	39.08	60.92	
	0.02	61.07	38.93	
	0.01	74.81	25.19	



**Figure S7.** The relationship between sample concentration (mg/mL) versus % Remaining DPPH.

### Antibacterial Activity

#### Minimum Inhibitory Concentration (MIC)



**Figure S8.** The images of the MIC test for Ag/SeO<sub>2</sub> NC against *K. pneumonia* and *S. aureus* pathogenic species.

## Minimum Inhibitory Concentration (MIC)

### 1. *Staphylococcus aureus*

**Table S9.** After 24 h of incubation at 37 °C, turbidity was noticed in test tube 3 whereas in tube 2 (MIC) no turbidity was seen, exhibiting inhibition of bacterial growth.

#### The measured O.D 600 for *Staphylococcus aureus*

Test tube no.	Concentration (mg/mL)	Ag/TiO <sub>2</sub> NC
1	16.58	0.008
2	8.29	<b>0.033</b>
3	4.145	1.043
4	2.0725	1.269
5	1.0363	1.415
6	0.5181	1.575
7	0.2591	1.570
8	0.1295	1.633
9	0.0648	1.708
10	0.0324	1.787

**MIC tube:** NO 2

**Table S10.** After 24 h of incubation at 37 °C, turbidity was noticed in test tube 7 whereas in tube 6 (MIC) no turbidity was seen, exhibiting inhibition of bacterial growth.

#### The measured O.D 600 for *Staphylococcus aureus*

Test tube no.	Concentration (mg/mL)	Ag/SeO <sub>2</sub> NC
1	9.46	0.000
2	4.73	0.002
3	2.365	0.001
4	1.1825	0.000
5	0.5913	0.006
6	0.2956	<b>0.034</b>
7	0.1478	0.196
8	0.0739	0.364
9	0.037	0.487
10	0.0185	0.684

**MIC tube:** NO 6

## 2. *Klebsiella pneumonia*

**Table S11.** After 24 h of incubation at 37 °C, turbidity was noticed in test tube 4 whereas in tube 3 (MIC) no turbidity was seen, exhibiting inhibition of bacterial growth.

### The measured O.D 600 for *Klebsiella pneumonia*

Test tube no.	Concentration (mg/mL)	Ag/TiO <sub>2</sub> NC
1	16.58	0.000
2	8.29	0.002
3	4.145	<b>0.050</b>
4	2.0725	0.832
5	1.0363	0.941
6	0.5181	1.421
7	0.2591	1.561
8	0.1295	1.781
9	0.0648	1.641
10	0.0324	1.521

**MIC tube: NO 3**

**Table S12.** After 24 h of incubation at 37 °C, turbidity was noticed in test tube 3 whereas in tube 2 (MIC) no turbidity was seen, exhibiting inhibition of bacterial growth.

### The measured O.D 600 for *Klebsiella pneumonia*

Test tube no.	Concentration (mg/mL)	Ag/SeO <sub>2</sub> NC
1	9.46	0.000
2	4.73	<b>0.002</b>
3	2.365	0.759
4	1.1825	1.095
5	0.5913	1.309
6	0.2956	1.307
7	0.1478	1.357
8	0.0739	1.441
9	0.037	1.520
10	0.0185	1.605

**MIC tube: NO 2**

### FTIR Spectral Charts of Fungal Species treated and untreated with Ag/SeO<sub>2</sub> NC

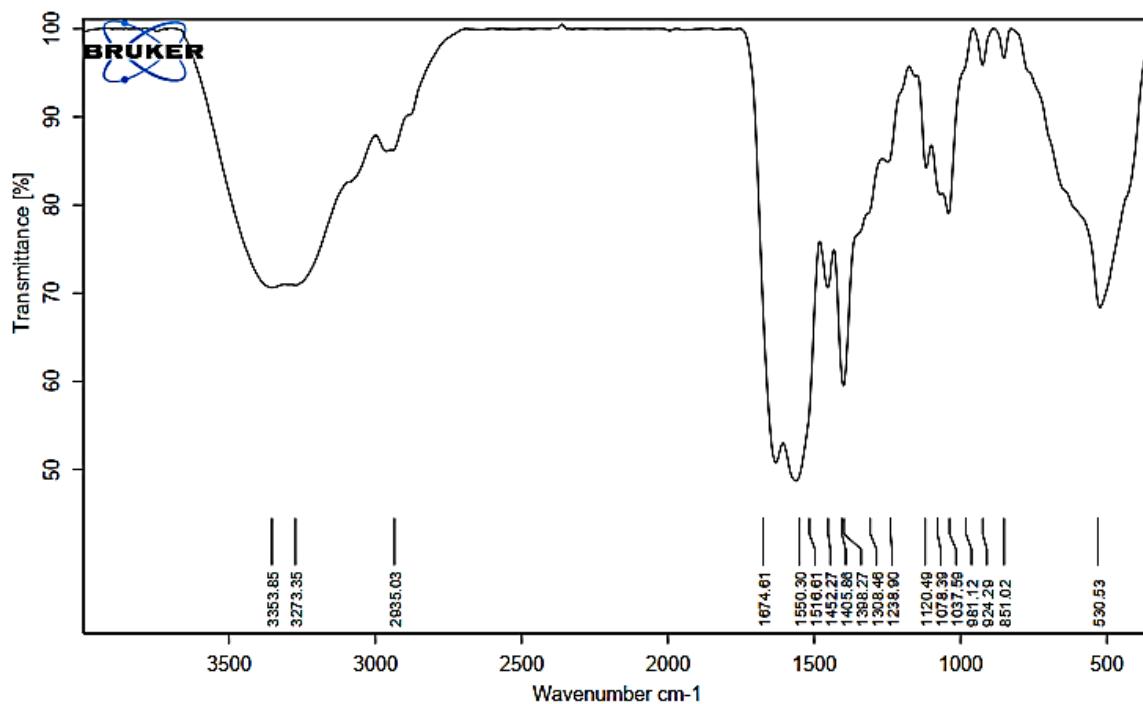


Figure S9. FTIR spectral chart of *C. glabrata* untreated with Ag-SeO<sub>2</sub> NC.

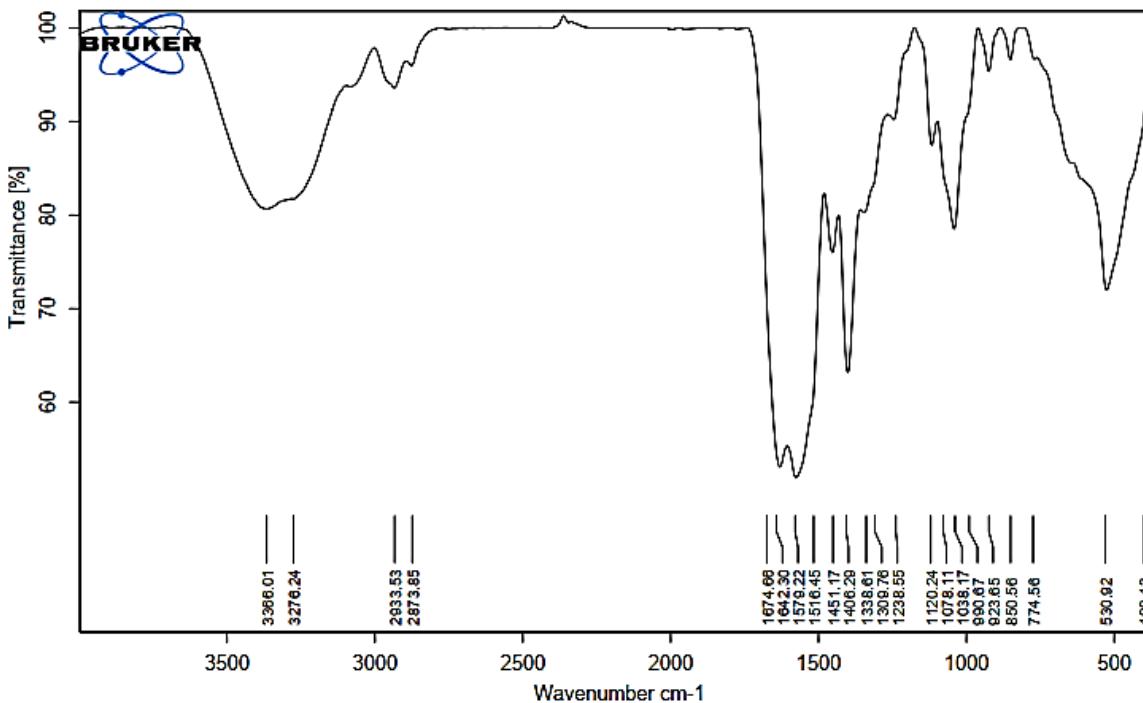
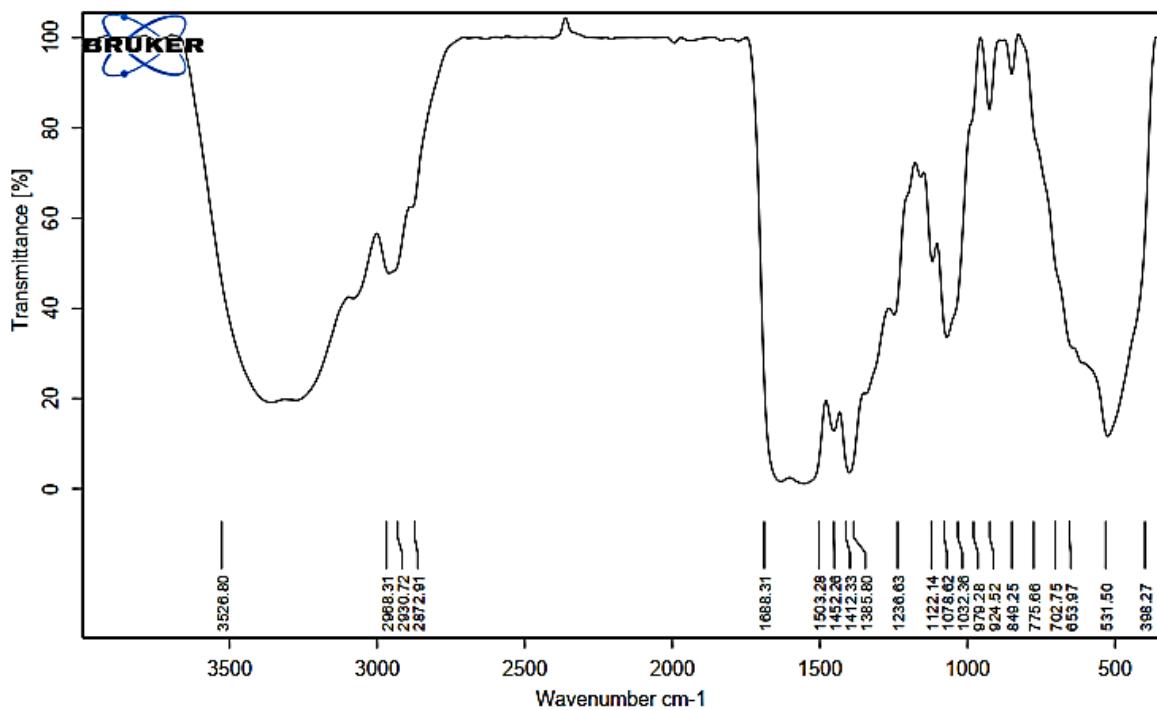
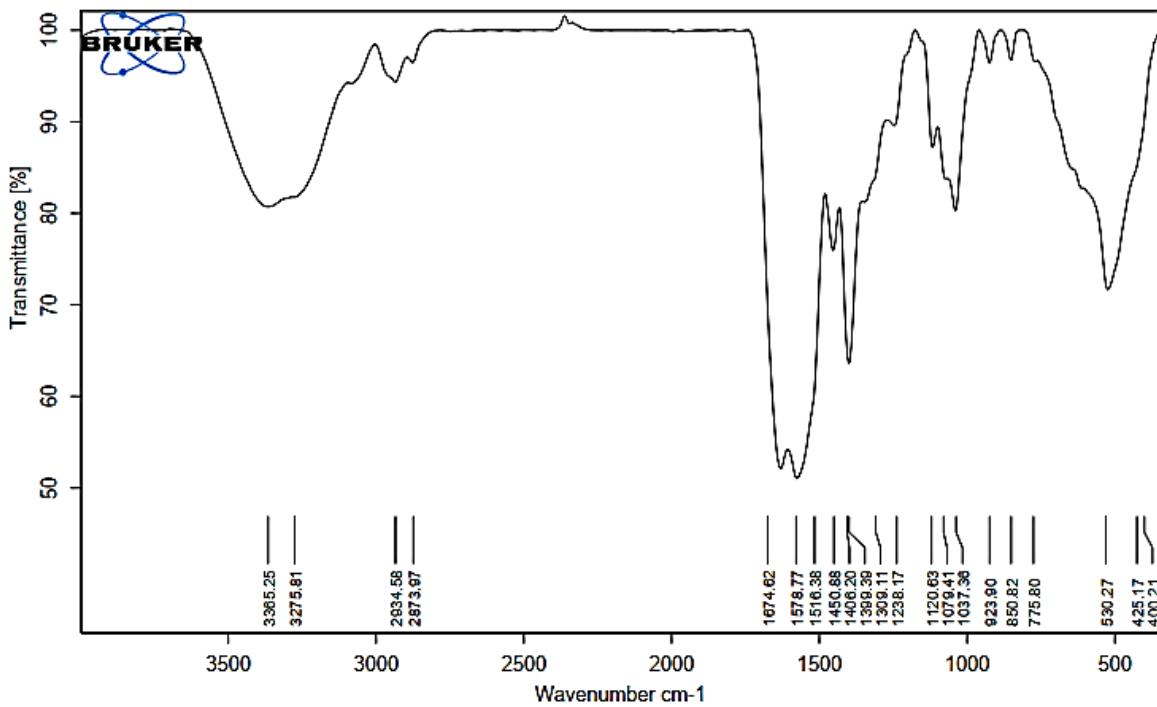


Figure S10. FTIR spectral chart of *C. glabrata* treated with Ag-SeO<sub>2</sub> NC.



**Figure S11.** FTIR spectral chart of *A. niger* untreated with Ag-SeO<sub>2</sub> NC.



**Figure S12.** FTIR spectral chart of *A. niger* control treated with Ag-SeO<sub>2</sub> NC.

## References

- [1] Sánchez-Rangel, J.C., Benavides, J., Heredia, J.B., Cisneros-Zevallos, L. and Jacobo-Velázquez, D.A., 2013. The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Analytical methods*, 5(21), pp.5990-5999.
- [2] Zhishen, J.; Mengcheng, T.; Jianming, W. Research on antioxidant activity of flavonoids from natural materials. *Food Chem* 1999, 64, e9.
- [3] Aberoumand, A. Nutritional evaluation of edible *Portulaca oleracea* as plant food. *Food Analytical Methods* 2009, 2, 204-207.