Supplementary Data

Polysaccharide-Stabilized Biogenic CuO/SeO_2 Nanocomposites: Green Synthesis, Physicochemical Characterization, and Enhanced Antioxidant, Antimicrobial, and Selective Anticancer Activities

Section S1: Materials and Methods

1. Reagents and Chemicals

Raw propolis samples were obtained from a certified local apiary and stored at 4 °C before use. Dried clove buds (*Syzygium aromaticum*) were purchased from a local herbal market, authenticated botanically, washed, air-dried, and finely powdered before extraction.

Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, ≥99.0%), selenium dioxide (SeO_2, ≥99.5%), gallic acid (≥98.0%), quercetin (≥98.0%), catechin (≥98.0%), glucose (≥99.5%), Folin-Ciocalteu reagent, aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$, ≥99.0%), vanillin (≥99.0%), anthrone (≥99.0%), 2,2-diphenyl-1-picrylhydrazyl (DPPH, ≥95.0%), ammonium molybdate tetrahydrate (≥99.0%), sodium phosphate monobasic and dibasic (≥99.0%), potassium ferricyanide (≥99.0%), ferric chloride hexahydrate (≥99.0%), trichloroacetic acid (≥99.0%), sulfuric acid (95-98%), hydrochloric acid (37%), and sodium carbonate (≥99.5%) were all obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

Absolute ethanol (≥99.9%), methanol (HPLC grade, ≥99.9%), and dimethyl sulfoxide (DMSO, ≥99.7%), were purchased from Merck (Darmstadt, Germany). Whatman No. 1 filter paper was obtained from GE Healthcare Life Sciences (UK). Dialysis tubing (molecular weight cut-off 12-14 kDa) was purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA).

Mueller-Hinton agar, Dulbecco's Modified Eagle Medium (DMEM, Cat# 11965-092), fetal bovine serum (FBS, Cat# 16000-044), penicillin-streptomycin solution (Cat# 15140-122), and MTT reagent (Cat# M6494) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Ciprofloxacin discs (5 µg) were obtained from Oxoid Ltd. (Basingstoke, UK).

All aqueous solutions were prepared using ultrapure deionized water (resistivity ≥18.2 MΩ·cm) produced by a Milli-Q purification system (Millipore, USA). All reagents were of analytical or cell culture grade and used without further purification.

2. Instruments

All physicochemical characterizations were carried out using standard analytical instruments. UV-visible absorption spectra of extracts, polysaccharides, and nanocomposites were recorded on a Spekol 11 UV-Vis spectrophotometer (Analytik Jena AG, Jena, Germany) in the range of 200-800 nm. Functional groups and surface chemical interactions were identified by FTIR spectroscopy (Thermo Scientific Nicolet IS10, USA) over 4000-400 cm^{-1} at a resolution of 4 cm^{-1}. Surface morphology and microstructural features were examined using field-emission scanning electron microscopy (FE-SEM, JEOL JSM-7610F, Japan), while elemental composition and spatial distribution were analyzed by energy-dispersive X-ray spectroscopy (EDX) and elemental mapping (Oxford Instruments X-Max 80, UK) coupled to the SEM. Particle size, lattice fringes, and crystallinity were further investigated by high-resolution transmission electron microscopy (HR-TEM, Thermo Scientific Talos F200i, USA) operated at 200 kV. Phase structure and crystallinity were determined by X-ray diffraction (XRD, PANalytical Philips diffractometer, Netherlands) using Cu Kα radiation (λ = 1.5406 Å) in the 2θ range of 10-80°. Surface chemical states and oxidation states were analyzed by X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha+, USA) using a monochromatic Al Kα source (1486.6 eV), with binding energies calibrated to the C 1s peak at 284.8 eV. Colloidal stability and surface charge were evaluated by zeta potential analysis (HORIBA SZ-100, Japan) at 25 °C. Ultrasonic treatment during synthesis and dispersion was performed using an ultrasonic bath (Elma Schmidbauer GmbH, Germany), and solid products were recovered using a Beckman Coulter Allegra X-15R refrigerated centrifuge (Beckman Coulter Inc., USA). Plant extraction was conducted in a horizontal water bath shaker (Memmert WB14, Germany), and all phytochemical and antioxidant assays were quantified spectrophotometrically using the Spekol 11 instrument.

Section S2: Results

Table S1. FTIR Vibrational Bands of Clove Extract, CuO/SeO_2, and CuO/SeO_2/Polysaccharide Nanocomposites.

Sample	Wavenumber (cm^{-1})	Characteristic Group / Vibration	Interpretation
Clove Extract	3058, 3025	Aromatic =C-H stretching	Confirms phenolic/aromatic constituents (eugenol).
	2923, 2848	Aliphatic C-H stretching	Methylene groups in phenylpropanoids.
	1766	C=O stretching	Trace carbonyl groups, possibly ester components.
	1601	Aromatic C=C stretching	Signature peak of eugenol-type compounds.
	1514, 1489, 1448	C-O and C-H bending	Typical of phenolic/phenylpropanoid structures.
	1367	O-H bending / C-H deformation	Correlates with polyphenolic content.
	1264-537	C-O, C-O-C, phenolic C-O; aromatic skeletal vibrations	Represents reducing and capping phytoconstituents active in nanoparticle formation.
CuO/SeO_2 NC	3360–3299	O-H stretching	Adsorbed water or residual plant-derived ligands.
	2921, 2851	Aliphatic C-H stretching	Remnants of organic stabilizers.
	1689, 1644	C=O, C=N stretching	Coordination of plant molecules with CuO/SeO_2 surface.
	1577, 1482, 1426	Aromatic C=C and C-O vibrations	Organic moieties bonded on nanoparticle surface.
	1369, 1343, 1322	C-H/C-O bending	Stabilizing organic residues.
	1265-990	C-O and C-O-C stretching	Plant-derived ligands interacting with oxide surface.
	918-698	Aromatic ring deformation	Surface-bound phytochemicals.
	613, 518, 469, 391	**M-O (Cu-O) and Se-O vibrations**	**Strong evidence of CuO and SeO_2 formation.**
CuO/SeO_2/Polysaccharide NC	3387	O-H stretching	Increased hydrogen bonding between polysaccharide and oxide surface.
	2924, 2851	Aliphatic C-H stretching	Carbohydrate and residual organic signals.
	1689-1635	C=O and amide-related stretching	Strong interactions between polysaccharide and CuO/SeO_2.
	1576, 1541, 1514	Aromatic C=C and N-H bending	Overlapped organic/polysaccharide vibrations.
	1484, 1423, 1371	Carbohydrate C-H / O-H bending	Polysaccharide backbone confirmation.
	1270, 1221, 1194, 1155, 1074, 1028	**C-O-C and C-O stretching of glycosidic linkages**	**Diagnostic polysaccharide bands confirming surface grafting.**
	994-917	C-O and ring vibrations	Polysaccharide chain interactions.
	826, 765, 692, 648, 569, 518	Metal-oxygen and Se-O vibrations with reduced intensities	Partial shielding of CuO/SeO_2 by the polysaccharide coating.

Characteristic aromatic and phenolic signals of eugenol derivatives were identified in the clove extract, confirming the presence of reducing and stabilizing phytochemicals. Thereafter, significant changes in the peak positions and intensities, with the appearance of diagnostic metal-oxygen and Se-O bands after the formation of the CuO/SeO_2 nanocomposite, verified the successful synthesis of the hybrid metal/oxide structure. Further spectral shifts, broadening of O-H stretching, appearance of glycosidic C-O-C vibrations, and partial attenuation of M-O/Se-O signals in the CuO/SeO_2/polysaccharide NC provided clear evidence for effective polysaccharide surface functionalization. All the combined spectral features confirm reduction, stabilization, and successful capping of CuO/SeO_2 nanoparticles through plant- and polysaccharide-mediated processes.

Table S2. Elemental composition of CuO/SeO_2 NC and CuO/SeO_2/polysaccharide NC determined by EDX analysis.

Sample	Element	Weight %	Atomic %	Error %
CuO/SeO_2 NC	O	47.04	80.24	9.32
	Se	35.59	12.30	6.87
	Cu	17.37	7.46	3.41
CuO/SeO_2/polysaccharide NC	C	28.17	52.06	13.54
	O	24.20	33.57	13.02
	Cu	14.37	5.02	4.70
	Se	33.26	9.35	5.06

Table S3. Major diffraction peaks of CuO/SeO_2 NC with phase assignments, crystallographic planes, corresponding JCPDS cards, and crystallite sizes.

No.	Observed 2θ (°)	d-spacing (Å)	Assigned Plane (hkl)	Phase Identification	JCPDS Card No.	Crystallite Size (nm)
1	24.5	3.63	(012)	SeO_2	70-2049	24.3
2	32.5	2.75	(110)	CuO	05-0661	22.8
3	33.6	2.66	(104)	SeO_2	70-2049	23.6
4	35.5	2.53	(111)	CuO	05-0661	21.9
5	36.2	2.48	(110)	SeO_2	70-2049	24.7
6	38.7	2.32	(111)	CuO	05-0661	22.1
7	41.5	2.18	(113)	SeO_2	70-2049	23.9
8	48.7	1.87	(202)	CuO	05-0661	20.8
9	50.2	1.82	(024)	SeO_2	70-2049	25.1
10	53.5	1.71	(020)	CuO	05-0661	21.4
11	54.8	1.67	(116)	SeO_2	70-2049	24.6
12	58.3	1.58	(202)	CuO	05-0661	22.5
13	61.5	1.51	(113)	CuO	05-0661	21.7

Note: Crystallite sizes were estimated using the Debye-Scherrer equation from the most intense diffraction peaks.

Table S4. Major diffraction peaks of CuO/SeO_2/polysaccharide NC with phase assignments, crystallographic planes, corresponding JCPDS cards, and crystallite sizes.

No.	Observed 2θ (°)	d-spacing (Å)	Assigned Plane (hkl)	Phase Identification	JCPDS Card No.	Crystallite Size (nm)
1	20-25	-	-	Polysaccharide amorphous halo	-	-
2	25.6	3.48	(012)	SeO_2	70-2049	18.9
3	32.5	2.75	(110)	CuO	05-0661	17.6
4	35.1	2.55	(104)	SeO_2	70-2049	18.4
5	35.5	2.53	(111)	CuO	05-0661	17.1
6	37.8	2.38	(110)	SeO_2	70-2049	19.2
7	38.7	2.32	(111)	CuO	05-0661	16.8
8	43.3	2.09	(113)	SeO_2	70-2049	18.5
9	48.7	1.87	(202)	CuO	05-0661	16.4
10	52.5	1.74	(024)	SeO_2	70-2049	19.0
11	53.5	1.71	(020)	CuO	05-0661	17.3
12	57.5	1.60	(116)	SeO_2	70-2049	18.7
13	58.3	1.58	(202)	CuO	05-0661	16.9
14	61.5	1.51	(113)	CuO	05-0661	17.5

Note: Crystallite sizes were estimated using the Debye-Scherrer equation from the most intense diffraction peaks.

Table S5. Phytochemical results of the investigated sample.

Sample	Phenolic Content (mg GAE/g)	Flavonoid Content (mg CE/g)	Tannin Content (mg TAE/g)	Carbohydrate content (mg GE/mL)
Clove	312.2±1.74	48.64±0.19	6.147±0.41	482.93±1.97
Cu/SeO_2 NC	225.4±1.98	45.25±0.08	6.705±0.16	431.22±2.91
Cu/SeO_2/polysaccharide NC	206.8±0.97	72.32±1.07	8.096±0.08	686.34±3.54

GAE: Gallic acid equivalents; **CE:** Catechin equivalents; **TAE:** Tannic acid equivalents; and **GE**: Glucose equivalents.
Std. Dev.: Standard deviation.
Values represent individual triplicate measurements.

Note: All values in the table are expressed as mean ± standard deviation.

Table S6. The antioxidant results by DPPH assay.

Sample	Concentrations (mg/mL)	% Remaining DPPH	% Scavenging Activity	IC_{50} (mg/mL)
Clove	0.19	9.801±0.08	90.2±0.08	0.013±0.002
	0.094	17.61±0.14	82.39±0.14	
	0.047	35.8±0.19	64.2±0.19	
	0.023	51.56±0.23	48.44±0.23	
Cu/SeO_2 NC (B)	0.15	9.787±0.17	90.21±0.17	0.0126±0.008
	0.077	22.87±0.31	77.13±0.31	
	0.039	34.66±0.54	65.34±0.54	
	0.019	49.72±0.81	50.28±0.81	
Cu/SeO_2/polysaccharide NC (C)	0.12	6.96±0.14	93.04±0.14	0.0096±0.001
	0.062	24.15±0.08	75.85±0.08	
	0.031	30.11±0.17	69.89±0.17	
	0.016	47.02±0.05	52.98±0.05	
Ascorbic acid	0.062	15.27 ± 0.08	84.73± 0.08	0.022± 0.003
	0.031	39.08± 0.23	60.92± 0.23	
	0.016	61.07± 0.04	38.93± 0.04	
	0.008	74.81± 1.27	25.19± 1.27	

Notes:

- SD: Standard deviation
- The table includes all replicates (triplicates) for each concentration and sample.

Table S7. Antioxidant activities of clove extract and nanocomposites evaluated by ferric reducing power and phosphomolybdate assays.

Sample	Absorbance	Reducing power (mg AAE/mL)	Absorbance	Total antioxidant capacity (mg AAE/mL)
Clove (A)	2.674	0.384 ± 0.10	1.734	0.561 ± 0.03
Cu/SeO_2 NC (B)	2.761	0.400 ± 0.08	1.756	0.568 ± 0.05
Cu/SeO_2/polysaccharide NC (C)	2.875	0.421 ± 0.16	1.912	0.619 ± 0.07

Ferric reducing power was determined using the potassium ferricyanide-trichloroacetic acid method, while total antioxidant capacity was assessed by the phosphomolybdate assay. Values are expressed as mean ± SD of triplicate measurements (n = 3). Results are reported as mg ascorbic acid equivalents (AAE) per mL of the sample.

Table S8. Time-dependent release of Cu^{2+} and Se^{4+} ions from CuO/SeO_2 NC and CuO/SeO_2/polysaccharide NC in aqueous medium.

Time (h)	Cu^{2+} release (µg/mL) CuO/SeO_2 NC	Cu^{2+} release (µg/mL) CuO/SeO_2/polysaccharide NC	Se^{4+} release (µg/mL) CuO/SeO_2 NC	Se^{4+} release (µg/mL) CuO/SeO_2/polysaccharide NC
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	1.12 ± 0.05	0.78 ± 0.04	0.48 ± 0.02	0.35 ± 0.01
3	2.36 ± 0.08	1.45 ± 0.06	0.97 ± 0.05	0.68 ± 0.03
6	3.88 ± 0.12	2.11 ± 0.09	1.63 ± 0.07	1.12 ± 0.04
12	5.12 ± 0.15	2.87 ± 0.11	2.45 ± 0.08	1.57 ± 0.05
24	6.03 ± 0.18	3.21 ± 0.12	3.21 ± 0.10	1.92 ± 0.06
48	6.75 ± 0.20	3.42 ± 0.14	3.82 ± 0.11	2.05 ± 0.07

Values are mean ± SD (n = 3).

Release studies were conducted in Mueller-Hinton broth at 37 °C.

Samples were centrifuged and filtered to quantify dissolved ions only.

Cu^{2+} and Se^{4+} release is lower for polysaccharide-coated NC, indicating controlled ion release.

Table S9. Antibacterial activity of clove extract, CuO/SeO_2 NC, and CuO/SeO_2/polysaccharide NC against different pathogenic bacteria, expressed as inhibition zone diameters (mm). Data are mean ± SD (n = 3), and ciprofloxacin (5 µg) was used as a positive control. NA refers to no activity. Superscript letters indicate statistically significant differences ($p < 0.05$) between groups.

Bacterial Species	Clove extract	CuO/SeO_2 NC	CuO/SeO_2/polysaccharide NC	Control	Ciprofloxacin (5 µg)
E. coli **(ATCC 10536)**	17 ± 0.55^{a}	18 ± 0.82^{ab}	16 ± 0.35^{a}	NA	30 ± 1.36^{c}
B. cereus **(EMCC1080)**	16 ± 0.30^{a}	19 ± 0.76^{b}	18 ± 0.06^{b}	NA	23 ± 1.20^{c}
B. subtilis **(DMS 1088)**	15 ± 0.69^{a}	20 ± 0.24^{b}	18 ± 0.39^{ab}	NA	28 ± 1.51^{c}
S. aureus **(ATCC 6538)**	19 ± 0.29^{a}	28 ± 0.59^{c}	23 ± 1.07^{b}	NA	29 ± 1.34^{c}
S. epidermidis **(EMCC1353t)**	15 ± 0.46^{a}	24 ± 0.73^{b}	27 ± 0.52^{c}	NA	30 ± 1.27^{d}
K. pneumoniae **(ATCC 10031)**	15 ± 0.61^{a}	38 ± 0.08^{c}	16 ± 0.60^{a}	NA	37 ± 0.08^{c}
E. cloacae **(DMS 30054)**	17 ± 0.48^{a}	37 ± 0.18^{c}	15 ± 1.53^{a}	NA	25 ± 0.11^{b}
S. Typhi **(ATCC 25566)**	14 ± 0.89^{a}	15 ± 0.43^{a}	15 ± 0.58^{a}	NA	27 ± 0.38^{b}

Superscript letters (a, b, c, d) reflect the results of one-way ANOVA with Tukey's post hoc test.
Identical letters across treatments indicate no significant difference, different letters indicate a statistically significant difference ($p < 0.05$).