

Article

Biological Activities and Host-Response Insights of *Ocimum basilicum* Flower-Derived Silver Nanoparticles

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Abstract

Antimicrobial resistance and the limitations of conventional antimicrobial therapies have intensified the search for multifunctional nanoscale agents. Plant flowers are effective and sustainable biological materials for the eco-friendly synthesis of stable nanoparticles. In this study, *Ocimum basilicum* flower (OBF) extracts were used for the synthesis of silver nanoparticles (OBF-AgNPs) and evaluated for antimicrobial and antibiofilm activities. Characterization by UV–Vis spectroscopy, FTIR, SEM, TEM, EDX, DLS, and zeta-potential analysis revealed an absorption maximum at 440 nm and predominantly spherical particles measuring 5–38 nm. The hydrodynamic diameter was 53.84 nm, with a PDI of 0.4123 and a zeta potential of −23.47 mV. OBF-AgNPs showed greater antimicrobial activity than the crude extract and AgNO₃, with MIC and MBC values of 97–194 and 388–776 µg/mL, respectively. Biofilm formation was inhibited against *S. aureus*, *P. aeruginosa*, and *C. tropicalis* in a concentration-dependent manner. Eight GC–MS-identified phytochemicals were separately examined through network pharmacology. Of 124 predicted targets, 34 overlapped with microbial-infection-related genes, and enrichment analysis highlighted inflammatory and immune-response pathways. Molecular docking against the network-derived host-target PTGS2 showed moderate predicted interactions compared with reference inhibitors, with τ -cadinol showing the lowest docking score of −7.7 kcal/mol among the tested phytochemicals. Overall, the synthesized OBF-AgNPs demonstrated antimicrobial and antibiofilm activities. The in silico analyses independently identified host-response-related computational insights from GC–MS-identified flower constituents.

Keywords: *Ocimum basilicum*; silver nanoparticles; antimicrobial activity; network pharmacology; molecular docking



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1. Introduction

Antimicrobial resistance is a major global health challenge that reduces the effectiveness of conventional antibiotics and increases the burden of infectious diseases [1,2]. Its spread is driven by factors such as inappropriate antibiotic use, inadequate sanitation, and poor adherence to infection-control and clinical practices. Consequently, alternative and sustainable strategies for controlling resistant pathogens are urgently required [3,4].

Silver nanoparticles (AgNPs) have attracted considerable attention for applications in medicine and biotechnology [5–9]. Their small dimensions and high surface-area-to-volume ratio promote interactions with biological systems [7]. Conventional physical and chemical synthesis methods can be energy-intensive and may rely on hazardous solvents, reducing agents, or stabilizers [10–12]. By contrast, green synthesis applies sustainable chemistry

principles to limit hazardous inputs and use renewable resources [13]. Plant-mediated synthesis has emerged as a simple, cost-effective, and environmentally friendly method [14]. Medicinal plants are rich in bioactive compounds such as phenolics, flavonoids, alkaloids, terpenoids, and proteins, which can act as natural reducing and capping agents [15]. These phytochemicals facilitate the reduction of metal ions and contribute to nanoparticle stability by limiting aggregation. In addition, many of these compounds possess intrinsic antimicrobial properties, which may further enhance the biological activity of synthesized nanoparticles [16].

Ocimum basilicum (basil) is a medicinal and aromatic plant valued for its diverse phytochemical composition and therapeutic properties [17,18]. Although native to tropical regions of Africa and Asia, it is now cultivated worldwide [19]. The present study uses *O. basilicum* flowers collected in Riyadh, Saudi Arabia, as the biological material for nanoparticle synthesis. Floral tissue contains volatile compounds, phenolics, flavonoids, and terpenoids that may participate in the reduction, capping, and stabilization of Ag-NPs [20]. Environmental and geographical conditions can alter the production of primary and secondary metabolites; consequently, nanoparticles produced from the same plant species in different regions may vary in size, morphology, surface properties, stability, and biological activity [21–26]. Regional variations in essential-oil yield, composition, and antibacterial activity have also been reported for Saudi-grown *O. basilicum* [27]. Although other parts of *O. basilicum* have been used to synthesize antimicrobial AgNPs [28,29], flower-mediated synthesis using Saudi-grown plants remains insufficiently studied. Therefore, the present study evaluates this floral source for nanoparticle synthesis, characterization, and biological activity.

Plant-mediated AgNPs can damage microbial membranes, promote reactive oxygen species generation, and interfere with intracellular components [30]. Nevertheless, the molecular basis of their biological activity is not fully resolved. Network pharmacology and molecular docking can offer exploratory information about relationships among phytochemicals, host targets, and signaling pathways. Accordingly, this study aimed to synthesize AgNPs using *O. basilicum* flower (OBF) extracts and evaluate their antimicrobial and antibiofilm activities. In addition, GC–MS-guided network pharmacology and molecular docking were conducted as a separate computational annotation of the major flower-derived phytochemicals to predict host-specific response mechanisms.

2. Results and Discussion

2.1. GC–MS Analysis of the Plant Extract

GC–MS analysis identified 26 constituents in the flower extract based on retention time, mass-spectral fragmentation, molecular weight, and comparisons with the NIST library. The profile was dominated by monoterpenes, sesquiterpenes, oxygenated terpenoids, and hydrocarbons. Estragole (37.18%) and linalool (34.50%) were the principal constituents, and together, they accounted for more than 70% of the total chromatographic area. Other notable compounds included trans- α -bergamotene (7.20%), τ -cadinol (3.31%), (+)-2-bornanone (1.75%), and eucalyptol (0.77%). α -Terpineol, borneol, citral, and several guaiene derivatives occurred at lower relative abundances. Table 1 lists the identified compounds, retention times, molecular weights, and relative areas.

Table 1. GC–MS profile of the ethanolic *Ocimum basilicum* flower extract.

S. No	RT (min)	Compound Name	Quality	Molecular Weight	Area %
1	6.181	beta. -Myrcene	96	136.125	0.65
2	6.886	D-Limonene	99	136.125	0.26
3	6.939	Eucalyptol	98	154.136	0.77
4	7.949	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	98	136.125	0.22
5	8.254	Linalool	97	154.136	34.50
6	8.949	(+)-2-Bornanone	98	152.12	1.75
7	9.338	endo-Borneol	97	154.136	0.58
8	9.748	alpha. -Terpineol	93	154.136	0.41
9	9.843	Estragole	97	148.089	37.18
10	10.864	Citral	96	152.12	0.29
11	11.106	Bicyclo [2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	99	196.146	1.30
12	12.621	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2. beta.,4.beta.)]-	94	204.188	1.81
13	13.179	trans-. alpha.-Bergamotene	99	204.188	7.20
14	13.242	alpha. -G uaiene	99	204.188	0.64
15	13.6	cis-Muurola-4(15),5-diene	98	204.188	0.49
16	13.842	(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	98	204.188	1.75
17	14.031	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo [8.1.0]undeca-2,6-diene	99	204.188	0.59
18	14.126	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	99	204.188	0.54
19	14.241	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	99	204.188	1.73
20	14.315	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	99	204.188	0.46
21	14.946	(1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethyldecahydro-1H-cyclopropa[a]naphthalen-7-ol	99	222.198	0.28
22	15.504	Epicubenol	99	222.198	0.45
23	15.809	.tau.-Cadinol	91	222.198	3.31
24	23.543	Phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-methyl-	97	340.24	0.94
25	27.446	Eicosane	98	282.329	0.67
26	30.477	Squalene	99	410.391	1.24

2.2. Characterization of Green-Synthesized Silver Nanoparticles (AgNPs)

2.2.1. UV–Visible Spectroscopy

The first indication of nanoparticle formation was a visible color change in the reaction mixture. After the *O. basilicum* flower extract was mixed with AgNO_3 , the solution changed from light yellow to dark brown within 15 min, consistent with the reduction of Ag^+ and the formation of Ag^0 nanoparticles (Figure 1a). The dark-brown color is associated with the surface plasmon resonance (SPR) of AgNPs [31,32]. The UV–visible spectrum showed a distinct absorption band at approximately 440 nm (Figure 1b), further supporting the formation of OBF-AgNPs.

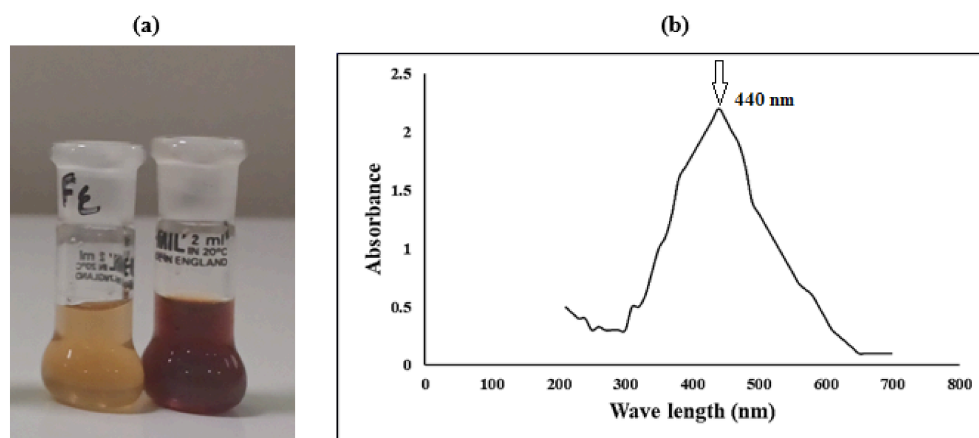


Figure 1. (a) Color change after silver nitrate was added to the *Ocimum basilicum* flower extract and (b) UV–visible absorption spectrum of the resulting OBF-AgNPs.

2.2.2. FTIR Analysis

The FTIR spectra of the *O. basilicum* flower extract (F–S) and the synthesized silver nanoparticles (F–N) are presented in Figure 2. FTIR analyses were used to identify functional groups in the extract and to assess their possible association with the nanoparticle surface. The extract exhibited peaks at 3394, 2931, 2368, 1620, 1386, 1265, 1072, 864, 771, 563, and 432 cm^{-1} , consistent with phytoconstituents such as phenolics, flavonoids, proteins, and other secondary metabolites. The broad band at 3394 cm^{-1} corresponds to O–H stretching of phenols/alcohols, while 2931 cm^{-1} is due to C–H stretching of aliphatic groups. The band at 1620 cm^{-1} is attributed to C=C or amide (C=O) stretching, suggesting the presence of proteins or aromatic compounds. Peaks at 1386 and 1265 cm^{-1} represent C–H bending and C–O stretching, respectively, and the peak at 1072 cm^{-1} indicates C–O/C–N stretching. Bands at 864 and 771 cm^{-1} correspond to aromatic C–H bending, whereas bands at 563 and 432 cm^{-1} are associated with skeletal vibrations. The nanoparticle spectrum (F–N) showed peaks at 3402, 2924, 2376, 1627, 1381, 1265, 1072, 825, 694, 598, 516, 429, and 324 cm^{-1} . Relative to the extract, shifts at 3394 $\rightarrow$ 3402, 2931 $\rightarrow$ 2924, and 1620 $\rightarrow$ 1627 cm^{-1} suggest possible associations of hydroxyl-, aliphatic-, and carbonyl/amide-containing biomolecules with the nanoparticle surface. Peaks at 1381, 1265, and 1072 cm^{-1} are consistent with phenolic, alcoholic, and amine-containing groups, whereas the additional low-frequency bands at 825, 694, 598, 516, and 324 cm^{-1} may reflect metal–biomolecule interactions and changes in the phytochemical environment after nanoparticle formation. Collectively, these spectral changes provide qualitative evidence that flower-derived biomolecules participate in the surface association and stabilization of OBF-AgNPs [33].

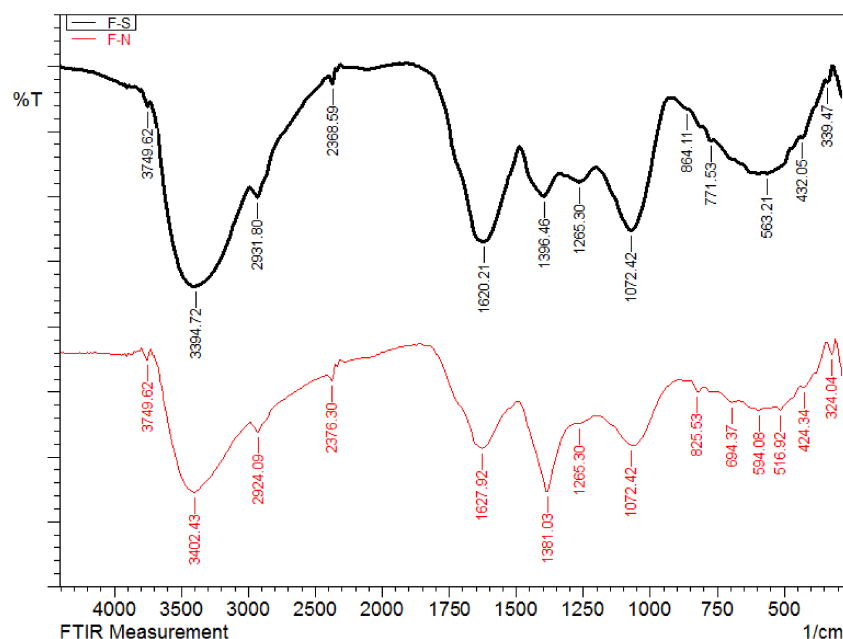


Figure 2. FTIR spectral analysis of *Ocimum basilicum* flower extract (F-S) and biosynthesized silver nanoparticles (F-N), indicating functional groups involved in reduction and capping.

2.2.3. Particle Size and Zeta Potential Analysis

Dynamic light scattering (DLS) was used to determine the hydrodynamic diameter, particle-size distribution, and polydispersity index (PDI) of OBF-AgNPs (Figure 3). The intensity-weighted mean hydrodynamic diameter was 53.84 nm, and the PDI of 0.4123 indicated a moderately polydisperse suspension. The larger hydrodynamic diameter relative to the TEM-derived core size may reflect surface-bound phytochemicals and modest aggregation in suspension. The zeta potential was -23.47 mV, indicating a negatively charged surface and moderate colloidal stability. The surface charge contributes to nanoparticle stability by generating electrostatic repulsion between similarly charged particles and thereby limiting aggregation [34]. Although absolute zeta-potential values greater than approximately 30 mV are commonly associated with high electrostatic stability, the value measured here indicates moderate stabilization. The negative charge may arise from phenolics, flavonoids, proteins, and other bioactive constituents adsorbed from the flower extract. Overall, the DLS and zeta-potential results support the formation of nanoscale moderately polydisperse OBF-AgNPs with moderate colloidal stability.

2.2.4. Morphological and Elemental Analysis

The morphology, size, and elemental composition of the OBF-AgNPs were analyzed using SEM, TEM, and EDX techniques (Figure 4). The TEM image (Figure 4a) provided a clearer visualization of individual nanoparticles, showing that they are predominantly spherical to quasi-spherical and tend to form small aggregates. The SEM micrograph showed densely distributed, nearly spherical particles arranged in clustered surface structures (Figure 4b). The micron-scale features observed in the SEM image are considered agglomerated nanoparticle clusters formed during sample drying [35]. Thus, SEM analysis was used mainly to observe surface distribution and aggregation patterns, while TEM provided clearer information on individual particle morphology and size distribution. The histogram of particle size distribution (Figure 4c) revealed a size range from 5 to 38 nm, with an average of 12 nm. The particle size observed from TEM analysis falls within the nanoscale range, supporting the formation of OBF-AgNPs [36]. The difference between the TEM-derived particle size and DLS hydrodynamic size can be explained by the different

measurement principles of the two techniques. TEM measures the dry metallic core of individual nanoparticles, whereas DLS measures the hydrodynamic diameter of particles dispersed in suspension. Since plant-derived biomolecules may remain adsorbed on the nanoparticle surface as capping and stabilizing agents, a larger hydrodynamic size compared with TEM is expected. The slight aggregation observed in TEM images may be attributed to interparticle interactions and the presence of organic compounds from the plant extract on the nanoparticle surface, which is common in plant-mediated nanoparticle synthesis due to biomolecules acting as capping agents. The elemental composition of the synthesized nanoparticles was evaluated by EDX analysis (Figure 4d, Table 2), which showed a characteristic peak corresponding to silver (Ag), supporting the presence of silver in the synthesized nanoparticles.

Carbon and oxygen signals were also detected and may originate from flower-derived organic constituents associated with the nanoparticle's surface. Comparable C and O signals have been reported for plant-mediated AgNPs and attributed to adsorbed secondary metabolites or other organic capping agents [37]. The relatively high C and O percentages in the present EDX spectrum suggest a phytochemical-associated surface coating, consistent with the FTIR results showing the involvement of biomolecules in reduction, capping, and stabilization. Thus, the quantitative EDX profile supports the presence of silver together with organic surface components in the synthesized OBF-AgNPs.

Table 2. Quantitative EDX elemental composition of OBF-AgNPs.

Element	Energy (keV)	Mass %	Atomic %
C	0.277	41.68	62.85
O	0.525	27.57	31.21
Cl	2.621	2.28	1.16
Ag	2.983	28.47	4.78
Total	-	100%	100%

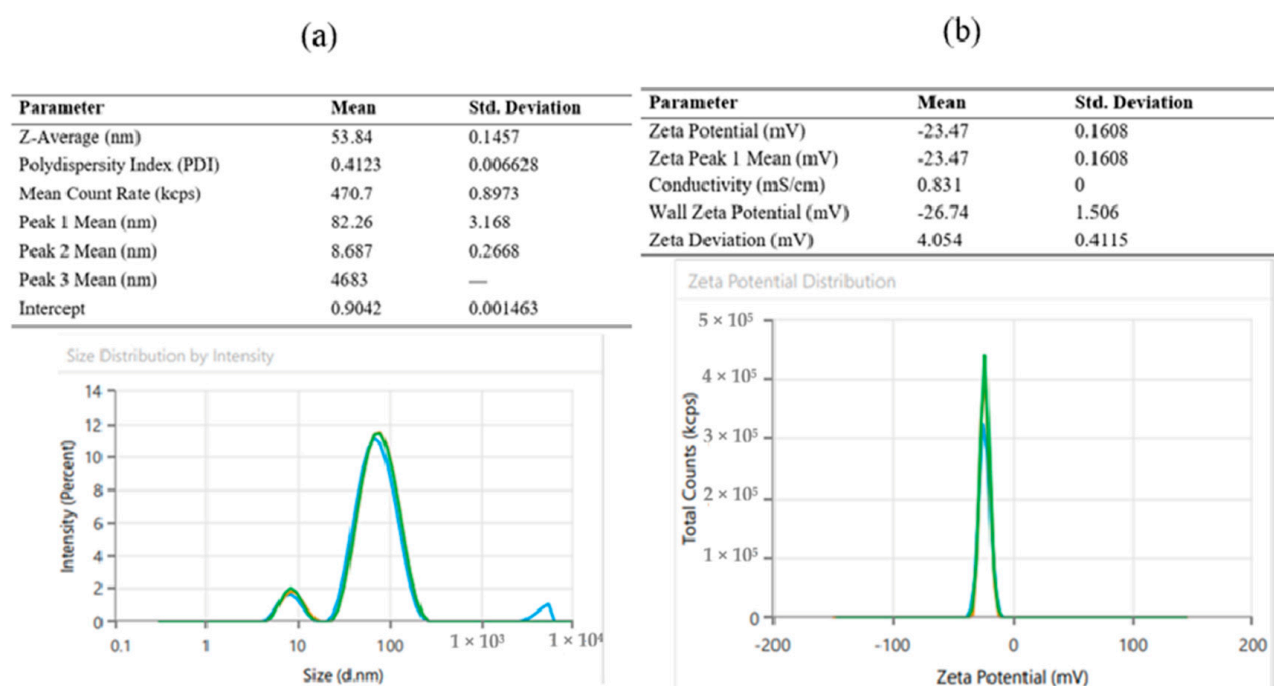


Figure 3. (a) Zeta size and (b) zeta potential of the Ag nanoparticles from the flower of *Ocimum basilicum* L.

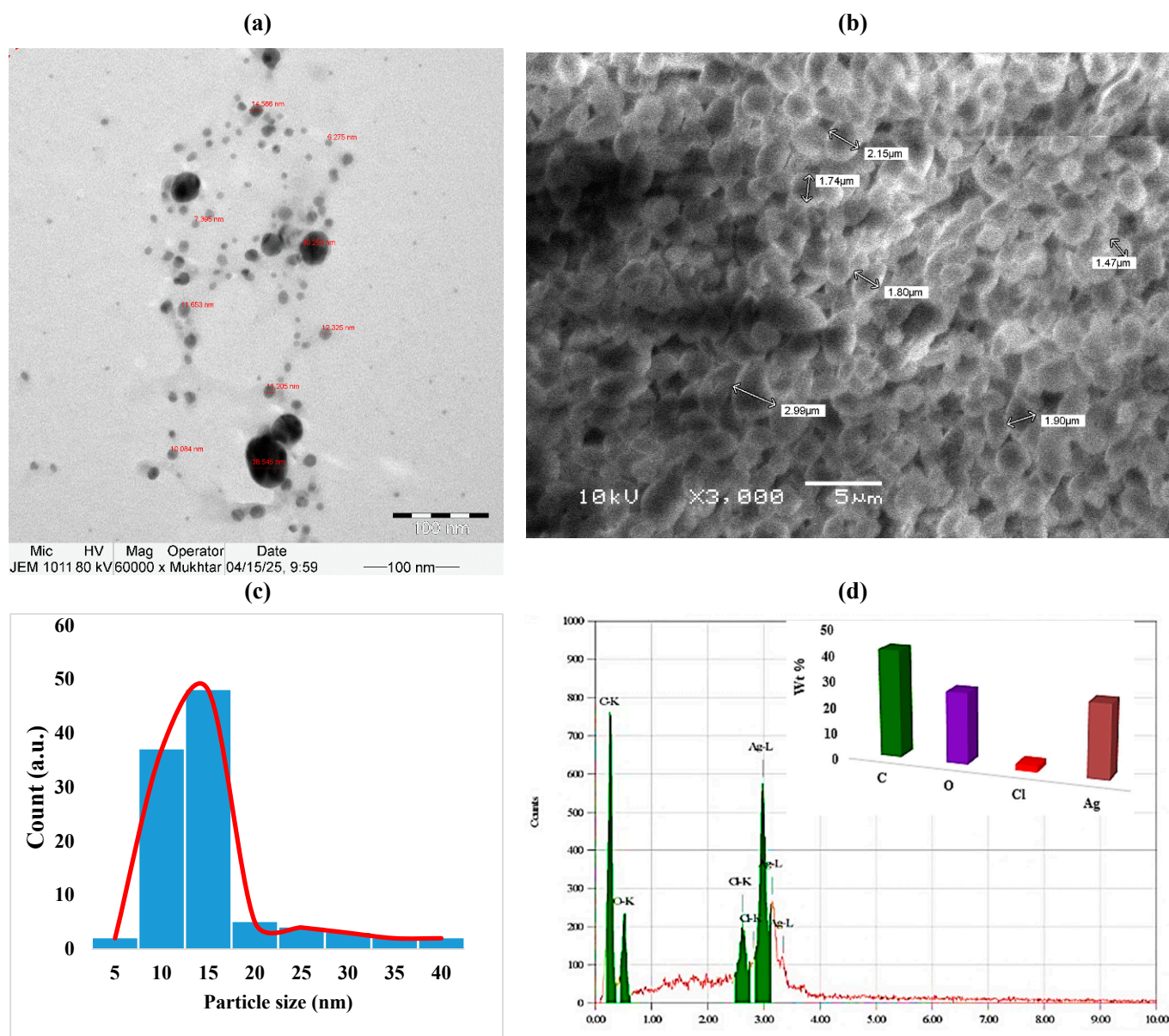


Figure 4. (a) TEM, (b) SEM at $\times 3000$ magnification, (c) particle size distribution histogram, and (d) EDX spectrum of biosynthesized Ag nanoparticles from the flower of *Ocimum*.

2.3. Antimicrobial Activity

The antimicrobial activities of the *O. basilicum* flower extract and OBF-AgNPs were evaluated against bacterial and fungal strains (Table 3). OBF-AgNPs produced inhibition zones of 12–16 mm, compared with approximately 9–10 mm for the crude extract. The water control showed no inhibition, whereas AgNO₃ produced only modest inhibition. *S. aureus*, *S. pneumoniae*, and *P. aeruginosa* were the most susceptible to OBF-AgNPs, with inhibition zones of up to 16 mm; *S. Typhi* showed the smallest zone (12 mm). OBF-AgNPs also inhibited *C. albicans* and *C. tropicalis*, demonstrating activity against both bacterial and fungal strains. Broth-microdilution testing (Table 4) yielded MIC values of 97–194 $\mu\text{g/mL}$ for OBF-AgNPs, substantially lower than those of the extract (25–50 mg/mL). The corresponding MBC values of 388–776 $\mu\text{g/mL}$ indicate bactericidal or fungicidal activity under the assay conditions. The enhanced activity of OBF-AgNPs may be related to their small size and large surface area, which favor interactions with microbial cell envelopes [38]. Reported actions of AgNPs include membrane disruption, silver-ion release, reactive oxygen

species generation, and interference with intracellular proteins and DNA [39,40]. Differences in susceptibility may reflect variations in cell-envelope structure and physiology. The presence of plant-derived phytochemicals, as suggested by FTIR analysis, may further enhance antimicrobial activity by acting synergistically as stabilizing and bioactive agents.

Table 3. Zones of inhibition produced by the *Ocimum basilicum* flower extract and OBF-AgNPs against the tested microorganisms.

Strains	Zone of Inhibition				
	Extract	AgNPs	Control		
			Water	AgNO ₃	Antibiotic
<i>S. epidermidis</i>	9 ± 0.0 ^c	14 ± 2.0 ^b	6 ^c	8.0 ± 0.71 ^c	18 ± 0.1 ^a
<i>E. faecalis</i>	9 ± 1.0 ^c	13 ± 1.0 ^b	6 ^c	7.5 ± 0.71 ^c	18 ± 0.11 ^a
<i>S. aureus</i>	9 ± 1.0 ^c	16 ± 1.5 ^b	6 ^c	8.5 ± 0.71 ^c	19 ± 0.61 ^a
<i>S. pneumoniae</i>	9 ± 1.5 ^c	16 ± 2.0 ^b	6 ^c	9.0 ± 0.71 ^c	19 ± 0.51 ^a
<i>B. subtilis</i>	9 ± 1.0 ^c	13 ± 1.0 ^b	6 ^c	8.0 ± 1.41 ^c	14 ± 0.1 ^a
<i>S. pyogenes</i>	9 ± 1.5 ^c	15 ± 1.0 ^b	6 ^c	7.0 ± 0.71 ^c	17 ± 0.1 ^a
<i>E. coli</i>	9 ± 1.5 ^c	15 ± 1.0 ^b	6 ^c	8.5 ± 0.71 ^c	15 ± 0.1 ^a
<i>E. cloacae</i>	10 ± 2.0 ^c	13 ± 2.0 ^b	6 ^c	7.5 ± 0.71 ^c	18 ± 0.1 ^a
<i>K. pneumoniae</i>	9 ± 1.5 ^c	15 ± 1.0 ^b	6 ^c	9.0 ± 0.00 ^c	18 ± 0.1 ^a
<i>P. aeruginosa</i>	9 ± 1.0 ^c	16 ± 2.0 ^b	6 ^c	8.5 ± 0.71 ^c	18 ± 0.1 ^a
<i>S. Typhi</i>	9 ± 2.0 ^c	12 ± 1.5 ^b	6 ^c	8.0 ± 0.71 ^c	15 ± 0.0 ^a
<i>C. albicans</i>	9 ± 1.0 ^c	14 ± 1.0 ^b	6 ^c	8.5 ± 0.6 ^b	18 ± 0.1 ^a
<i>C. tropicalis</i>	10 ± 1.0 ^c	15 ± 1.0 ^b	6 ^c	9.0 ± 0.5 ^b	19 ± 0.1 ^a

Values are expressed as mean ± SD of three independent replicates. Different superscript letters within the same row indicate statistically significant differences among treatment groups according to one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Gentamicin for bacterial strains and fluconazole for *Candida* species as positive controls. Distilled water and AgNO₃ as negative controls.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Ocimum basilicum* flower extract and synthesized Ag nanoparticles.

Strains	Extract		Biogenic NP	
	MIC mg/mL	MBC mg/mL	MIC µg/mL	MBC µg/mL
<i>S. aureus</i>	25	100	194	776
<i>P. aeruginosa</i>	50	200	97	388
<i>C. tropicalis</i>	25	100	194	776

2.4. Antibiofilm Activity of OBF-AgNPs

OBF-AgNPs inhibited biofilm formation by *S. aureus*, *P. aeruginosa*, and *C. tropicalis* in a concentration-dependent manner (Figure 5). The strongest inhibition occurred at 4×MIC and 2×MIC, whereas activity progressively decreased at MIC, ½×MIC, and ¼×MIC. These results show that the antibiofilm effect of OBF-AgNPs increased with concentrations in all three tested strains.

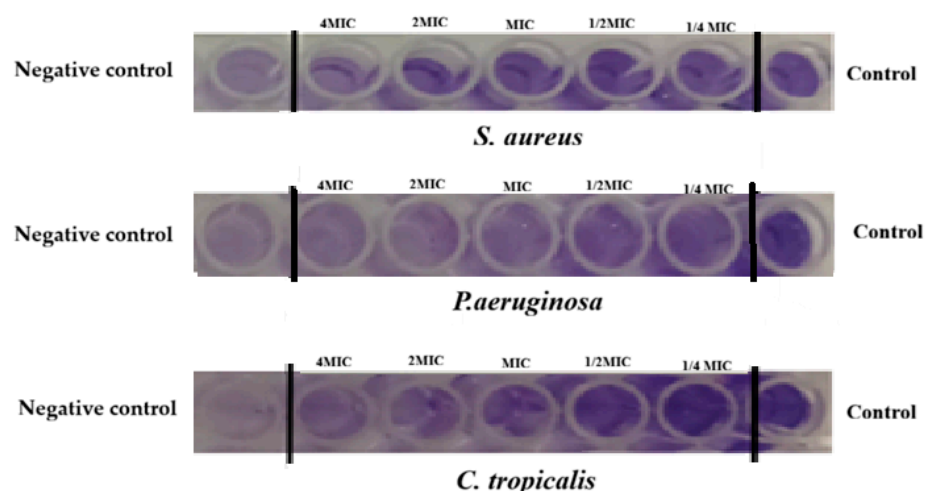


Figure 5. Concentration-dependent inhibition of biofilm formation by OBF-AgNPs against *S. aureus*, *P. aeruginosa*, and *C. tropicalis*.

2.5. In Silico Studies

Eight phytochemicals detected by GC–MS were selected for network pharmacology analysis based on their abundance and reported biological relevance.

2.5.1. Network Pharmacology Analysis of Host-Response-Related Targets

Canonical SMILES strings for the selected compounds were used for target prediction (Figure 6 and Supplementary Table S1). SwissTargetPrediction yielded 124 unique potential human protein targets after standardization and duplicate removal (Supplementary Table S2). GeneCards provided 2218 genes associated with the term “microbial infection” (Supplementary Table S3). Intersection analysis using a Venn diagram (Figure 7) identified 34 common targets (Supplementary Table S4). These overlapping targets were designated as exploratory host-response-associated targets. Functionally, many of these genes are involved in inflammatory signaling (PTGS2 and JAK family), immune regulation (IDO1 and CD81), oxidative stress response (HMOX1 and NQO1), and metabolic processes (PPARA, PPARG, and HMGCR), indicating that the selected flower constituents may be associated with host inflammatory and immune-response pathways. The involvement of JAK–STAT signaling, oxidative stress pathways, and immune-related targets suggests that the GC–MS-identified flower constituents may be associated with host-response pathways relevant to microbial infection [41].

2.5.2. Protein–Protein Interaction (PPI) Network and Hub Gene Analysis

A protein–protein interaction (PPI) network was constructed to examine functional relationships among the 34 overlapping host-response-associated targets. The network contained 34 nodes and 117 edges, with a mean node degree of 6.88 and a mean local clustering coefficient of 0.515 (Figure 8a). This connectivity indicates extensive predicted functional relationships among proteins involved in inflammatory, immune, oxidative-stress, and metabolic responses. Hub genes were ranked by degree centrality using the cytoHubba plugin (Figure 8b), and the 10 highest-ranked genes are listed in Table 5. PTGS2 (COX-2) had the highest degree, suggesting a central position within this host inflammatory-response network. JAK2 participates in JAK–STAT-mediated immune signaling; HMOX1 and NQO1 are associated with oxidative-stress responses; PPARG and HMGCR contribute to metabolic regulation; PARP1 and PTPN11 are involved in DNA repair and signal transduction, respectively. These network properties support potential associations between the selected phytochemicals and multiple host-response processes but do not establish biological activity in vivo.

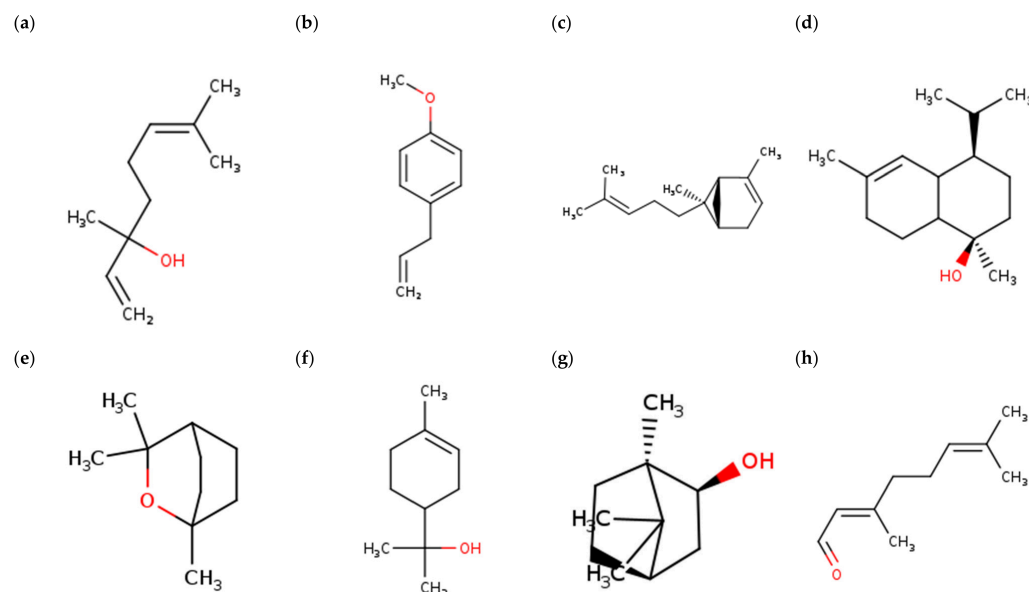


Figure 6. Structures of selected phytochemicals used for network pharmacology analysis: (a) linalool, (b) estragole, (c) trans- α -bergamotene, (d) tau-cadinol, (e) eucalyptol, (f) α -terpineol, and (g) endo-borneol (h) citral.

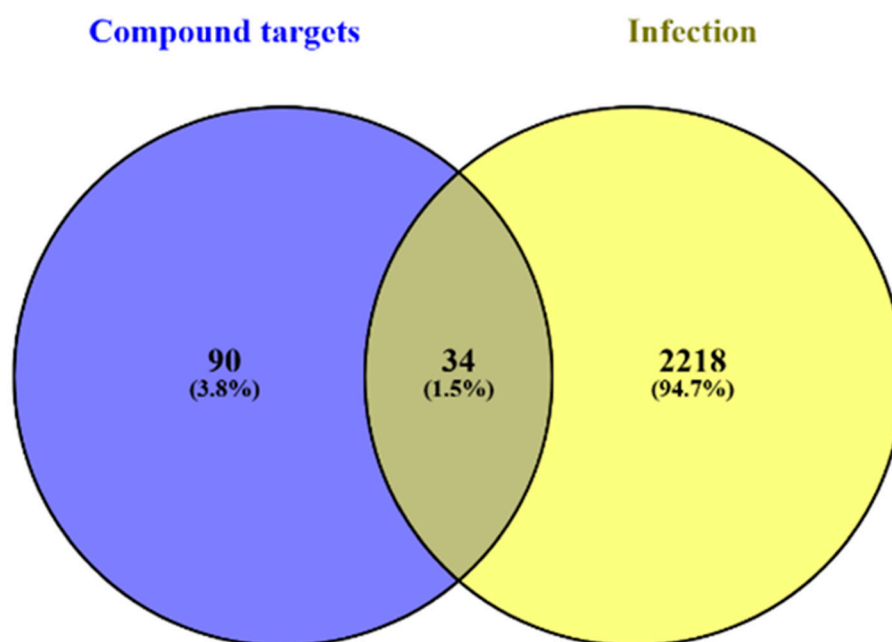


Figure 7. Venn diagram showing overlap between compound targets and infection-related genes.

2.5.3. Functional Enrichment Analysis (GO and KEGG)

Functional enrichment analysis was conducted to explore the biological functions and pathways associated with the identified targets (Figure 9). Gene Ontology (GO) biological-process terms included signal transduction, responses to chemical stress, regulation of cell death, and cellular homeostasis, consistent with roles in host defense and stress responses during infection. Cellular-component terms were enriched for the plasma membrane, receptor complexes, and membrane microdomains, suggesting involvement in membrane-associated signaling and host–pathogen interactions. Molecular-function terms included receptor binding, oxidoreductase activity, nuclear-receptor activity, and protein-kinase binding, indicating potential roles in enzymatic and intracellular signaling processes.

KEGG pathway analysis showed enrichment in pathways such as NF- κ B signaling, JAK-STAT signaling, AMPK signaling, adipocytokine signaling, and necroptosis, as well as metabolic pathways including tryptophan and phenylalanine metabolism. The top enriched KEGG pathways based on FDR, gene count, and fold enrichment analysis are presented in Supplementary Table S5. These pathways are mainly associated with inflammation, immune regulation, oxidative stress, and metabolic adaptation during infection. PTGS2, a key inflammatory mediator [42–44], was identified as the top-ranked hub gene in the PPI network and was therefore selected for exploration of molecular docking analyses.

Table 5. Top 10 hub genes identified from the PPI network using CytoHubba (degree method).

Rank	Gene Symbol		Score
1	PTGS2	Prostaglandin-endoperoxide synthase 2 (Cyclooxygenase-2, COX-2)	36
2	PPARG	Peroxisome proliferator-activated receptor gamma	34
3	ESR1	Estrogen receptor 1	30
4	JAK2	Janus kinase 2	28
5	PARP1	Poly(ADP-ribose) polymerase 1	24
5	HMOX1	Heme oxygenase 1	24
7	HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase	20
7	PTPN11	Protein tyrosine phosphatase non-receptor type 11	20
9	MAOA	Monoamine oxidase A	18
9	IDO1	Indoleamine 2,3-dioxygenase 1	18

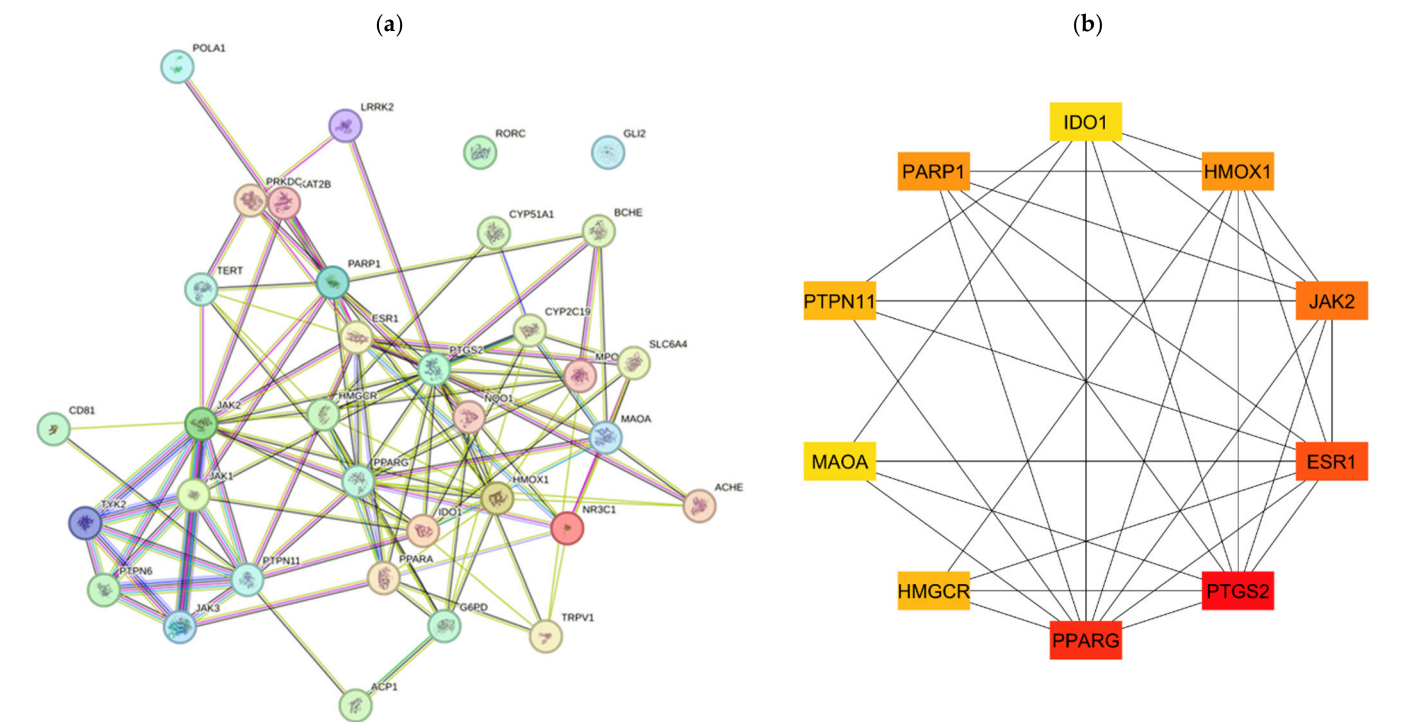
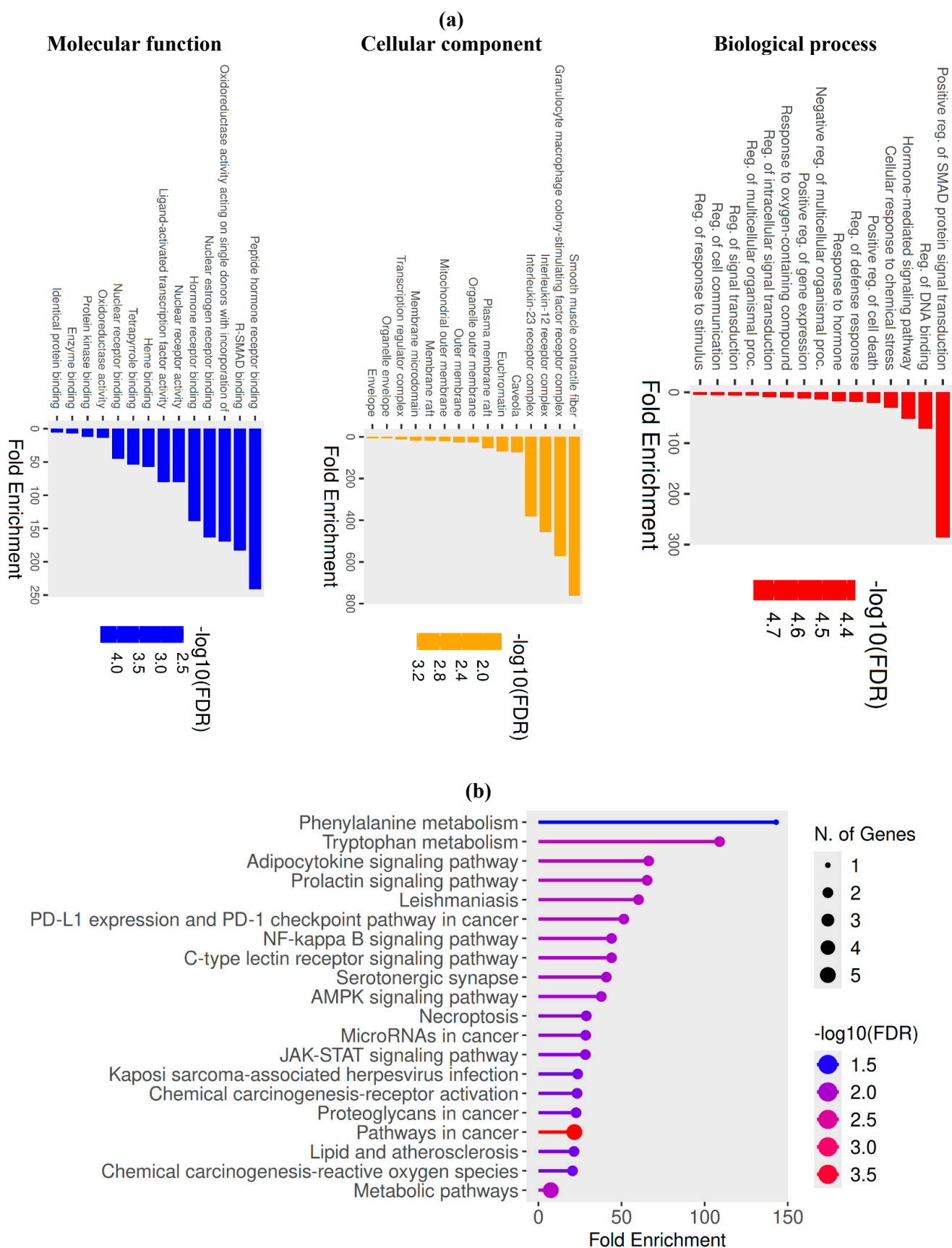


Figure 8. (a) Protein–protein interaction (PPI) network of overlapping targets constructed using the STRING database; (b) top 10 Hub gene network identified using CytoHubba (degree method). Colors indicate degree ranking, from red (highest) to yellow (lowest).



2.5.4. Molecular Docking

Molecular docking was performed as an exploratory assessment of interactions between the selected phytochemicals and PTGS2. Predicted binding affinities ranged from -5.1 to -7.7 kcal/mol (Table 6). Among the phytochemicals, τ -cadinol produced the most favorable score (-7.7 kcal/mol), followed by trans- α -bergamotene (-6.6 kcal/mol) and α -terpineol (-6.2 kcal/mol). Celecoxib and mefenamic acid, included as reference PTGS2 inhibitors and docked under the same conditions, yielded scores of -9.1 and -8.7 kcal/mol, respectively (Figure 10). Visualization of the predicted poses suggested contacts with residues in the PTGS2 binding pocket, including Ser530, Tyr385, and Leu352 (Figure 11). PTGS2 is a host inflammatory enzyme involved in prostaglandin synthesis and inflammation-related signaling during infection [45,46]. The docking results suggest moderate predicted interactions between selected flower-derived phytochemicals and PTGS2. The docking results suggest moderate predicted interactions between selected flower-derived phytochemicals and PTGS2.

Table 6. Molecular docking results of selected phytochemicals against PTGS2 showing binding affinity, cavity size, and docking parameters.

Gene	Compound Name	Cur Pocket ID	Vina Score (Binding Affinity)	Cavity Size	Centre (x, y, z)	Docking Size (x, y, z)
PTGS2	Celecoxib	C1	-9.1	3625	28, 26, 67	28, 31, 22
PTGS2	Mefenamic acid	C1	-8.7	3734	45, 35, 93	20, 30, 30
PTGS2	Linalool	C1	-5.8	3734	45, 35, 93	25, 30, 30
PTGS2	Estragole	C1	-6.1	2146	23, 9, 53	18, 26, 18
PTGS2	trans- α -Bergamotene	C1	-6.6	5489	49, 21, 64	35, 35, 35
PTGS2	τ -Cadinol	C1	-7.7	5489	49, 21, 64	35, 35, 35
PTGS2	Eucalyptol	C1	-5.8	3734	49, 21, 64	35, 35, 35
PTGS2	α Terpineol	C1	-6.2	3734	45, 35, 93	25, 30, 30
PTGS2	endo-Borneol	C1	-5.1	5489	49, 21, 64	35, 35, 35
PTGS2	Citral	C1	-5.8	3734	45, 35, 93	25, 30, 30



Figure 10. Molecular docking visualization of reference PTGS2 inhibitors. (a) Celecoxib docked. (b) Mefenamic acid docked with PTGS2.



Figure 11. Molecular docking interactions of selected phytochemicals with PTGS2 showing binding conformations and key amino acid interactions (a–h).

3. Materials and Methods

3.1. Plant Material

Fresh *O. basilicum* flowers were collected in Riyadh, Saudi Arabia, in May 2025. The plant material was taxonomically authenticated by Dr. Mona S. Alwhibi. A voucher specimen was deposited in the King Saud University herbarium under accession number KSU-24640.

3.1.1. Preparation of Flower Extract

The flowers were thoroughly rinsed with distilled water to remove dust and other impurities and were then shade-dried at room temperature. The dried material was ground to a fine powder using a mechanical grinder. Approximately 100 g of powdered material was extracted by maceration with 80% ethanol (ethanol/water, 80:20, *v/v*). Three consecutive 48 h extractions were performed with occasional stirring. The extracts were combined, filtered, and concentrated under reduced pressure at 40–45 °C using a rotary evaporator. The resulting crude extract was weighed and stored at 4 °C until use.

3.1.2. Gas Chromatography–Mass Spectrometry Analysis

The chemical profile of the *O. basilicum* flower extract was determined using a Thermo TRACE GC Ultra system coupled to a Thermo DSQ II mass spectrometer (Waltham, MA, USA). Compounds were identified by comparing the acquired mass spectra with reference spectra in the NIST/EPA/NIH Mass Spectral Library (2014 edition), as implemented in the MassHunter GC–MS software (NIST/EPA/NIH Mass Spectral Library (NIST 14)).

3.2. Green Synthesis of Silver Nanoparticles (AgNPs)

A 20 mL aliquot of 0.5 mM AgNO₃ was added to 80 mL of aqueous *O. basilicum* extract (1 g/100 mL), and the mixture was kept in the dark. Nanoparticle formation was monitored visually by recording color changes at regular intervals. After completion of the reaction, the mixture was centrifuged at 3000 rpm for 30 min to collect the nanoparticles. The pellet was washed three times with deionized water and then with absolute ethanol to remove residual impurities. The purified nanoparticles were dried at room temperature and stored until characterization.

3.3. Characterization of Silver Nanoparticles

3.3.1. Physical Observation

A change in the color of the reaction mixture was recorded as an initial qualitative indicator of AgNP formation.

3.3.2. UV–Visible Spectroscopy

AgNP formation was further assessed by UV–visible spectrophotometry using a Thermo Scientific instrument (Waltham, MA, USA). Absorbance was recorded from 300 to 600 nm, and the characteristic surface plasmon resonance (SPR) band was used as spectroscopic evidence of nanoparticle formation.

3.3.3. Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was used to identify functional groups potentially involved in nanoparticle reduction and stabilization. Spectra of the plant extract and synthesized nanoparticles were recorded using a PerkinElmer Spectrum BX FTIR spectrometer (Perkin Elmer, Yokohama, Japan). Each sample was scanned three times over 4000–400 cm^{−1}, and the mean transmittance spectrum was evaluated.

3.3.4. Particle-Size and Zeta-Potential Analyses

The hydrodynamic diameter and surface charge of the synthesized nanoparticles were measured by dynamic light scattering (DLS) and electrophoretic light scattering (ELS), respectively, using a Malvern Panalytical Nano series instrument (Malvern Instruments Ltd., Malvern, UK) under standard conditions. Zeta potential was used to assess dispersion stability, with larger absolute values indicating stronger electrostatic repulsion between particles.

3.3.5. Morphological and Elemental Analysis

The morphology and size distribution of the nanoparticles were examined using Transmission Electron Microscopy (TEM, JEOL Ltd., Tokyo, Japan). Samples were prepared by placing a drop of the nanoparticle suspension onto carbon-coated copper grids and allowing them to dry before imaging. Analyses were performed using a JEOL JEM-1400 Plus (JEOL Ltd., Tokyo, Japan) to obtain high-resolution images at the nanoscale. The surface morphology and size characteristics of the synthesized silver nanoparticles were examined using Scanning Electron Microscopy (SEM, JEOL Ltd., Tokyo, Japan). Imaging was performed using a JSM-7610F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan), where the sample surface was scanned by a focused electron beam to generate detailed, high-resolution images. Elemental composition of the nanoparticles was further analyzed using energy-dispersive X-ray (EDX) spectroscopy (JEOL Ltd., Tokyo, Japan) coupled with the SEM system. This technique enabled the identification of the elemental constituents and confirmed the presence of silver within the synthesized nanostructures.

3.4. Microbial Strains and Culture Conditions

Thirteen microbial strains used in this study were obtained from the Central Research Laboratory, King Saud University, Riyadh, Saudi Arabia. The tested microorganisms comprised both standard ATCC reference strains and clinical isolates. The Gram-positive bacteria included *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (clinical isolate), *Streptococcus pneumoniae* (ATCC 49610), *Bacillus subtilis* (ATCC 6633), and *Streptococcus pyogenes* (ATCC 19615). The Gram-negative group consisted of *Salmonella Typhi* (clinical isolate), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35218), *Enterobacter cloacae* (ATCC 13047), and *Klebsiella pneumoniae* (clinical isolate). Two fungal strains, *Candida albicans* (ATCC 66027) and *Candida tropicalis* (ATCC 66029), were also included in this study. Prior to antimicrobial assays, bacterial strains were cultured on Mueller–Hinton agar (MHA), while fungal strains were maintained on Sabouraud dextrose agar (SDA).

3.4.1. Antimicrobial Activity (Agar Well-Diffusion Method)

Antimicrobial activity was evaluated by the agar well-diffusion method. Microbial cultures were incubated in nutrient broth for approximately 20 h and adjusted to about 10^6 CFU/mL. A 20 μ L aliquot of each culture was distributed on sterile MHA plates using a sterile swab. After the inoculated plates had dried, 6 mm wells were prepared and filled with 100 μ L of plant extract or nanoparticle suspension. Plates were incubated at 37 °C for 18 h, and inhibition-zone diameters were measured in millimeters. Gentamicin discs (10 μ g/disc; 6 mm diameter; Condalab, Madrid, Spain) served as positive controls for bacterial strains, and fluconazole discs (25 μ g/disc; 6 mm diameter; Condalab) served as positive controls for *Candida* strains. Distilled water and 1 mM AgNO₃ were used as negative controls. All assays were performed in triplicate.

3.4.2. Determination of MIC and MBC

The MIC and MBC values of *O. basilicum* flower extract and OBF-AgNPs were determined by broth microdilution in 96-well plates. Serial dilutions of each test material were prepared in broth and inoculated with microbial suspensions adjusted to approximately 10^6 CFU/mL. After incubation at 37 °C for 24 h, growth was evaluated visually and by measuring optical density at 600 nm (OD₆₀₀). The MIC was defined as the lowest concentration that produced no visible growth and no increase in OD₆₀₀ relative to the control. For MBC determination, aliquots from wells without visible growth were plated on agar and

incubated. The MBC was defined as the lowest concentration yielding no visible colonies, corresponding to approximately 99.9% microbial killing.

3.4.3. Determination of Biofilm Formation

The antibiofilm activity of OBF-AgNPs was evaluated against *S. aureus*, *P. aeruginosa*, and *C. tropicalis*, selected as the most sensitive strains based on MIC and MBC results. Overnight microbial cultures were adjusted to approximately 10^6 CFU/mL and added to 96-well microtiter plates containing OBF-AgNPs at $4\times$ MIC, $2\times$ MIC, MIC, $\frac{1}{2}\times$ MIC, and $\frac{1}{4}\times$ MIC. Untreated microbial cultures and medium-only wells served as positive and negative controls, respectively. After incubation at 37 °C for 72 h, the wells were washed with sterile phosphate-buffered saline and stained with 0.1% crystal violet for 15 min. The retained stain was dissolved using 30% glacial acetic acid, and biofilm formation was quantified by measuring absorbance at 570 nm. The wells were also photographed for qualitative evaluation.

3.5. Statistical Analysis

For antimicrobial assays, statistical comparisons were performed separately for each microbial strain among the extract, OBF-AgNPs, AgNO₃, negative control, and positive control groups. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Different superscript letters within the same row indicate statistically significant differences at $p < 0.05$.

3.6. In Silico Studies

In silico approaches were applied to evaluate the potential biological relevance of phytochemicals identified from the *O. basilicum* flower extract.

3.6.1. Integrated Network Pharmacology of Host-Target Prediction for the Selected Phytochemicals

Phytochemicals were selected according to their relative abundance and reported biological relevance. Potential human molecular targets were predicted using SwissTargetPrediction. Canonical SMILES strings were submitted for each compound, with the organism restricted to *Homo sapiens*, and predictions with probabilities greater than zero were retained. Target lists for all selected compounds were combined, duplicate entries were removed, and identifiers were standardized to official gene symbols using UniProt.

3.6.2. Identification of Microbial-Infection-Related Targets

Genes associated with microbial infection were retrieved from GeneCards using the search term "microbial infection." The database was accessed on 19 April 2026, and genes with relevance scores of ≥ 5 were retained. The predicted phytochemical targets were intersected with the GeneCards set, and the overlap was visualized using a Venn diagram. The shared genes were designated as exploratory host-response-associated targets for subsequent network and enrichment analyses.

3.6.3. Protein–Protein Interaction (PPI) Network Analysis

The overlapping targets were submitted to STRING (version 11.5) for protein–protein interaction analyses. The organism was restricted to *Homo sapiens*, and interactions with confidence scores >0.9 were retained. The network was visualized using Cytoscape version 3.10.3, and hub genes were identified and ranked by node degree using the cytoHubba plugin version 0.1, with a higher degree indicating greater connectivity within the network.

3.6.4. Functional and Pathway Enrichment Analysis

Functional and pathway enrichment analyses of the overlapping host-response-associated targets were performed using ShinyGO (<https://bioinformatics.sdstate.edu/go/>, accessed on 20 April 2026). Enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms were evaluated across the biological-process, molecular-function, and cellular-component categories.

3.6.5. Molecular Docking Analysis

Molecular docking was performed to investigate the predicted binding interactions between selected GC–MS-identified phytochemicals and PTGS2. The crystal structure of human PTGS2 was retrieved from the Protein Data Bank (PDB ID: 5IKR). The three-dimensional structures of the selected phytochemicals were retrieved from PubChem, including linalool (CID: 6549), estragole (CID: 8815), trans- α -bergamotene (CID: 6429302), τ -cadinol (CID: 6429185), eucalyptol (CID: 2758), α -terpineol (CID: 17100), endo-borneol/borneol (CID: 64685), and citral (CID: 638011). Celecoxib (PubChem CID: 2662) and mefenamic acid (PubChem CID: 4044), both known PTGS2 inhibitors, were included as reference ligands for comparative docking under the same conditions. Docking simulations were carried out using the CB-Dock2 online platform to evaluate predicted binding affinities and ligand–protein interaction patterns. The best binding poses were selected based on docking scores and interaction profiles. The docking results were interpreted only as predicted ligand–target interactions.

4. Conclusions

Silver nanoparticles were successfully synthesized using *Ocimum basilicum* flowers and exhibited antimicrobial activity against the tested bacterial and fungal strains. UV–Vis, FTIR, SEM, TEM, DLS, zeta-potential, and quantitative EDX analyses supported the formation of nanoscale OBF-AgNPs with a phytochemical-associated surface layer and moderate colloidal stability. However, the absence of XRD analysis remains a limitation in confirming the crystalline metallic silver phase. GC–MS-guided network pharmacology identified host-response-related targets with moderate predicted interactions.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/inorganics14080216/s1>. Table S1: Phytochemicals identified from *Ocimum basilicum* flower extract used for network pharmacology analysis; Table S2: Predicted targets of selected phytochemicals; Table S3: Microbial infection-related targets (GeneCards); Table S4: Common targets between phytochemicals and infection; Table S5: Top enriched KEGG pathways based on FDR, gene count, and fold enrichment analysis.

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