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The immunomodulation *of Nelumbo nucifera* seed extract on rabbits infected with *Enterobacter cloacae complex*

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Abstract

Objective

In this study, isolation and identification of members of the *Enterobacter cloacae complex* (ECC) from domestic cats in Baghdad, Iraq, and determination of their susceptibility patterns to various antibiotics were conducted. This study also assessed the effect of Nelumbo nucifera (lotus) seed extract as an immunomodulator in an experimental animal model.

Materials and Methods

Seventy five clinical specimens from domestic cats (feces, blood, and urine) were obtained. Preliminary isolation and identification were done based on morphological characteristics and fermentation pattern of ECC strains to lactose in select media. Confirmatory test included automatic system analysis using VITEK 2 Compact System and identification through PCR amplification of the 16S rRNA gene (1250 bp). Antibiotic susceptibility was determined according to guidelines. GC-MS analysis was done for ethanolic extract of *N. nucifera* seed. Immunomodulatory activity of lotus seed extract was tested using rabbits as the experimental animal through determination of hematological parameters and levels of interleukins IL-1β and IL-8 using ELISA.

Results

The isolation rate of ECC was 2.7% among all cultures collected, with a higher prevalence in the feces (5.7%) than in blood and urine. Susceptibility testing showed high sensitivity to carbapenems, aminoglycosides, and fluoroquinolones, with partial resistance to β-lactam/β-lactamase inhibitor combinations. In the GC-MS analysis of the lotus seed extract, linoleic acid was found to be the main active constituent. After administration of the extract in vivo at a preventive dose (200 mg/mL), there were remarkable decreases in leukocytosis and granulocytosis, as well as increases in the lymphocyte count. Moreover, the levels of pro-inflammatory cytokines IL-1β and IL-8 were significantly reduced according to the ELISA results.

Conclusions
Based on the results, domestic cats within the study population in Baghdad might constitute a limited source of ECC. The ethanolic extract of *N. nucifera* seeds exhibits promising immunomodulatory properties through the regulation of inflammatory responses and promoting immune balance.
Keywords: 16S rRNA, antimicrobial resistance, domestic cats, *Enterobacter cloacae* complex, VITEK 2

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Introduction

Enterobacter species are Gram-negative, facultatively anaerobic, bacilli that are abundant in environmental and host-associated environments. *Enterobacter cloacae* complex (ECC) is becoming a significant opportunistic pathogen with an increasing role in hospital-acquired infections and significant plasticity of its genome, which supports the acquisition of multidrug resistance (MDR) (Mohammed et al., 2026; Davin-Regli et al., 2019; Mezzatesta et al., 2012). It is linked to urinary tract, bloodstream and respiratory infections, especially in immunocompromised hosts (Annavajhala et al., 2019; Salimiyan rizi et al., 2020), and the presence of ESBLs and AmpC β-lactamases only complicates treatment (Elshobary et al., 2025; Adams et al., 2018). The issue of companion animals, particularly cats and dogs, is becoming a recognized risk of MDR *Enterobacter*iaceae, which may be used in the possible transmission of the zoonotic infection due to close contact with a person (Sadeq & Lafta, 2024; Guardabassi et al., 2004; Scott Weese, 2008). This underscores the importance of constant monitoring and use of alternative therapies. Phytobiotics and medicinal plants have received significant attention in recent years because of their possibility as a substitute for synthetic additives in nutrition (Amirteymoori et al., 2021). Such natural sources are highly effective and contain bioactive substances, including essential oils, alkaloids, flavonoids, and phenolic acids (Mohammadabadi et al., 2025a). Therefore, phytobiotics are considered highly valuable as health, productivity, and quality enhancing factors (Safaei et al., 2025). Utilizing phytobiotics and medicinal plants as natural growth promoters against microbial infections without resorting to antibiotics in the diet

provides numerous advantages (Khezri et al., 2025). This includes optimizing zootechnical efficiency parameters, preventing certain diseases (Mohammadabadi et al., 2025b), antimicrobial and antioxidative properties, hypocholesterolemic effects, digestive enzyme stimulation, and increased liver activity (Roudbar et al., 2015). Furthermore, the use of phytobiotics allows for better modulation of gut microbiota that ensures more effective nutrient utilization and improves immune functions (Vahabzadeh et al., 2021). It was also found that including these plants in nutrition increases feed intake, improves feed efficiency, and stimulates performance (Vahabzadeh et al., 2020). Moreover, phytobiotics have been linked to mitigating the adverse effects of stress, enhancing the quality of health, and minimizing the ecological footprint by maximizing nutrient efficiency (Alhasoon et al., 2026; Mohammadabadi et al., 2025c). In light of the rising global worries about antibiotic resistance and the need for a safer and healthier existence, the integration of phytobiotics and medicinal plants in animal feeds can be considered an innovative approach (Mohammadabadi et al., 2022). In this respect, *Nelumbo nucifera* (lotus) seed extracts, which are rich in bioactive polyphenols and fatty acids have been shown to have promising pharmacological effects (Mohammed, 2025; Mukherjee et al., 2009). To this end, the study was aimed at identifying the prevalence, characterization, and antimicrobial resistance profiles of ECC in domestic cats in Baghdad, Iraq and to evaluate the in vivo antibacterial, hematological, and immunomodulatory potentials of *N. nucifera* seed extract in a controlled rabbit infection model.

Materials and methods

Study design and sample collection: The research was a cross-sectional epidemiological study that took place between October 2025 and January 2026. The samples comprised of 75 clinical samples (feces, blood, and urine) of domestic cats in veterinary clinics in Baghdad, Iraq. Microbial viability was preserved by all the specimens being aseptically collected and transported to the microbiology laboratory under cold-chain conditions as (Table 1).

Table 1. Sample distribution in Baghdad, Iraq

Type of sample	Numbers	Percentage
Urine	20	26.7%
Blood	20	26.7%
Feces	35	46.6%
Total	75	100

Isolation and phenotypic identification: Clinical samples underwent the bacterial isolates cultured on MacConkey and Eosin Methylene Blue (EMB) agar and cultured aerobically at 37 C. The initial identification was done on the morphology of the colonies and the lactose fermentation patterns. This was confirmed by additional Gram staining and microscopy, confirming the presence of Gram-negative bacilli (O'Hara, 2005; Jorgensen et al., 2015).

Automated biochemical identification and AST: Definitive identification and antimicrobial susceptibility testing (AST) were done through automated VITEK 2 Compact system (bioMerieux, France). The DensiChek™ system was used to standardize the pure colonies

(18-24 h) to 0.50-0.63 McFarland. To identify it, Gram-negative (GN) cards with 64 biochemical tests were automatically inoculated and recognized after approximately 6.35 h providing a biochemical profile that identified *Enterobacter cloacae* complex with a confidence of 94 percent. In the case of AST, GN AST cards were read and analyzed by the Advanced Expert System (AES) which computed the MIC values after 15-18 h incubation. The reported resistance phenotypes and categories of susceptibility (S, I, SDD, R) were based on the CLSI guidelines (CLSI, 2023).

Molecular confirmation: The DNA of the phenotypically confirmed ECC isolates was moved to genomic DNA using a commercial DNA extraction kit (Genesig Easy, Primerdesign Ltd., Southampton, UK) according to the guidelines of the manufacturer. Molecular confirmation was done through PCR analysis of the highly conserved 16S rRNA gene with the help of primers that were specific to members of the *Enterobacter cloacae* complex. Amplicons were then run on a 1.5% gel of agarose, stained and viewed under ultraviolet transillumination to verify the anticipated target size of the amplicon of 1250 base pairs (Kaplan et al., 2025; Hu et al., 2023).

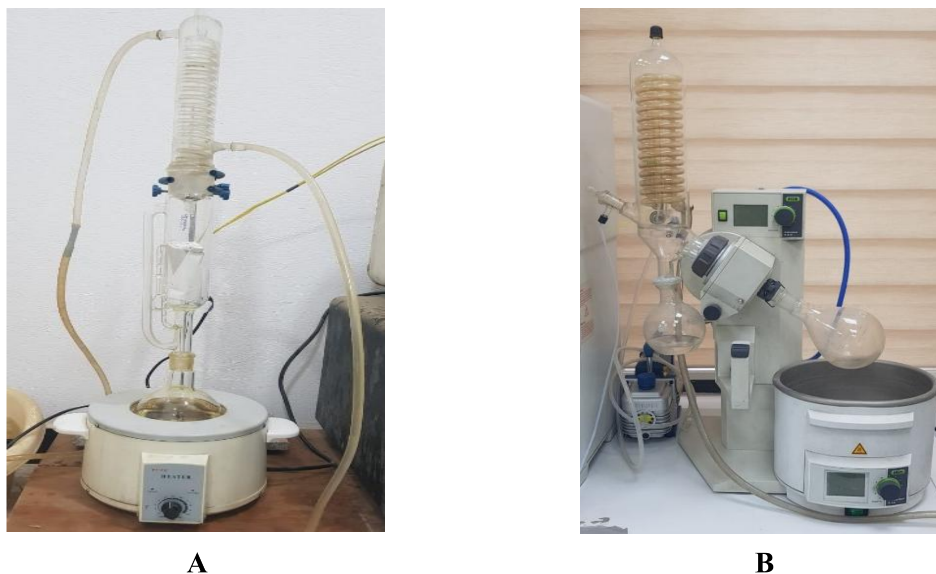
Lotus seeds extract: Bioactive compounds of *N. nucifera* seeds (Figure 1) were obtained by a continuous hot solvent extraction (Figure 2). The seeds were dried and milled into fine powder and subjected to Soxhlet extraction with absolute ethanol. (Luque de Castro & Priego-Capote, 2010). A rotary evaporator was used to concentrate the ethanolic extract under a lower pressure to achieve a solvent-free crude extract. This dried extract was reconstituted with sterile distilled water to obtain three working concentrations (100, 200, and 300 mg/mL) to be used in in vivo experiments. (Mukherjee et al., 2009).

A B

Figure 1. Processing of *Nelumbo nucifera* (Lotus) seeds. (A) Whole unprocessed dark lotus seeds. (B) Seeds with a homogenous, fine powder using an electrical grinder to facilitate an efficient solvent extraction

Gas chromatography-mass spectrometry analysis (GC-MS): After being extracted in DCM, phytochemical constituents were examined using GC-MS. The separation was done on an Agilent 7890B GC column with a 5977A mass spectrometer using a 1.0 mL/min helium (99.999) carrier gas at 1.0 mL/min. The oven temperature was set to 50 C-280 C and retention times and

mass spectra were compared to the NIST library to identify the presence of important bioactive metabolites (Fan et al., 2018).



A B

Figure 2. Ethanolic extraction of *Nelumbo nucifera* (Lotus) seed powder (A) Soxhlet extractor apparatus over a heating mantle to carry out the extraction of the hot solvent. B) Büchi rotary evaporator was employed in the effective evaporation of ethanol and concentration of the crude lotus extract under a vacuum

Experimental design and challenge pathogen In Vivo: Forty-eight rabbits were acclimatized, randomly assigned to six groups of equal number (n=8). The prophylaxis was 21-days and extract was orally administered twice per week in Groups 1 (100 mg/mL), Group 2 (200 mg/mL), Group 3 (300 mg/mL), and Group 5 (200 mg/mL extract, uninfected control). The positive control (Group 4) was the infected, no treatment, and the negative control (Group 6) was the PBS only (Table 2). On the 21 st -day, Groups 1-4 were orally challenged using a standardized inoculum of *Enterobacter cloacae* complex after the baseline blood collection. To obtain the final infective dose, the bacterial suspension was brought to 0.5 McFarland (approximately 1.5 x 10 8 CFU/mL), and the Reed-Muench method was used (Reed and Muench, 1938).

Table 2. Experimental grouping design of rabbits

Group	Treatment	Infection Status
G1	Lotus extract 100 mg/mL	Infected with ECC
G2	Lotus extract 200 mg/mL	Infected with ECC
G3	Lotus extract 300 mg/mL	Infected with ECC
G4	No treatment	Infected positive control
G5	Lotus extract 200 mg/mL only	Uninfected control
G6	PBS only	Negative control

Hematological profiling: In order to assess the systemic physiological effect of the bacterial challenge and the immunomodulatory effects of the *N. nucifera* extract, the blood samples in EDTA-coated tubes were collected in all groups of the experiment (G1-G6). Two sampling points (baseline (day 0) and post-challenge (day 31) were done. An automated veterinary hematology

analyzer (HT-300 Vet) was used to measure dynamic changes in total White Blood Cell (WBC) counts, percentages of differential lymphocytes and granulocytes to assess changes in the count.

Immunological analysis of pro-inflammatory cytokines: To measure systemic inflammation, serum IL-1β and IL-8 levels were measured. Serum was obtained by collecting blood in serum separator tubes, allowing them to clot, and centrifugation (3000 × g, 10 min, 4 C) (Lee et al., 2016). Cytokines were determined by the rabbit-specific sandwich ELISA kits according to the conventional protocols. Antibody-coated plates were incubated with samples and standards (0-120 pg/mL), and then conjugated with HRP, washed and then TMB substrate developed. The reaction was stopped and absorbance measured at 450 nm. Linear regression was used to obtain concentrations based on standard curves.

Statistical analysis: GraphPad Prism and SPSS were used to analyze the data in terms of mean ± SD. Comparisons were carried out by one-way ANOVA with the post hoc test offered by Tukey and paired t-test. Results of ELISA were determined based on standard curves (R 2 > 0.99), and a significance level was set at $p < 0.05$.

Results

Epidemiological prevalence of isolates: During the surveillance period, 75 clinical specimens were analytically handled. Only 2 samples were isolated to confirm the presence of the target pathogen, and this is an overall prevalence rate of 2.7%. The rest of the specimens (97.4%) were culturally negative to ECC. Notably, the positive isolates were only found in fecal samples (with a 5.7% specific prevalence of feces) but no targeted growth was found in all tested blood and urine samples (Table 3).

Table 3. Isolation of *Enterobacter cloacae* complex by clinical specimen type

Sample	Total number of samples	Number of Positive isolates	Percentage of positive (%)
Blood	20	0	0%
Feces	35	2	5.7%
Urine	20	0	0%
Total	75	2	2.7%

Cultural characteristics: The isolates had a very characteristic phenotypic expression on the selective media, which was typical of *Enterobacter*iaceae. The colonies were pink in colour on MacConkey agar, which showed a strong ability to ferment lactose (Figure 3). Growth on Eosin Methylene Blue (EMB) agar resulted in large colonies with a distinct mucoid look and a dark purple pigmentation (Figure 4).

Automated biochemical confirmation using VITEK 2 system: To validate the results of the phenotypic study and eliminate the taxonomic ambiguity, the isolates were tested using the VITEK 2 automated system (bioMerieux, France) with GN identification cards. With a high confidence level of 94%, the system was able to identify the *Enterobacter cloacae* complex within 6.35-6.85 hours (Figures 6 and 7).

Figure 3. ***Enterobacter cloacae*** **complex colonies on the MacConkey agar**

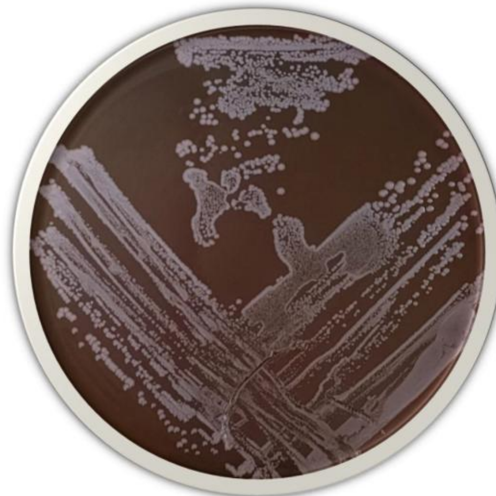


Figure 4. ***Enterobacter cloacae*** **complex morphologic colony on EMB agar**

Microscopic examination: The Gram-negative, rod-shaped bacillus microscopic observation, which was confirmed with Gram staining, perfectly matched the morphological profile of *Enterobacter* species (Figure 5).

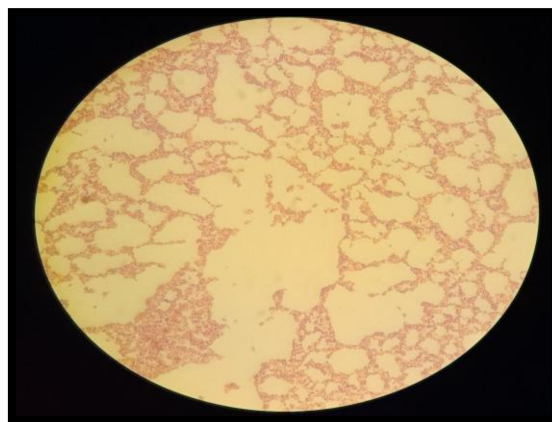


Figure 5. Gram staining of ***Enterobacter cloacae*** **complex that illustrates Gram-negative bacilli**

The biochemical profiles indicated a positive assimilation of important substrates such as β-galactosidase, D-maltose, D-mannitol, D-mannose, D-xylose, D-sorbitol, and sucrose. The activity of β-glucosidase varied across isolates, demonstrating heterogeneity of the strains at strain level. All the isolates were negative in APPA, pyrrolidonyl arylamidase, urease and H2S production. This broad metabolic profile was a sound identification scheme that diminished confusion in the classification of *Enterobacter*iaceae.

Organism Quantity:
Selected Organism : Enterobacter cloacae complex

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:** 6.85 hours	**Status:** Final
Selected Organism	94% Probability **Enterobacter cloacae complex** **Bionumber:** 0627631553533010	
ID Analysis Messages		

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	(-)	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	+	45	PHOS	-
46	GlyA	+	47	ODC	+	48	LDC	-	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Figure 6. VITEK2 automated biochemical identification report of the feline *Enterobacter cloacae* isolate (Time taken to analyze: 6.35 hours) with a 94 percent probability and positive response in the BGLU test

Organism Quantity:
Selected Organism : Enterobacter cloacae complex

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:** 6.85 hours	**Status:** Final
Selected Organism	94% Probability **Enterobacter cloacae complex** **Bionumber:** 0627631553533010	
ID Analysis Messages		

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	(-)	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	+	45	PHOS	-
46	GlyA	+	47	ODC	+	48	LDC	-	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Figure 7. VITEK 2 automated biochemical identification report of the isolated individual *Enterobacter cloacae* (Analysis time: 6.85 hours). It confirms that the probability is 94% and shows a phenotypic variation with a negative BGLU reaction

Antimicrobial susceptibility profiling and resistance patterns: Antimicrobial susceptibility testing (AST) in vitro was done in the VITEK 2 system, and the MIC values were interpreted as per CLSI guidelines. Both isolates were fully susceptible to carbapenems (imipenem, meropenem), aminoglycosides (amikacin, gentamicin), fluoroquinolones (ciprofloxacin) and colistin, indicating retained efficacy of these classes of drugs (Figures 8 and 9). On the contrary, distinct variations were noticed in cephalosporin and β-lactam/ β-lactamase inhibitor responses. Sample A exhibited pan-susceptible phenotype, and it was highly sensitive to cefepime, cefotaxime and ceftolozane/tazobactam. Sample B, however, exhibited a multidrug-resistant pattern, being resistant to ceftazidime/avibactam and ceftolozane/tazobactam, SDD to ceftepime, and showing intermediate susceptibility to cefotaxime. The trend indicates that there was probable overexpression of AmpC β-lactamase or resistance due to ESBL.

Organism Quantity:
Selected Organism : Enterobacter cloacae complex

Source: **Collected:**

Comments:	

Susceptibility Information	**Analysis Time:** 18.07 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin/Sulbactam			Meropenem	<= 0.25	S
Piperacillin/Tazobactam	8	S	Amikacin	<= 1	S
Cefotaxime	<= 0.25	S	Gentamicin	<= 1	S
Ceftazidime	0.5	S	Ciprofloxacin	<= 0.06	S
Ceftazidime/Avibactam	8*	S	Tigecycline	1	S
Ceftolozane/Tazobactam	<= 0.25	S	Colistin	<= 0.5	S
Cefepime	<= 0.12	S	Trimethoprim/ Sulfamethoxazole	<= 20	S
Imipenem	1	S			

***= AES modified **= User modified**

Figure 8. VITEK 2 automated antimicrobial susceptibility test of *Enterobacter cloacae* isolate (Sample A). This report took 18.07 hours of kinetic analysis to demonstrate a very vulnerable and wild-type phenotype that is susceptible to high doses of cephalosporins and combinations of inhibitors

PCR molecular identification: The phenotypic taxonomy was conclusively supported by genomic amplification of the targeted 16S rRNA marker. Electronic separation of the PCR products on a 1.5 percent agarose gel produced prominent, high-intensity bands in Lanes 1 and 2 at about 1250 bp, which were the appropriate molecular weight of the *Enterobacter cloacae* complex (Figure 10).

Phytochemical profiling of *Nelumbo nucifera* extract: The *N. nucifera* extract was analytically deconvoluted to show it had a complex biochemical profile. The UV-Vis spectra revealed the presence of absorption peaks at 463, 490, 501 and 505 nm, indicating the existence of polyphenolic compounds that contain a high number of conjugates (Figure 11). The FTIR spectroscopy revealed general O-H stretching peaks and classic peaks of aliphatic C-H and

carbonyl (C=O) groups indicating the presence of different functional moieties in the extract (Figure 12).

Organism Quantity:
Selected Organism : Enterobacter cloacae complex

Source: **Collected:**

Comments:	

Susceptibility Information	**Analysis Time:** 15.60 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin/Sulbactam			Meropenem	<= 0.25	S
Piperacillin/Tazobactam	8	S	Amikacin	<= 1	S
Cefotaxime	2	I	Gentamicin	<= 1	S
Ceftazidime	0.5	S	Ciprofloxacin	<= 0.06	S
Ceftazidime/Avibactam	>= 16*	R	Tigecycline	1	S
Ceftolozane/Tazobactam	16	R	Colistin	<= 0.5	S
Cefepime	4	SDD	Trimethoprim/ Sulfamethoxazole	<= 20	S
Imipenem	<= 0.25	S			

***= AES modified **= User modified SDD= Susceptible-dose dependent (SDD) implies that susceptibility of an isolate is dependent on the dosing regimen used. For further information, please refer to CLSI Performance Standards, or Product information.**

Figure 9. VITEK 2 automated antimicrobial susceptibility report of *Enterobacter cloacae* isolate (Sample C). This report is determined in 15.60 hours and it indicates a concerning multidrug-resistant (MDR) profile, in which there is a marked resistance to both Ceftazidime/Avibactam and Ceftolozane/Tazobactam

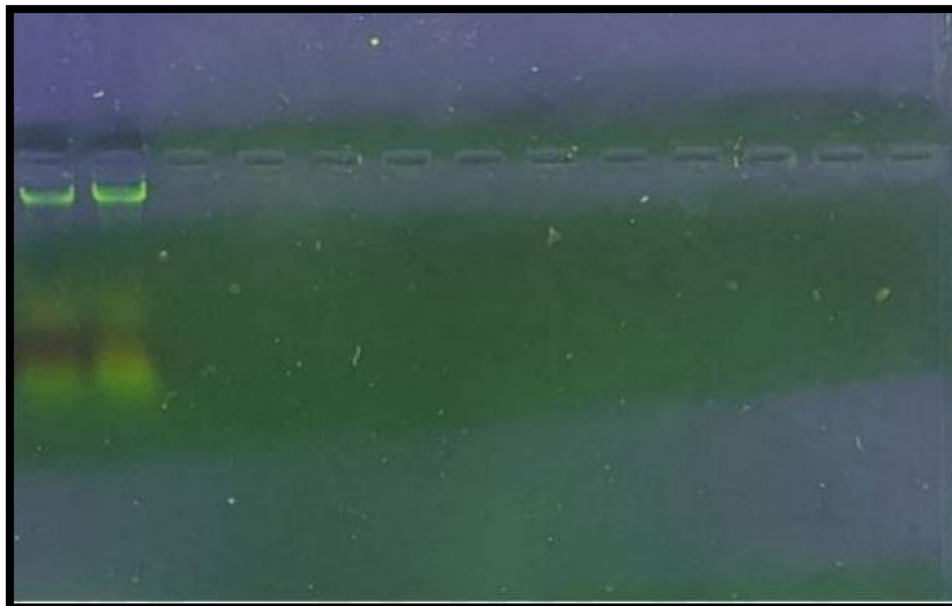


Figure 10. PCR amplification of a target gene in two bacterial isolates in a 1.5% agarose gel electrophoresis. Distinct bands were detected at around 1250 bp. Lanes 1-2: positive isolates

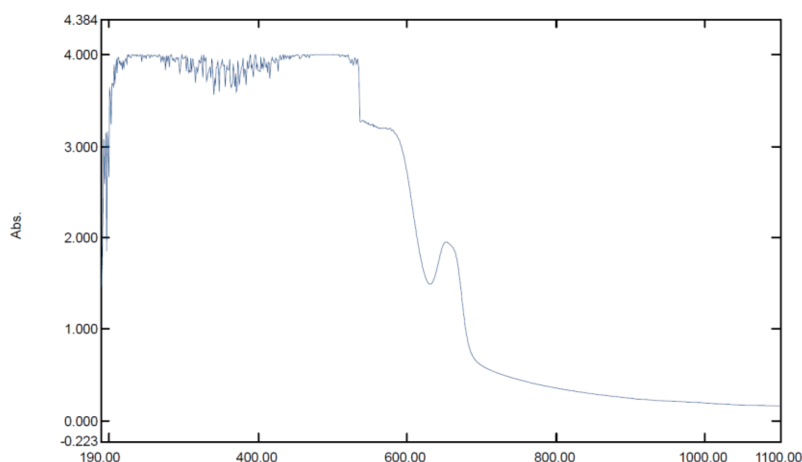


Figure 11. UV-Visible absorption spectrum of *Nelumbo nucifera* (Lotus) alcoholic extract with the highest maxima in the visible region

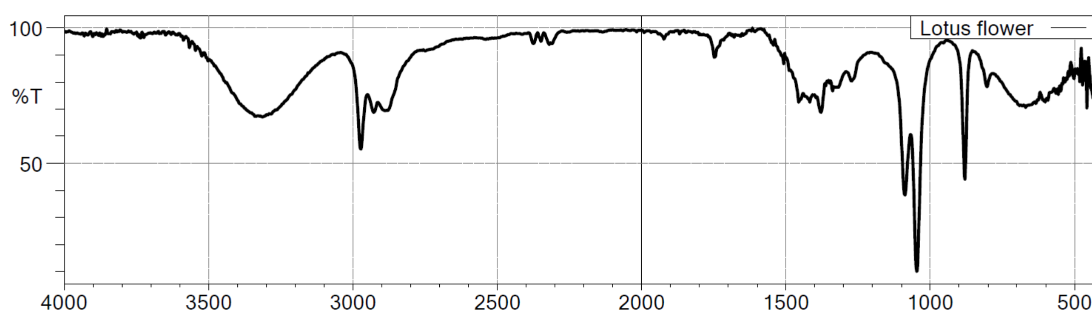


Figure 12. FTIR spectrum of alcoholic extract of *Nelumbo nucifera* seeds

Analysis by GC-MS also revealed an abundance of bioactive compounds, one of which was the polyunsaturated fatty acid 9,12-Octadecadienoic acid (linoleic acid) at RT 26.334 min, and other compounds such as phenylethyl alcohol and 1,2-cyclopentanedione, which reveals significant pharmacologically relevant chemical diversity (Table 4).

In Vivo hematological modulation (complete blood count): The hematological results in vivo revealed that *N. nucifera* extract had a tremendous immunomodulatory effect on the experimental models. The untreated positive control (G4) as indicated in Table 3-3 showed a severe inflammatory response after bacterial challenge with a significant leukocytosis of the sample ($15.05 \pm 0.98 \times 10^3/\mu L$), a strong granulocytosis (60.34 ± 3.35), and lymphopenia (35.11 ± 3.25). Conversely, these parameters were significantly stabilized by the prophylactic administration of the extract in groups G1, G2 and G3. The most effective one was the medium dose (G2, 200 mg) that had the lowest level of WBC elevation ($8.99 \pm 0.17 \times 10^3/\mu L$), the most desirable immune balance with the highest percentage of lymphocytes ($69.00 \pm 0.82\%$) and the lowest percentage of granulocytes ($23.54 \pm 0.71\%$). Significantly, the control groups (G5 and G6) were all within normal physiological limits. The stability of G5 (extract only) is a sign that *N. nucifera* is safe, because it did not cause any abnormal inflammatory markers, in the absence of infection as (Table 5).

Table 4. GC-MS results of the major bioactive compounds of *Nelumbo nucifera* alcoholic extract, indicate their retention time (RT) and percentage relative peak areas

Compound Label	RT	Name	DB Formula	Hits (DB)
Cpd 1: 1,3-Benzenediol, monobenzoate	4.215	1,3-Benzenediol, monobenzoate	C13H10O3	10
Cpd 2: Ethanol, 2-(trimethylsilyl)-	5.405	Ethanol, 2-(trimethylsilyl)-	C5H14OSi	10
Cpd 3: 2-Cyclopenten-1-one, 2-hydroxy-	8.049	2-Cyclopenten-1-one, 2hydroxy-	C5H6O2	10
Cpd 4: Pentane, 1,1-diethoxy-	8.483	Pentane, 1,1-diethoxy-	C9H20O2	10
Cpd 5: Benzeneacetaldehyde	10.27	Benzeneacetaldehyde	C8H8O	10
Cpd 6: Benzenemethanol, .alpha.-methyl-	11.577	Benzenemethanol, .alpha.methyl-	C8H10O	10
Cpd 7: Camphor	11.926	Camphor	C10H16O	10
Cpd 8: Nonane, 4,5-dimethyl-	16.82	Nonane, 4,5-dimethyl-	C11H24	10
Cpd 9: 1R,2c,3t,4t-Tetramethyl-cyclohexane	18.966	1R,2c,3t,4t-Tetramethylcyclohexane	C10H20	10
Cpd 10: Borane, diethyl(decyloxy)-	19.139	Borane, diethyl(decyloxy)-	C14H31BO	10
Cpd 11: 1-Propanol, 3-(diethylamino)-2,2-dimethyl-, p-aminobenzoate (ester)	21.458	1-Propanol, 3-(diethylamino)-2,2-dimethyl-, paminobenzoate (ester)	C16H26N2O2	10
Cpd 12: Tetradecanoic acid, 10,13-dimethyl-, methyl ester	21.514	Tetradecanoic acid, 10,13dimethyl-, methyl ester	C17H34O2	10
Cpd 13: n-Hexadecanoic acid	21.931	n-Hexadecanoic acid	C16H32O2	10
Cpd 14: Hexadecanoic acid, ethyl ester	22.162	Hexadecanoic acid, ethyl ester	C18H36O2	10
Cpd 15: Norbornane, 2isobutyl-	23.13	Norbornane, 2-isobutyl-	C11H20	10
Cpd 16: Oxazole, 4,5-dihydro-2-pentadecyl-	23.552	Oxazole, 4,5-dihydro-2pentadecyl-	C18H35NO	10
Cpd 17: cis-7,cis-11-Hexadecadien-1-yl acetate	23.567	cis-7,cis-11-Hexadecadien-1yl acetate	C18H32O2	10
Cpd 18: 1R,2c,3t,4t-Tetramethyl-cyclohexane	23.605	1R,2c,3t,4t-Tetramethylcyclohexane	C10H20	10
Cpd 19: Linoleic acid ethyl ester	23.733	Linoleic acid ethyl ester	C20H36O2	10
Cpd 20: 9-Octadecenoic acid, ethyl ester	23.772	9-Octadecenoic acid, ethyl ester	C20H38O2	10
Cpd 21: Octadecanoic acid, ethyl ester	23.989	Octadecanoic acid, ethyl ester	C20H40O2	10
Cpd 22: 2-((8Z,11Z)-Heptadeca-8,11-dien-1-yl)4,5-dihydrooxazole	25.059	2-((8Z,11Z)-Heptadeca-8,11dien-1-yl)-4,5-dihydrooxazole	C20H35NO	10

Table 4. continued

Cpd 23: Carbonic acid, 2dimethylaminoethyl ethyl ester	26.191	Carbonic acid, 2dimethylaminoethyl ethyl ester	C7H15NO3	10
Cpd 24: Cyclododecyne	26.331	Cyclododecyne	C12H20	10
Cpd 25: 1,2,3,4-Tetrahydro-3-(phenylacetamido)quinoline	26.508	1,2,3,4-Tetrahydro-3-(phenylacetamido)quinoline	C17H18N2O	10
Cpd 26: 4-Cyanophenol, TBDMS derivative	26.907	4-Cyanophenol, TBDMS derivative	C13H19NOSi	10
Cpd 27: cis-7,cis-11-Hexadecadien-1-yl acetate	28.022	cis-7,cis-11-Hexadecadien-1yl acetate	C18H32O2	10
Cpd 28: Fumaric acid, di(2,2,3,3,4,4,5,5octafluoropentyl) ester	29.713	Fumaric acid, di(2,2,3,3,4,4,5,5octafluoropentyl) ester	C14H8F16O4	10
Cpd 29: .beta.-Tocopherol	30.505	.beta.-Tocopherol	C28H48O2	10
Cpd 30: Nonacosan-10-ol	30.714	Nonacosan-10-ol	C29H60O	10
Cpd 31: (2R)-2,8-Dimethyl-2-[(3E,7E)-4,8,12trimethyltrideca-3,7,11trienyl]-3,4-dihydrochromen6-ol, methyl ether	31.378	(2R)-2,8-Dimethyl-2-[(3E,7E)-4,8,12-trimethyltrideca3,7,11-trienyl]-3,4dihydrochromen-6-ol, methyl ether	C28H42O2	10
Cpd 32: .alpha.-Ergostenol	31.913	.alpha.-Ergostenol	C28H48O	10
Cpd 33: 4-t-Butyl-1-(1methylallyl)cyclohexanol	32.386	4-t-Butyl-1-(1methylallyl)cyclohexanol	C14H26O	10
Cpd 34: .gamma.-Sitosterol	32.684	.gamma.-Sitosterol	C29H50O	10
Cpd 35: Phosphorus P4	34.08	Phosphorus P4	P4	10
Cpd 36: 2-Oxatricyclo[20.2.2.1(3,7)]heptacosa-3,5,7(27),22,24,25hexaene-5,24,25-triol	34.09	2-Oxatricyclo[20.2.2.1(3,7)]heptacosa-3,5,7(27),22,24,25hexaene-5,24,25-triol	C26H36O4	10

In Vivo immunological assessment (ELISA): The presence of the inflammatory response and therapeutic effect was verified by ELISA-based cytokine profiling. The untreated infected group (G4) presented an impressive cytokine surge in which IL-1β extract (Figure 13) and IL-8 extract (Figure 14) were 513.07/21.64 and 374.75/21.64 pg/mL, respectively, which is indicative of severe hyper-inflammation (Table 6). On the other hand, there was significant reduction in the levels of both IL-1β and IL-8 in case of Nelumbo nucifera extracts, which occurred in a dose-dependent manner. G2 group, at 200 mg/mL concentration, had more significant inhibition of inflammation, while G3 at 300 mg/mL concentration had similar inhibitory effects, with relatively high cytokines. The non-infected groups had low levels of cytokines.

Discussion

The present work is composed of the integrated examination of the present epidemiological state of the *Enterobacter cloacae complex* (ECC) in domestic cats and of the potential of phytochemically-based immunomodulatory strategy to break the mechanisms of resistance of the

ECC. Isolation rates were mostly low (2.7%) and recovery was restricted to fecal samples (5.7%) which then validates the conclusion that domestic cats serve as minor and intermittent sources in Baghdad.

Table 5. Comparative systemic hematological profile (Mean ± SD) of experiment rabbit models challenged with ECC and treated with *N. nucifera* extract

Group	Parameter	Before	After	p-value
G1 (100mg + ECC)	WBC ($10^3/\mu L$)	7.06 ± 0.20	10.19 ± 0.25	<0.0001
	Lymphocytes (%)	32.91 ± 0.40	62.09 ± 0.83	<0.0001
	Granulocytes (%)	58.74 ± 0.67	33.30 ± 0.40	<0.0001
G2 (200mg + ECC)	WBC ($10^3/\mu L$)	7.09 ± 0.18	8.99 ± 0.17	<0.0001
	Lymphocytes (%)	32.85 ± 0.65	69.00 ± 0.82	<0.0001
	Granulocytes (%)	57.39 ± 0.81	23.54 ± 0.71	<0.0001
G3 (300mg + ECC)	WBC ($10^3/\mu L$)	7.06 ± 0.19	9.36 ± 0.27	<0.0001
	Lymphocytes (%)	32.48 ± 0.58	64.96 ± 1.07	<0.0001
	Granulocytes (%)	58.45 ± 0.49	26.44 ± 0.89	<0.0001
G4 (Positive Control)	WBC ($10^3/\mu L$)	5.28 ± 0.11	15.05 ± 0.98	<0.0001
	Lymphocytes (%)	50.77 ± 2.59	35.10 ± 3.25	0.0002
	Granulocytes (%)	43.88 ± 0.79	60.34 ± 3.35	<0.0001
G5 (Lotus Only)	WBC ($10^3/\mu L$)	7.89 ± 0.47	8.24 ± 0.38	0.0408
	Lymphocytes (%)	51.60 ± 0.66	45.25 ± 5.36	0.0358
	Granulocytes (%)	36.53 ± 0.81	37.80 ± 1.18	0.0072
G6 (Negative Control)	WBC ($10^3/\mu L$)	6.71 ± 0.63	4.07 ± 0.37	0.0002
	Lymphocytes (%)	53.19 ± 2.12	73.02 ± 2.36	<0.0001
	Granulocytes (%)	38.38 ± 2.17	20.02 ± 1.40	<0.0001

Table 6. The serum concentration of IL-1 2 and IL-8 (pg/mL) illustrating the powerful dose-dependent anti-inflammatory impact of *N. nucifera* extract

Group	IL-1β (pg/mL) (Mean ± SD)	IL-8 (pg/mL) (Mean ± SD)
G1 (100 mg)	298.59 ± 8.12	191.91 ± 5.04
G2 (200 mg)	40.69 ± 12.35	43.21 ± 9.68
G3 (300 mg)	151.04 ± 20.86	131.05 ± 17.02
G4 (Positive Control)	513.07 ± 21.64	374.75 ± 21.64
G5 (Lotus Only)	17.79 ± 21.66	29.32 ± 14.73
G6 (Negative Control)	12.13 ± 7.91	28.09 ± 14.15

These results are in line with the local reports with a prevalence of 3.5% in pet otitis by Zaid Ali and Al-Yassari (2023), and 4% in goats by (Al-Darraji, 2012). The latter was performed to improve the diagnostic accuracy by applying selective culture media, automated identification (VITEK 2), and molecular confirmation by the use of 16S rRNA amplification (~1250 bp) that is needed to differentiate closely related enterobacteriaceae (Mohammed et al., 2025; Davin-Regli et al., 2019; Jorgensen et al., 2015). The antimicrobial susceptibility profile was very sensitive to the carbapenems, amino-glycosides and fluoroquinolones. However, sensitivity to certain combinations of β-lactam/ β-lactamase inhibitors (e.g. ceftolozane/tazobactam) demonstrate the existence of chromosomal AmpC enzymes or plasmid-mediated ESBLs, which promotes the ongoing therapeutic dilemmas (Sadeq et al., 2025; Elshobary et al., 2025).

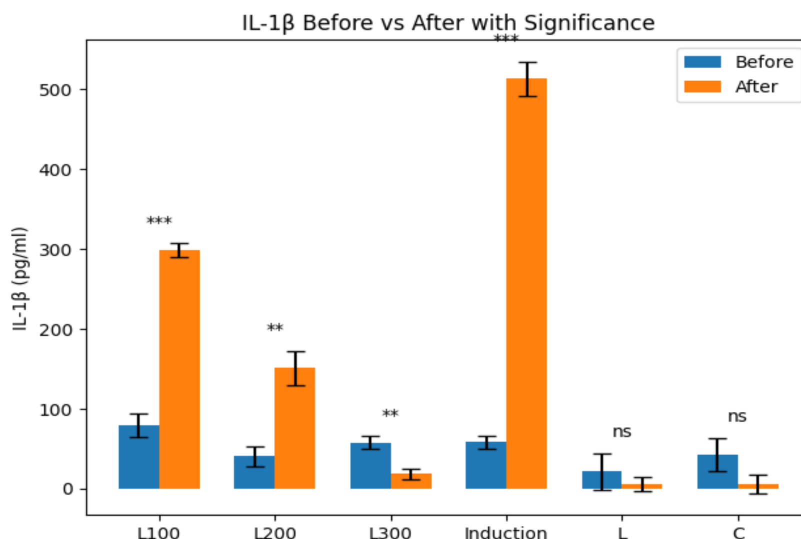


Figure 13. Expression of IL-1β treatment: the response of IL-1β levels in the various experimental groups differed. Induction group had the greatest significant change as compared to the baseline with the L300 group having a significant change of reduction, which was examined using ANOVA ($F=308.20$, $p<0.001$)

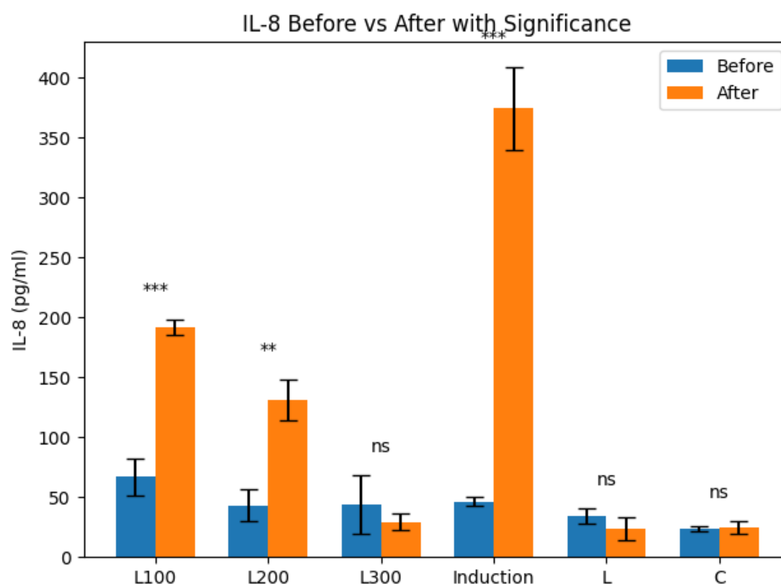


Figure 14. IL-8 expression (treated): illustrating the response differences in IL-8. Induction group was the most significantly increased over the baseline, which was evaluated through ANOVA ($F = 142.88$, $p < 0.001$)

Nelumbo nucifera seed extract was tested in vivo where it was found to have important antibacterial and immunomodulatory effects. Linoleic acid was revealed as one of the key components of GC-MS analysis, and this acid has the ability to disrupt the Gram-negative bacterial membranes and cause cell lysis (Qasim et al., 2023; Al-Mariri and Safi, 2014; Band and Weiss, 2015; Desbois & Smith, 2010). Also, phenolics with FTIR and UV-Vis properties added antioxidant properties that alleviated oxidative stress related to endotoxemia (Mukherjee et al., 2009; Paudel and Panth, 2015). ECC-related endotoxemia is pathophysiologically mediated by

the activation of the TLR4/NF-κB pathway by lipopolysaccharide (LPS), which induces excessive production of IL-1β and IL-8 and leads to significant leukocytosis and granulocytosis (Holmes et al., 2021; Mohammed & Al-Samarraae, 2021; Bennett et al., 2023; Annavajhala et al., 2019; Mezzatesta et al., 2012). These cytokines were significantly downregulated by the administration of the extract especially at 200 mg/mL, revealing effective control of this pathway. This treatment minimized pathological inflammation which is innate and maintained adaptive immune responses as shown by the elevation of lymphocyte levels. It may be mediated by blocking LPS-induced signaling and reactive oxygen species scavenging (Hemaiswarya et al., 2008; Hashemi and Davoodi, 2011). Taken together, these results indicate that cats do not play an epidemiological role in the spread of ECC and that *N. nucifera* seed extract is a promising complementary therapeutic option that can regulate the host immune system and prevent multidrug-resistant infections.

Limitations: The small number of positive isolates is a limitation of this study as they limit the extrapolation of antimicrobial resistance pattern. Also, the small geographical coverage and sample size need to be subjected to more large-scale, multicentric longitudinal research to confirm these results.

Conclusion: The research found a low rate (2.7%) of the *Enterobacter cloacae* complex among domestic cats in Baghdad, implies a minor reservoir role among the studied group. Although the isolates in general were vulnerable to major antimicrobials, there is a concern that there is some emerging resistance to some combinations of β-lactamase and β-lactam. The seed extract of *Nelumbo nucifera* was found to be as a promising immunomodulatory agent with antimicrobial properties, which can be explained by involvement of certain inflammation signal pathway, such as that related to TLR4/NF-κB by linoleic acid and phenolic compounds. This also led to the decreased IL-1β and IL-8 levels, controlled inflammation, and improved lymphocytic response, which indicates its possibility as a complementary medicine against multidrug resistant diseases.

Authors Contributions

No particular author contributions are made to this work.

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Data availability statement

The data contributing to the findings of this study are available from the investigating researcher upon request.

Ethical considerations

Approval was obtained locally by the institutional animal care and use committee at the College of Veterinary Medicine, University of Baghdad (P-G\112,21\2\2026).

Conflict of Interest

Authors state that there are no conflicts of interest.

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تعدیل سیستم ایمنی توسط عصاره بذر *Nelumbo nucifera* در خرگوش‌های آلوده به کمپلکس *Enterobacter cloacae*

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چکیده

هدف: در این مطالعه، جداسازی و شناسایی اعضای کمپلکس *Enterobacter cloacae* (ECC) از گربه‌های خانگی در بغداد، عراق، و تعیین الگوی حساسیت آن‌ها به آنتی‌بیوتیک‌های مختلف انجام شد. همچنین، اثر عصاره بذر *Nelumbo nucifera* (نیلوفر آبی) به‌عنوان یک تعدیل‌کننده سیستم ایمنی در یک مدل حیوانی آزمایشی مورد ارزیابی قرار گرفت.

مواد و روش‌ها: تعداد ۷۵ نمونه بالینی از گربه‌های خانگی شامل مدفوع، خون و ادرار جمع‌آوری شد. جداسازی و شناسایی اولیه بر اساس ویژگی‌های مورفولوژیک و الگوی تخمیر لاکتوز توسط سویه‌های ECC در محیط‌های انتخابی انجام گرفت. آزمون‌های تأییدی شامل آنالیز با سیستم خودکار VITEK 2 Compact و شناسایی از طریق تکثیر ژن 16S rRNA (۱۲۵۰ جفت باز) به روش PCR بود. آزمون حساسیت آنتی‌بیوتیکی بر اساس دستورالعمل‌های استاندارد انجام شد. همچنین، آنالیز GC-MS برای عصاره اتانولی بذر *N. nucifera* صورت گرفت. فعالیت تعدیل‌کننده ایمنی عصاره بذر نیلوفر آبی با استفاده از خرگوش‌ها به‌عنوان حیوان آزمایشگاهی و از طریق ارزیابی شاخص‌های خونی و اندازه‌گیری سطوح اینترلوکین‌های IL-1β و IL-8 با روش ELISA بررسی شد.

نتایج: میزان جداسازی ECC در میان تمام کشت‌ها ۲/۷٪ بود و بیشترین شیوع در نمونه‌های مدفوع (۵/۷٪) نسبت به خون و ادرار مشاهده شد. آزمون حساسیت آنتی‌بیوتیکی نشان داد که سویه‌ها حساسیت بالایی به کارباپنم‌ها، آمینوگلیکوزیدها و فلوروکینولون‌ها داشتند، در حالی که مقاومت نسبی نسبت به ترکیبات β-لاکتام/مهارکننده-β لاکتاماز مشاهده شد. در آنالیز GC-MS عصاره بذر نیلوفر آبی، اسید لینولئیک به‌عنوان مهم‌ترین ترکیب فعال شناسایی شد. پس از تجویز عصاره به‌صورت درون‌تنی با دوز پیشگیرانه ۲۰۰ میلی‌گرم بر میلی‌لیتر، کاهش قابل توجهی در لکوسیتوز و گرانولوسیتوز مشاهده شد و تعداد لنفوسیت‌ها افزایش یافت. علاوه بر این، نتایج ELISA کاهش معنی‌دار سطوح سایتوکاین‌های پیش‌التهابی IL-1β و IL-8 را نشان داد.

نتیجه‌گیری: بر اساس نتایج این مطالعه، گربه‌های خانگی در جمعیت مورد بررسی در بغداد می‌توانند منبع محدودی از کمپلکس *Enterobacter cloacae* باشند. عصاره اتانولی بذر *Nelumbo nucifera* دارای ویژگی‌های امیدوارکننده‌ای در تعدیل سیستم ایمنی است که از طریق تنظیم پاسخ‌های التهابی و تقویت تعادل ایمنی عمل می‌کند.

کلمات کلیدی: کمپلکس *Enterobacter cloacae*، گربه‌های خانگی، مقاومت ضدمیکروبی، 16S rRNA، VITEK 2

نوع مقاله: پژوهشی

استناد: فیروز عباس حسین، اکرام عباس السمراء (۱۴۰۵) تعدیل سیستم ایمنی توسط عصاره بذر Nelumbo nucifera در خرگوش‌های آلوده به کمپلکس Enterobacter cloacae. *مجله بیوتکنولوژی کشاورزی*، ۱۸(۳)، ۴۳۱-۴۵۲.

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