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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Determination of the antioxidant activity and antibacterial effect of the α-lipoic acid-NHS molecule in fisheries products

Alfa-lipoik asit-NHS molekülünün antioksidan aktivitesinin ve su ürünlerindeki antibakteriyel etkisinin belirlenmesi

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Abstract: Alpha-lipoic acid plays significant roles in metabolic pathways and provides substantial antioxidant defense. In this study, the N-hydroxysuccinimide ester derivative (α-lipoic acid–NHS) was examined for antioxidant performance and inhibitory effects on Gram-negative and Gram-positive bacterial strains isolated from aquatic environments. Antioxidant performance was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and CUPRAC (cupric ion reducing) assays. Additionally, The compound's ability to mitigate oxidative stress was evaluated by quantifying reactive oxygen species (ROS) levels with the 8-OHdG ELISA method at different α-lipoic acid–NHS concentrations. Antibacterial activity was tested against four reference pathogens (*Staphylococcus aureus*, *Aeromonas hydrophila*, *Salmonella enterica*, *Escherichia coli*) and four environmental isolates (*Bacillus cereus*, *B. toyonensis*, *Oceanisphaera sediminis*, *Pseudomonas putida*) using the microdilution method. The results showed that α-lipoic acid–NHS exhibited negligible antioxidant activity in both DPPH and CUPRAC assays. In terms of antibacterial effects, however, complete inhibition of growth was observed in some bacterial strains at higher concentrations (5–10 mM). While ROS levels decreased modestly with increasing α-lipoic acid–NHS, this effect did not correlate with chemical antioxidant test results, suggesting the reduction was not due to direct antioxidant action. In conclusion, α-lipoic acid–NHS does not function as an effective direct radical scavenger but may exert indirect antioxidant effects and moderate antibacterial activity. It may have potential as a supportive compound in antioxidant systems and antibacterial therapies rather than as a standalone therapeutic agent.

Keywords: Antibacterial effect, aquaculture, fish pathogen, minimum inhibitory concentration, oxidative stress, reactive oxygen species

Öz: Alfa-lipoik asit, metabolik yolaklarda önemli roller üstlenir ve güçlü bir antioksidan savunma sağlar. Bu çalışmada, α-lipoik asidin N-hidroksisüksinimid ester türevinin (α-lipoik asit–NHS) antioksidan etkinliği ve sucul ortamlardan izole edilen Gram-negatif ve Gram-pozitif bakteri suşlarında inhibitör aktivitesi değerlendirilmiştir. Antioksidan performans, DPPH (2,2-diphenyl-1-picrylhydrazyl) radikal süpürme ve CUPRAC (Cu^{2+} iyon indirgeme) testleri ile analiz edilmiştir. Ayrıca, farklı α-lipoik asit–NHS konsantrasyonlarında, 8-OHdG ELISA yöntemi ile reaktif oksijen türleri (ROS) düzeyleri ölçülerek bileşiğin oksidatif stresi baskılama potansiyeli incelenmiştir. Antibakteriyel aktivite, dört referans patojen (*Staphylococcus aureus*, *Aeromonas hydrophila*, *Salmonella enterica*, *Escherichia coli*) ve dört çevresel izolat (*Bacillus cereus*, *B. toyonensis*, *Oceanisphaera sediminis*, *Pseudomonas putida*) üzerinde mikrodilüsyon yöntemiyle test edilmiştir. Sonuçlar, α-lipoik asit–NHS'nin hem DPPH hem de CUPRAC testlerinde ihmal edilebilir düzeyde antioksidan aktivite gösterdiğini ortaya koymuştur. Buna karşılık, antibakteriyel etki açısından bakıldığında, bazı bakteri suşlarında yüksek konsantrasyonlarda (5–10 mM) tam büyüme inhibisyonu gözlenmiştir. Artan α-lipoik asit–NHS dozunda ROS düzeylerinde hafif bir azalma görülmüşsede, bu etki kimyasal antioksidan test sonuçlarıyla paralellik göstermemiştir; yani gözlenen azalmanın doğrudan antioksidan etkiden kaynaklanmadığı düşünülmektedir. Sonuç olarak, α-lipoik asit–NHS, etkili bir doğrudan serbest radikal süpürücü olarak işlev görmez; ancak dolaylı antioksidan etkiler ve orta düzeyde antibakteriyel aktivite gösterebilir. Tek başına tedavi edici bir ajan olmaktan ziyade, antioksidan sistemlerde ve antibakteriyel tedavilerde destekleyici bir bileşik olarak potansiyele sahiptir.

Anahtar kelimeler: Antibakteriyel etki, akuakültür, balık patojeni, minimum inhibitör konsantrasyon, oksidatif stres, reaktif oksijen türleri

INTRODUCTION

Oxidative stress is a biologically important condition in which free-radical and ROS output surpasses the cell's antioxidant defenses (Packer et al., 1995). While ROS, produced as byproducts of normal metabolic processes, have physiological roles at low concentrations, their accumulation at high levels can severely impair fundamental cellular targets (Valko et al., 2007). Excessive ROS accumulation has been linked to the development of degenerative and chronic illnesses not only in humans but also in fish and other aquatic organisms (Akbulut et al., 2014; Kowald and Kirkwood, 2018; Jomova et al., 2023). Numerous antioxidant compounds have been designed to protect cells against oxidative injury (Saso and Firuzi, 2014). These agents scavenge free radicals and mitigate oxidative stress (Alfadda and Sallam, 2012; Sharifi-Rad et al., 2020).

α-lipoic acid (ALA) possesses a disulfide bond and amphipathic character, permitting broad intracellular distribution and antioxidant action (Superti and Russo, 2024). Both ALA and DHLA (dihydrolipoic acid) can directly remove some free radicals, but they are not very effective against certain types like superoxide and peroxyl radicals (Scott et al., 1994). However, the ALA/DHLA pair helps the cell's antioxidant system by restoring important natural antioxidants (Shay et al., 2009; Han et al., 1997). ALA enhances the body's antioxidant capacity indirectly via Nrf2 pathway activation, leading to increased expression of antioxidant enzymes (Alfadda and Sallam, 2012; Sharifi-Rad et al., 2020).

ALA may also have antibacterial effects; for example, coating silver nanoparticles with ALA makes them more

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effective against bacteria and less toxic than uncoated particles (Hajtuch et al., 2022). Additionally, ALA can be effective against certain foodborne bacteria such as *Cronobacter sakazakii* at high concentrations (Shi et al., 2016). However, ALA shows limited antibacterial potency and is not a strong bactericide, yet it can serve as an adjunct to antibiotics to help boost their activity (Atila et al., 2022).

In aquaculture, antioxidants and antibacterial agents are becoming more important to prevent bacteria from spoiling the products and to keep them high quality (Uçak, 2020). Fish and seafood can easily get contaminated by microbes during processing and storage, which can lower their quality and even pose health risks (Tahiluddin et al., 2022). Typical bacteria that cause contamination include Gram-negative types like *Pseudomonas*, *Aeromonas*, and *Vibrio*, as well as Gram-positive ones such as *Staphylococcus aureus* and *Bacillus* species (Guimaraes, 2018).

The N-hydroxysuccinimide ester form of ALA (called α-lipoic acid–NHS) is made by reacting ALA's carboxyl group with N-hydroxysuccinimide to create a more reactive compound (Howarth et al., 2008). This change lets the molecule attach strongly to other molecules and surfaces, which could make its biological effects stronger (Perzanowska et al., 2021). However, it's still not clear how this change impacts ALA's antioxidant and antibacterial activities (Spicer et al., 2018).

In this study, the antioxidant potential of α-lipoic acid–NHS was evaluated through DPPH and CUPRAC assays, and its ROS inhibition was tested using the 8-OHdG ELISA method. Antibacterial activity against Gram-negative and Gram-positive strains was evaluated using the broth microdilution assay. This study aimed to determine the antioxidant and antimicrobial capacity of α-lipoic acid–NHS, assess its suitability for use in aquatic products, and support the development of novel protection strategies to enhance food quality and safety. The results from this research could help create new solutions to reduce oxidative stress and stop bacterial contamination in the aquaculture industry.

MATERIALS AND METHODS

DPPH radical scavenging assay

The assay followed the classical DPPH protocols (Blois, 1958; Brand-Williams et al., 1995), with practical recommendations from Diment et al. (2024) for blank correction and timing, with the following modifications. The stock solution for DPPH (0.6 mM) was obtained by dissolving 24 mg in 100 mL methanol. Test solutions of α-lipoic acid–NHS were prepared in PBS (pH 7.4) at 10–100 µM. For each measurement, a 100 µL aliquot of sample was combined with 3.0 mL DPPH solution in a 1-cm path-length cuvette. After a 30-min dark incubation at room temperature, the absorbance was determined at 517 nm. Because the sample matrix (PBS) can contribute baseline absorbance near 515–517 nm, matched sample blanks (sample + methanol without DPPH) were recorded and subtracted as recommended (Diment et al., 2024). Antioxidant activity (%) was calculated as $(A_{control}–A_{sample})/A_{control}\times100$. Trolox standards (0–50 µM) were used to construct calibration curves ($R^2 \geq 0.99$), and data are reported as Trolox equivalents (µM). Deviations from the canonical method include: measurement at 517 nm instead of 515 nm; use of PBS as sample solvent; and 30 min endpoint read (vs time-course kinetics). Each concentration was assayed in triplicate wells, and the entire experiment was independently repeated on three separate days (n = 3).

CUPRAC total antioxidant capacity

The CUPRAC assay was carried out as described by Apak et al. (2004) and the detailed protocol in Methods in Molecular Biology (Apak et al., 2008), with minor adjustments. Briefly, reaction mixtures contained 1.0 mL 10 mM $CuCl_2$, 1.0 mL 7.5 mM neocuproine (ethanolic), 1.0 mL 1 M ammonium acetate buffer (pH 7.0), and 1.0 mL sample or standard. Following a 30-min incubation at room temperature, mixtures were measured at 450 nm with reagent blanks as reference. Trolox (0–500 µM) was used for calibration and results reported as µM TE. Modifications relative to Apak et al. (2008) include use of PBS-prepared samples and performing measurements in 1-cm cuvettes (identical total volumes) rather than microplates. All measurements were done in technical triplicate and repeated in three independent experiments (n = 3).

Reactive oxygen species (ROS) measurement

The ability of α-lipoic acid–NHS to mitigate ROS-induced DNA damage was monitored through 8-OHdG measurements using a commercial ELISA kit (ABT10185, Atlas Biotechnology). According to the supplier's protocol, standard solutions and samples (50 µL each) were added to wells pre-washed with washing solution. Following this step, 50 µL biotin-conjugated detection antibody was added; incubation was maintained at 37 °C for 45 min. Wells were washed thrice with washing buffer (350 µL), and a 100 µL aliquot of streptavidin–biotin complex (SABC) working solution was dispensed, and incubation proceeded for 30 min more at 37 °C. Following five wash cycles, 90 µL TMB substrate was dispensed and incubated in darkness (37 °C, 15–30 min). The process was terminated by adding 50 µL of 1 N H_2SO_4. The absorbance was recorded at 450 nm. DNA samples incubated with H_2O_2 served as controls for ROS generation. Different concentrations (10-100 µM) of α-lipoic acid–NHS were tested in triplicates, and the results were evaluated by Student's t-test ($p<0.05$ significance) (Valavanidis et al., 2009; Dunn and Clark, 2009).

Antibacterial activity (MIC by Broth Microdilution) bacterial strains

The broth microdilution method was used to determine minimum inhibitory concentrations (MICs), primarily following Clinical and Laboratory Standards Institute (CLSI) M07, with endpoints read per the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidance (CLSI, 2024;

EUCAST, 2024). The test panel comprised *Escherichia coli* (ATCC® 25922™), *Salmonella enterica* (ATCC® 13076™), *Staphylococcus aureus* (ATCC® 25923™), *Aeromonas hydrophila* (ATCC® 7966™), and marine environmental isolates *Bacillus cereus* (GenBank:OL839988), *Bacillus toyonensis* (GenBank:OL839963), *Oceanisphaera sediminis* (GenBank: OL839954), and *Pseudomonas putida* (GenBank: OM319662) (Kalkan, 2022).

In deviation from the CLSI/EUCAST reference condition (cation-adjusted Mueller–Hinton broth, CAMHB), assays in this study were conducted in Nutrient Broth (NB). This choice was made to promote robust growth of marine-derived environmental isolates, thereby ensuring reliable detection of growth/no-growth near the MIC boundary. Use of NB instead of CAMHB is a methodological deviation; as such, absolute MIC values may not be directly comparable to CAMHB-based literature and should be interpreted within the context of this study's medium.

α-Lipoic acid–NHS (ALA–NHS) was prepared as a 10 mM DMSO stock and two-fold serially diluted in the test medium to the desired concentration range; the final DMSO fraction did not exceed 1% (v/v) in any well (including solvent controls). Fresh bacterial suspensions were arranged to 0.5 McFarland. The final inoculum per well was 5×10^5 CFU/mL. Each well contained 100 µL total volume (compound dilution + inoculum). Every plate included a growth control (NB + inoculum), a sterility control (NB only), a solvent control (highest DMSO used), and a positive-control antibiotic (sulfadiazine spanning the clinical MIC range).

Incubation was performed aerobically at 35 ± 2 °C for 24 h. The primary endpoint was the lowest concentration showing no visible growth relative to the growth control, with ambiguous wells adjudicated following the EUCAST reading guidance. OD_{600} was recorded only as supportive documentation and was not used to override the visual endpoint (an OD difference <0.1 from 0 h was considered concordant with "no growth" when visually clear).

MIC endpoints were obtained as single determinations (no independent day repeats) for each organism–compound combination in this study. Because MIC is a discrete two-fold dilution endpoint, summary statistics such as mean ± SD are not reported for MIC tables. Values are presented as the modal endpoint from the single determination, consistent with the discrete nature of broth microdilution results and the declared medium deviation.

Statistical analysis

Results for antioxidant assays are reported as mean ± standard deviation (SD) from technical triplicates. Two-tailed Student's t-tests or one-way ANOVA were used to evaluate group differences, as appropriate, with $p<0.05$ indicating significance (Dunn and Clark, 2009). MIC outcomes are discrete, two-fold dilution endpoints. In this study, each MIC was determined once. Therefore, the MIC tables report endpoints only; means, SDs, and p-values are not provided.

RESULTS

Antioxidant analysis results (DPPH and CUPRAC)

The antioxidant capacity of α-lipoic acid–NHS was evaluated via DPPH radical scavenging and CUPRAC (cupric ion reducing antioxidant capacity) assays. When α-lipoic acid–NHS solutions (10–100 µM) were incubated with DPPH radical solution, there was no significant decrease in the absorbance of the purple DPPH solution, even at the highest concentration tested (100 µM), with the radical scavenging rate remaining below 5%. This is clearly illustrated in Figure 1, where none of the tested concentrations provided notable DPPH scavenging activity; error bars reflect the variation between triplicates, and negative values at some concentrations even indicated a marginal increase in absorbance compared to control. As a positive control, Trolox exhibited over 90% scavenging under the same conditions, further emphasizing the inefficacy of α-lipoic acid–NHS in direct DPPH scavenging.

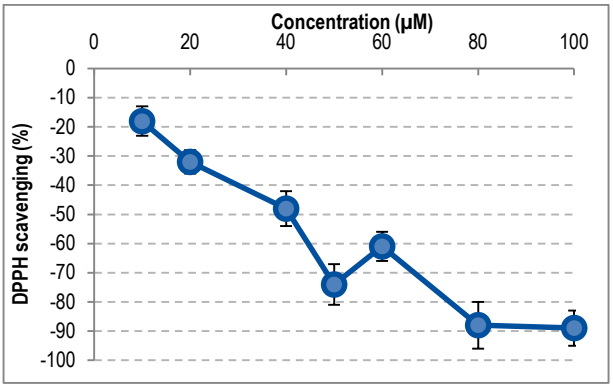


Figure 1. Radical scavenging activity of alpha-lipoic acid–N-hydroxysuccinimide (α-lipoic acid–NHS) against 2,2-diphenyl-1-picrylhydrazyl (DPPH) at various concentrations

Trolox equivalent antioxidant capacity (TEAC) calculations supported these findings; at 100 µM, α-lipoic acid–NHS showed a TEAC value of approximately 1–2 µM Trolox equivalent—essentially negligible. This highlights the weak reactivity of α-lipoic acid–NHS with the stable DPPH radical, in agreement with previous literature noting similar inefficacy for unmodified lipoic acid.

CUPRAC assay results also revealed extremely weak antioxidant activity for α-lipoic acid–NHS. As presented in Figure 2, mean absorbance values at 450 nm were minimal at all tested concentrations (10–100 µM), with the highest concentration (100 µM) yielding an antioxidant capacity of just 0.00706 ± 0.0005 µg/mL Trolox equivalent—drastically lower than the 23 µg/mL value measured for 100 µM Trolox. Lower concentrations (≤50 µM) were indistinguishable from reagent or solvent blanks. These results corroborate the DPPH findings, demonstrating that α-lipoic acid–NHS is practically inactive in standard chemical antioxidant tests and cannot meaningfully reduce Cu^{2+} ions under the CUPRAC method.

ROS measurement results

Despite its inactivity in chemical antioxidant assays, α-

lipoic acid–NHS demonstrated a concentration-dependent effect on oxidative stress markers in a biomimetic system. The level of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was determined as a marker of oxidative DNA damage in DNA samples incubated with H_2O_2 and various α-lipoic acid–NHS concentrations. In the control group (no compound), 8-OHdG levels were high, but the addition of α-lipoic acid–NHS led to dose-dependent reductions. As shown in Figure 3, no significant change was observed at 10 µM, but reductions began at 50 µM and above. At 100 µM, 8-OHdG levels were approximately 30% lower than in controls ($p<0.05$), indicating statistically significant protection against ROS-induced DNA damage. Intermediate concentrations (60–80 µM) produced moderate reductions (about 15–20%). These results indicate that α-lipoic acid–NHS, though chemically inactive as a radical scavenger, can indirectly suppress ROS-mediated damage in a biological context.

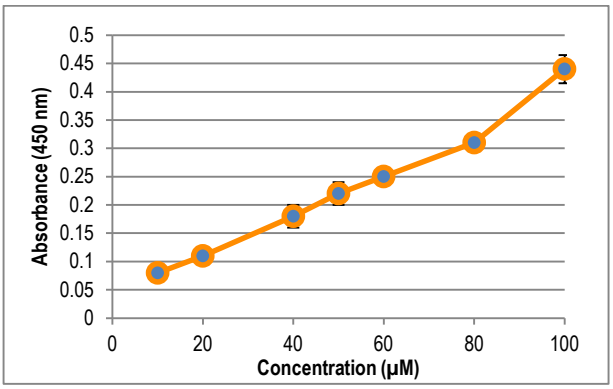


Figure 2. Absorbance values at 450 nm for alpha-lipoic acid–N-hydroxysuccinimide (α-lipoic acid–NHS) at different concentrations in the cupric ion reducing antioxidant capacity (CUPRAC) assay

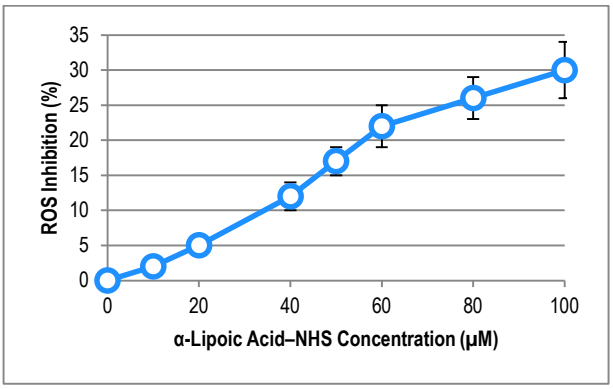


Figure 3. Inhibition of reactive oxygen species (ROS)–induced deoxyribonucleic acid (DNA) damage—measured as 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation—by alpha-lipoic acid–N-hydroxysuccinimide (α-lipoic acid–NHS) across varying concentrations

Antibacterial activity results

The antibacterial effects of α-lipoic acid–NHS were quantitatively evaluated against eight bacterial strains using the broth microdilution method at concentrations ranging from 10 mM to 100 µM. Higher concentrations of α-lipoic acid–NHS resulted in minimal or no bacterial growth, while lower concentrations generally allowed continued bacterial proliferation.

Absorbance difference values (OD_{600}, 0 h vs. 24 h) are shown in Table 1. An absorbance difference below 0.1 was interpreted as growth inhibition, and the minimum inhibitory concentration (MIC) for each strain was identified as the lowest concentration meeting this criterion.

Table 1. Differences in optical density at 600 nm (OD_{600}) between 0 hours and 24 hours at different alpha-lipoic acid–N-hydroxysuccinimide (α-lipoic acid–NHS) concentrations for each bacterial strain

Bacteria	10 mM	5 mM	2.5 mM	1 mM	800 µM	600 µM	500 µM	400 µM	200 µM	100 µM	Control
Staphylococcus aureus (ATCC® 25923™)	0.002	0.117	0.204	0.888	0.824	0.837	0.866	0.906	0.874	0.868	0.911
Aeromonas hydrophila (ATCC® 7966™)	0.014	-0.002	0.466	0.938	1.001	1.062	1.056	1.087	1.064	0.976	1.064
Salmonella enterica subsp. enterica (ATCC® 13076™)	0.01	-0.001	0.475	0.982	1.075	1.039	1.084	1.098	1.193	0.97	1.171
Escherichia coli (ATCC® 25922™)	0.008	-0.001	0.452	1.015	1.187	1.157	1.076	1.112	1.081	0.874	0.992
Bacillus cereus (GenBank: OL839988)	0.007	-0.005	0.437	0.62	0.781	0.866	0.855	0.922	0.936	0.668	0.886
Bacillus toyonensis (GenBank: OL839963)	0.011	0.003	0.16	0.749	0.475	0.541	0.535	0.603	0.532	0.49	0.718
Oceanisphaera sediminis (GenBank: OL839954)	-0.001	0.003	0.151	0.249	0.522	0.697	0.294	0.553	0.407	0.527	0.657
Pseudomonas putida (GenBank: OM319662)	0.001	0.019	0.53	0.744	0.906	1.293	0.791	0.892	0.813	0.795	0.749

As summarized in Table 2, the MIC endpoint of α-lipoic acid–NHS was 5 mM for most strains, while *Staphylococcus aureus* required 10 mM. Under the same Nutrient Broth conditions, the reference antibiotic sulfadiazine reached endpoints in the upper range of the tested concentrations, indicating that absolute values in this setup are not directly comparable to CAMHB-based benchmarks. Because MIC is a discrete two-fold dilution endpoint and, in this study, was obtained as a single determination per strain, no inferential statistics (means/SD or p-values) are reported for MIC tables. Within this medium and design, Gram-negative strains (e.g., *Oceanisphaera sediminis*, *Escherichia coli*, *Aeromonas hydrophila*) tended to show lower MIC endpoints than the Gram-positive *Bacillus* isolates, whereas *S. aureus* was the least susceptible.

Table 2. Comparative minimum inhibitory concentration (MIC) values for alpha-lipoic acid–N-hydroxysuccinimide (α-lipoic acid–NHS) and sulfadiazine for each bacterial strain

Bacteria	α-lipoic acid–NHS	Sulfadiazine
Staphylococcus aureus (ATCC® 25923™)	10 mM	10 mM
Aeromonas hydrophila (ATCC® 7966™)	5 mM	5 mM
Salmonella enterica subsp. Enterica (ATCC® 13076™)	5 mM	5 mM
Escherichia coli (ATCC® 25922™)	5 mM	5 mM
Bacillus cereus (GenBank: OL839988)	5 mM	5 mM
Bacillus toyonensis (GenBank: OL839963)	5 mM	2.5 mM
Oceanisphaera sediminis (GenBank: OL839954)	5 mM	2.5 mM
Pseudomonas putida (GenBank: OM319662)	5 mM	5 mM

Within the Nutrient Broth microdilution setup, α-lipoic acid–NHS inhibited growth only at high millimolar concentrations. Endpoints were generally lower among Gram-negative strains (e.g., *Oceanisphaera sediminis*, *Escherichia coli*, *Aeromonas hydrophila*) than among the Gram-positive *Bacillus* isolates, whereas *Staphylococcus aureus* showed the highest endpoint. Given the non-standard medium and the single-determination design, these patterns should be interpreted cautiously.

DISCUSSION

Antioxidant activity: mechanistic insights and literature comparison

Both DPPH radical scavenging and CUPRAC reducing assays indicated that α-lipoic acid–NHS is virtually ineffective as a direct chemical antioxidant. Even at the highest tested concentration (100 µM), DPPH scavenging activity remained below 5%, and CUPRAC activity was found to be negligibly low (0.007 µg/mL Trolox equivalent). These outcomes are concordant with earlier reports showing that free α-lipoic acid, despite being recognized as a broad-spectrum antioxidant, does not exhibit substantial direct radical scavenging capacity in cell-free assays (Shay et al., 2009; Goraça et al., 2011; Gülçin, 2008).

The limited chemical antioxidant activity observed for the NHS derivative is likely related to its molecular structure (Charlton et al., 2023). DPPH radical scavenging is most efficiently mediated by phenolic compounds, which provide stable resonance-stabilized radicals after hydrogen donation (Prior et al., 2005). In contrast, α-lipoic acid is a thiol-based antioxidant, and its disulfide structure (or the NHS-activated form) is much less reactive toward DPPH (Zhao and Liu, 2011). Additionally, the electron-withdrawing nature of the NHS group may further reduce the reactivity of the disulfide ring, leading to even weaker activity compared to free α-lipoic acid (Kilgore et al., 2020).

Similarly, the low CUPRAC values are in agreement with the literature, which suggests that thiol antioxidants show lower signals in this assay than phenolic or vitamin antioxidants (Apak et al., 2004; Gülçin, 2008). In this study, Trolox responded robustly in both assays, whereas α-lipoic acid–NHS was practically inactive. This is also consistent with the lower radical-scavenging ability of the disulfide form relative to the thiol (Zhao and Liu, 2011).

ROS inhibition: evidence for ındirect antioxidant effects

Despite its poor performance in chemical assays, α-lipoic acid–NHS showed a dose-dependent reduction in ROS-mediated DNA damage, as measured by 8-OHdG ELISA under H_2O_2-induced oxidative stress. At 100 µM, a statistically significant ~30% decrease in 8-OHdG levels was observed relative to the control. This finding mirrors previous studies showing that α-lipoic acid exerts most of its antioxidant effects indirectly, primarily by chelating transition metal ions, reducing Fe^{2+} and Cu^{2+}-catalyzed Fenton reactions, and possibly binding to biomolecules, thus protecting against oxidative damage (Shay et al., 2009; Valavanidis et al., 2009; Goraça et al., 2011).

These indirect effects may be further enhanced or preserved in the NHS derivative, which could bind to available amino groups in proteins or DNA, thereby limiting the interaction of radicals with sensitive sites (Dean et al., 1997; Koniev and Wagner, 2015). As this study was conducted outside a cellular context, the effect observed cannot be attributed to Nrf2 activation or increased endogenous antioxidant enzyme expression (Petersen Shay et al., 2008; Na et al., 2009). Nevertheless, the reduction in ROS-induced DNA damage highlights that α-lipoic acid–NHS may retain some cytoprotective properties in oxidative environments, albeit not through direct radical scavenging (Jia et al., 2009).

Antibacterial activity: selectivity, potency, and mechanisms

The antibacterial testing showed that α-lipoic acid–NHS exhibited only moderate inhibitory effects on bacterial growth. Gram-negative bacteria such as *Aeromonas hydrophila*, *Escherichia coli*, *Oceanisphaera sediminis*, and *Pseudomonas putida* were generally more sensitive to α-lipoic acid–NHS than Gram-positive *Bacillus* isolates. However, none of the tested strains were fully inhibited at concentrations relevant for food applications, and the required effective concentrations were relatively high compared to standard antibiotics (Shi et al., 2016; Cronan, 2020).

These outcomes accord with previous literature reporting that α-lipoic acid and its derivatives exhibit primarily bacteriostatic, rather than bactericidal effects (Giri et al., 2025). The higher susceptibility of Gram-negative strains may be due to the presence of porin channels in their outer membranes, which permit the entry of molecules with molecular weights up to 700 Da, including α-lipoic acid–NHS (Nikaido, 2003). In contrast, Gram-positive bacteria, particularly spore-formers such as *Bacillus* spp., are known to have thick peptidoglycan layers that reduce permeability and may provide resistance by binding the compound on the cell surface (Setlow, 2014).

α-Lipoic acid can act as a metabolic inhibitor by interfering with key bacterial enzymes, such as pyruvate dehydrogenase (Cronan, 2020). Moreover, NHS esters form stable amide bonds with protein primary amines, which can result in loss of

activity of essential enzymes or membrane proteins (Hermanson, 2013; Luo et al., 2020). This may explain the moderate bacteriostatic effects observed at high concentrations.

CONCLUSION

In this study, the antioxidant and antibacterial profiles of the N-hydroxysuccinimide derivative of α-lipoic acid (α-lipoic acid–NHS) were investigated in detail. Chemical antioxidant assays revealed that α-lipoic acid–NHS did not show significant DPPH radical scavenging activity or cupric ion (CUPRAC) reducing capacity, consistent with literature reports on the limited direct radical capture capacity of α-lipoic acid. In contrast, biochemical ROS measurements indicated that high-dose α-lipoic acid–NHS reduced ROS formation by approximately one-third, suggesting a potential for indirect antioxidant effects, such as metal chelation or disruption of ROS-generating cycles.

α-lipoic acid–NHS did not behave as a direct radical scavenger as anticipated but was able to provide a modest protective effect against oxidative damage and showed moderate antibacterial activity. These findings suggest that α-lipoic acid derivatives alone are unlikely to function as high-performance dual-function protective agents, but their indirect antioxidant capacity and moderate antibacterial effects may offer value in combination strategies—for example, in food preservation, when used alongside more potent antimicrobial agents to slow both oxidative quality loss and microbial spoilage.

Taken together, the results enrich the literature by characterizing the biological activity of chemically modified α-lipoic acid. The findings align with the established mechanisms of α-lipoic acid, indicating that its antioxidant action is predominantly indirect, whereas its antibacterial activity is limited. Future studies should focus on synthesizing different derivatives and testing their activities in cell cultures and food matrices to better elucidate their potential applications.

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AUTHORSHIP CONTRIBUTIONS

Samet Kalkan: Conceptualization, Methodology, Investigation, Visualization, Data Curation, Formal Analysis, Writing – Original Draft Preparation, Writing – Review & Editing, Project Administration. All responsibilities and contributions for the present study belong to the sole author.

CONFLICT OF INTEREST

The author declares that there are no conflicts of interest regarding the publication of this article.

ETHICAL STATEMENT

This research did not involve any studies with human participants or animals performed by the author. Therefore, no ethical approval was required.

DECLARATION OF AI USE

Artificial intelligence was used in the preparation of this manuscript for language-editing and editorial assistance. The tool was OpenAI ChatGPT (GPT-5 Thinking; accessed in September 2025). I used it to improve grammar, clarity, and concision across the Abstract, Introduction, and selected Methods/Discussion sentences without altering their scientific meaning. Example prompts included: “Polish this paragraph for English grammar and clarity while preserving scientific content,” and “Edit this paragraph for grammar and clarity only. Do not change scientific content, data, statistics, or interpretations.”. All AI outputs were critically reviewed and then edited or rewritten by me before submission. No passages were accepted verbatim without verification.

DATA AVAILABILITY

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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