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Genomic and Cellular Insights into Lepidium sativum Extract: Protection and Nutrigenomic Potential

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ABSTRACT: The current study examines Lepidium sativum extract as an anticancer agent with potential activity against CAL51 breast cancer cells by investigating antioxidant, antibacterial, and molecular analyses. The phytochemical screening characterised flavonoids, phenolic acids, and glucosinolates, implying the extract's richness in bioactive compounds. The DPPH radical-scavenging assay was employed to assess antioxidant activity; the findings indicated strong dose-dependent activity for neutralising free radicals. The antibacterial examination showed comparable inhibition zones against S. aureus and E. coli, ranging between 6 and 20 mm ($p<0.01$). MTT assay findings demonstrated a dose-dependent cytotoxic effect, with CAL51 cell viability decreasing significantly as concentration. Compared to the control group ($p < 0.05$–0.001). Gene expression analysis revealed overexpression of TP53 and OGG1 ($p < 0.01$), which aligns with apoptosis and the activation of DNA repair, while leading to NRF2 downregulation ($p < 0.05$), suggesting suppression of antioxidant defences. The current findings underscore the importance of the phytochemicals in L. sativum extract as a natural source with antioxidant, antibacterial and anticancer potential. Future studies should evaluate the biological activities of L. sativum in vivo.

1. INTRODUCTION

Nutritional genomics is the field that studies the interaction between dietary bioactive compounds and the genome to regulate gene expression, maintain DNA stability, and preserve cellular homeostasis (Brennan, 2021 and Ferguson, 2019). Understanding the relationship between nutrition and molecular biology provides a comprehensive insight into the mechanisms by which food-derived molecules affect overall health and help prevent disease (Shakti, 2022). Among the Brassicaceae family members, *Lepidium sativum,* the common name is garden cress, has drawn great attention due to its various phytochemicals profile involving glucosinolates, flavonoids, and phenolic compounds (Das, 2020; Mali, 2024; Yildirim, 2024; and Al-Issawi, 2025), which act as antioxidants and suppress oxidative stress and stabilise the genome (Al-sayed *et al*., 2020 and Shakti *et al*., 2022).

Experimental evidence demonstrates that *Lepidium sativum* extracts preserve mitochondrial function and glutathione levels against oxidative toxicity and stimulate antioxidant enzymes (Abdelmonem, 2014; Das, 2020; Paolillo, 2023; and Lesco *et al*., 2025). Beyond its antioxidant potential, *Lepidium sativum* has demonstrated significant antimicrobial activity. Ethanolic and methanolic seed extracts inhibited pathogenic microorganisms such multidrug-resistant P. aeruginosa, S. aureus, and E. coli (Kamal & Nafi, 2018; Khan and Ali, 2020), Grape seed oil shown antifungal properties against Aspergillus niger and Candida

albicans. (Al-Sheikh, 2021; Alzahrani *et al*., 2023). These findings validate its dual role as both an antioxidant and antimicrobial agent, strengthening its relevance as a nutraceutical candidate.

Lepidium sativum extract also demonstrated activity against cancer cell viability, providing direct evidence of its cytotoxic effects. Rajasekaran and Suresh (2021) reported that methanolic seed extracts of *Lepidium sativum* caused cytotoxicity in MCF-7 breast cancer cells that was dose-dependent, with significant cell death confirmed by MTT and PI-based assays. Similarly, the in vitro efficacy of garden cress (*L. sativum*) extracts against the HuH7 and HEPG2 hepatocellular cancer cell lines was evaluated by Nazir *et al*. (2021), with the normal hexane extract being the most effective against both cell lines. This cytotoxicity was confirmed by the marked decrease in the levels of BCL2 and EGFR gene expression and the and the marked HuH7 and HEPG2 cell lines showed elevated levels of SMAD3, BAX, and p53 gene expression. The previous findings support the use of *L. sativum* as an antioxidant and antibacterial agent, suggesting its importance as a nutraceutical candidate. The extract of L. sativum is considered a promising anticancer agent with cell viability reduction potential in several types of cancer cells. A previous study by Rajasekaran and Suresh (2012) investigated the methanolic *L. sativum* seed extract; their findings revealed the extract's influence on breast cancer (MCF-7) cell death. This capacity increased with increasing the extract dose and was assessed via MTT and PI-based assays. Another study by Aslani *et al*. (2014) documented promising antiproliferative activity against K562 leukaemia cells using the MTT assay. In general, several studies have demonstrated the anticancer capacity of *L. sativum* extract against various cancer types, suggesting its role as a promising candidate in nutrigenomics and in suppressing multiple types of tumours. On the other hand, *L. sativum* has shown promising improvements in genomic stability and modulation of DNA repair pathways under harsh oxidative stress conditions (Ben Wattian, 2025). Several studies reported the essential role of the extract in reducing chromosomal defects and generating micromolecules, suggesting its potential as a nutraceutical in protecting the genome (Bin Watyan *et al*., 2025). Thus, the current work explored key DNA repair regulation and the genes responding to stress. In particular, OGGIU, XRCC, and TP53 are characterised by the preservation of genome stability. Notably, TP53 regulates cell-cycle arrest in response to genotoxic stress conditions. OGGI contributes to repairing DNA defects caused by oxidative stress through base excision repair, while NRF2 controls the mechanisms of antioxidant defence. Generally, the contribution of these genes to genomic stability under oxidative stress offers an overview of the importance of *L. sativum* extract as a protective factor and nutrigenomic (Ferrarini, 2011 and Kaur, 2017). However, most previous studies have not explored *L. sativum* extract in genomic protection and nutrigenomics. The current study aims to address this gap by elucidating the extract's phytochemical profile and providing mechanistic proof for its potential as an antioxidant, antibacterial agent and genome protector. The study seeks to examine the effects of the extract on repairing DNA and stress-response genes.

2. MATERIALS AND METHODS

2.1. Plant Extract Preparation

Garden cress (*Lepidium sativum* L.) seeds were obtained from certified local markets, thoroughly cleaned, dried, and ground into a fine powder. Twenty grams of the powder were dissolved in 1000 milliliters of a mixture of solvents that 20% distilled water and 80% ethanol, and the solution was incubated for 3 hours at 70 °C to make it easier to extraction the bioactive compounds. the extract was filtered, concentrated using a rotary evaporator at low pressure, then freeze-dried to produce a stable powder, and it was stored at −20 °C until use.

2.2. Phytochemical Screening

Three characterisation methods were employed to identify the chemical compositions *of L. sativum* extract. The dissolved and ultrasonicated extract (to ensure complete dissolution) was filtered, then subjected to GC-MS. Deionised water was utilised for recording UV-Vis spectra. The dried extract was directly analysed by FTIR for functional group characterisation and identification of chemical bonds from observed absorption peaks.

2.3. **Oxidative stress**

DPPH test (2,2-diphenyl-1-picrylhyrazyl) (Sreejayan and Rao 1996; Okawa, 2021) was employed for the antioxidant assessment of *L. sativum* extract. One hundred milliliters of ethanol were used to dissolve 0.002 grams of powdered DPPH. A magnetic stirrer was used to agitate the mixture for five minutes. The mixture was kept at 4 °C in a foil-wrapped flask to avoid degradation. Ascorbic acid, the positive control, was produced in concentrations of 12.5, 25, 50, and 100 µg/mL. The

extract was mixed with one milliliter of DPPH solution, which was incubated for 30 minutes in the dark at room temperature. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The following formula was used to determine radical scavenging activity (%):

Radical scavenging activity (%) = (A_Control-A_(Sample))/A_(Contro;) × 100

where the absorbance of the DPPH solution without extract is shown by (*A*Control), and (*A*sampl) the absorbance when the extract is present.

2.4. Antimicrobial Activity

The agar well diffusion assay was used to assess the antibacterial activity of Lepidium sativum extract against strains of S. aureus and E. coli. (Khashan, 2020; Bahjat, 2021). Muller–Hinton (M–H) medium was made by dissolving 38 grams of dried powder in one liter of distilled water, then heating while stirring constantly. Autoclaving the medium at 121 °C for 15 minutes and cooling it to 50 °C, and transferred into sterile Petri dishes. The plates were inverted and kept at 4°C after solidification until use (Aryal, 2022). Bacterial inocula were extracted from their original cultures using a sterile wire loop, and 20 mL of M–H medium was added to sterile Petri dishes for the test. (Khashan, 2021). 100 µL of Lepidium sativum extract at several doses (12.5, 25, 50, and 100 µg/mL) was added to wells with a diameter of 6 mm that were aseptically punched into the agar. The negative control was sterile distilled water. Following a 24-hour incubation period at 37 °C, the diameters of the inhibitory zones were measured and noted. The following formula was used to determine the percentage inhibition in comparison to the positive control:

$$\text{Inhibition } (\%) = \frac{Z\text{sample} - Z\text{control}}{Z\text{standard} - Z\text{control}} \times 100$$

where (*Z*sample) represents the inhibition zone of the extract, (*Z*control) represents the negative controls inhibitory zone, and (*Z*standard) symbolizes the positive control's inhibition zone.

2.5. Cell Culture and Treatment

The Iraqi Center for Cancer Research and Medical Genetics, Al-Mustansiriya University's Cell Bank Unit of the Experimental Therapy department provided the tested CAL51 breast cancer cell line. The cell line was grown and dispersed in RPMI-1640 media using L-glutamine, HEPES buffer, 10% fetal calf serum (FCS), and antibiotics (1% streptomycin and ampicillin). To enable exponential development, the cultures were maintained at 37 oC in an incubator with 5% CO2 and humidity. Subculturing was carried out at 70-80% confluence for cytotoxic and genomic evaluation. A different extract concentration was added to the seeded cells at suitable densities. Untreated cells were used as negative controls.

2.6. MTT Cytotoxicity Assay

The MTT assay was employed to evaluate the *L. sativum* extract cytotoxicity; it is a colourimetric procedure for assessing cell viability (Mosmann, 1983). In 96-well plates, the CAL51 breast cancer cell line was grown at a density of 5×105 cells/mL in RPMI-1640 media supplemented with 200 µL/well of 10% fetal bovine serum. The culture media were kept in a humid atmosphere with 5% CO2 for the entire nite at 37 oC. The wells were filled with various extract concentrations ranging from 12.5 to 100 µL/ml, and they were then incubated for a whole day. As a negative control, a serum-free medium was employed Following the culture media incubation period, 150 µl was taken out of each well, and 50 µl of MTT solution (5 mg/mL; Bio-World GeneLinx International, USA). The plates containing the solution were incubated for 4 hours under similar conditions to enable viable cells and mitochondrial dehydrogenase to reduce MTT to formazan crystals are insoluble. Dimethyl sulfoxide (DMSO) was added to each well to solubilise crystals, and the wells were incubated for 2 hours. A microplate reader (Human, Germany) was employed to calculate the optical density at 570 nm. In comparison to untreated controls, cell viability was displayed as a percentage, introducing a quantitative cytotoxicity assessment. The growth inhibition % was computed using the following formula.

$$\text{Inhibition } (\%) = \frac{A_{Control} - Areated}{A_{Contro;}} \times 100$$

where (*A*control) and (*A*treated) represent the absorbance values of control and treated wells, respectively (Stockert *et al.*, 2012; Präbst *et al.*, 2017).

2.7. Analyzing Gene Expression

Following the manufacturer's instructions, total RNA was extracted from CAL51 cells treated with Lepidium sativum extract using the RNeasy Mini Kit (QIAGEN, Germany). NanoDrop spectrophotometry (A260/A280) was used to assess RNA purity, and all samples exhibited high purity values within the acceptable range (1.8–2.0). The RNA concentration was approximately 900 ng per sample.

cDNA synthesis was performed immediately after RNA extraction using the 5× RT PCR Master Mix (4 µL) combined with RNase-free water (7 µL), 10 µL of RNA template is then added to each reaction. The combination (poly(A) RNA templates) was incubated for 15 minutes at 42 °C, by a final 5-second enzyme inactivation at 85 °C. The cDNA that was produced was either used directly for qPCR or kept at -20 °C to prevent frequent cycles of freezing and thawing (Damgaard and Treebak, 2022 and Stránský *et al*., 2025).

The PCM08 Master SYBR Mixture was used to perform quantitative real-time PCR (RT-qPCR) (Tinzyme Co., Limited), it includes dNTPs, SYBR Green I dye, Fast Taq DNA Polymerase, and Mg^{2+}. 12.5 µL of 2×1 SYBR Master Mix, 0.5 µL of forward primer (10 µM), 0.5 µL of reverse primer (10 µM), cDNA template (1–10 ng), and nuclease-free water to volume were all included in each 25 µL reaction. ROX Reference Dye was added according to instrument requirements (Pereira-Gómez *et al*., 2020; Souza *et al*., 2022). The following primers were used:

- House-keeping gene (TBP):

Forward primer (Pr-F): 5'-AGAGGTGCTGGAAGTGACTGCT-3', and

Reverse primer (Pr-R): 5'-GTAGAGTGGAAGTGGTGTTGT-3'

- NRF2 gene

Forward primer (Pr-F): 5'-CACCGTAAAGAGACAGCAGAA-3', and

Reverse primer (Pr-R): 5'-TTGACATCTTGCAGGCGACCTA-3'

- OGG1 gene

Forward primer (Pr-F): 5'-CTGGAGATGGGTAGTACAGGGGA-3', and

Reverse primer (Pr-R): 5'-CTAGCGTGGTCTGTGTCTCT-3'

- TP53 gene

Forward primer (Pr-F): 5'-TGACTTCAAGAGCCAGGCAGGT-3', and

Reverse primer (Pr-R): 5'-AGGATAGGAAAGGACAAGGC-3'

The amplification was conducted using these cycling parameters: an initial denaturation step at 95 °C for 30 seconds, then 40 cycles of denaturation at 95 °C for 5 seconds and annealing/extension at 60 °C for 30 seconds. Melt curve analysis was incorporated to validate specificity. Relative gene expression levels of OGG1, XRCC1, and TP53 were normalised to GAPDH and computed with the 2^−ΔΔCt method. To ensure reproducibility, all reactions were conducted three times. (Livak and Schmittgen, 2001).

2.8. Statistical Evaluation

Data are expressed as mean ± SD, with all experiments conducted in triplicate. Using one-way ANOVA followed by Tukey's post hoc test (GraphPad Prism X), statistical significance was determined, with $p < 0.05$ deemed significant.

3. RESULTS

3.1. Phytochemical Examination

A comprehensive insight into *Lepidium sativum* extract chemical compositions were screened employing GC- MS, UV-Vis spectroscopy and FTIR analysis. The UV-Vis image shows a distinct peak in the visible and UV regions, thereby identifying phenolic compounds and flavonoids by characterising conjugated double bonds and aromatic systems (Fig.1).

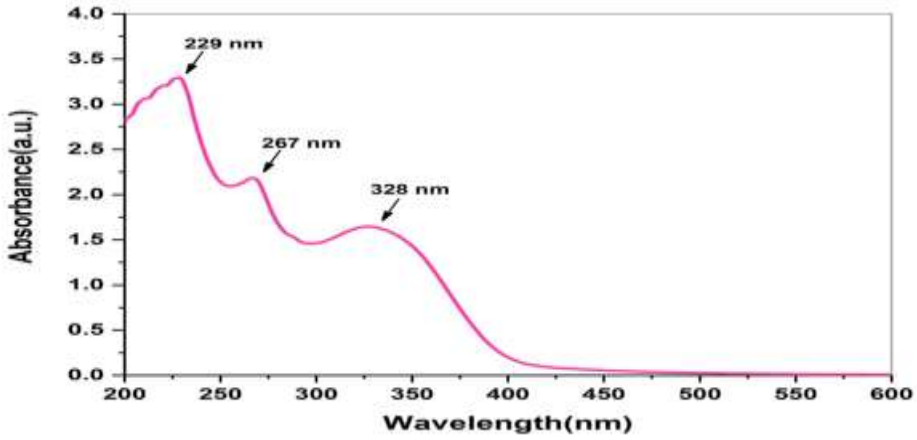


Figure 1: UV–Vis spectrum of *Lepidium sativum* extract

The FTIR spectrum exhibited a strong band of C–H stretching vibrations in aliphatic chains at 2922 cm^{-1}, whereas the peaks at 1512 and 1579 cm^{-1} are attributed to C=C stretching vibrations of aromatic rings. Furthermore, the absorption peak at 1058 cm^{-1} is attributed to C–O stretching vibrations, characteristic of alcohols or esters. The minor peaks observed at 823 and 696 cm^{-1}, however, are ascribed to aromatic C–H bending (Fig. 2).

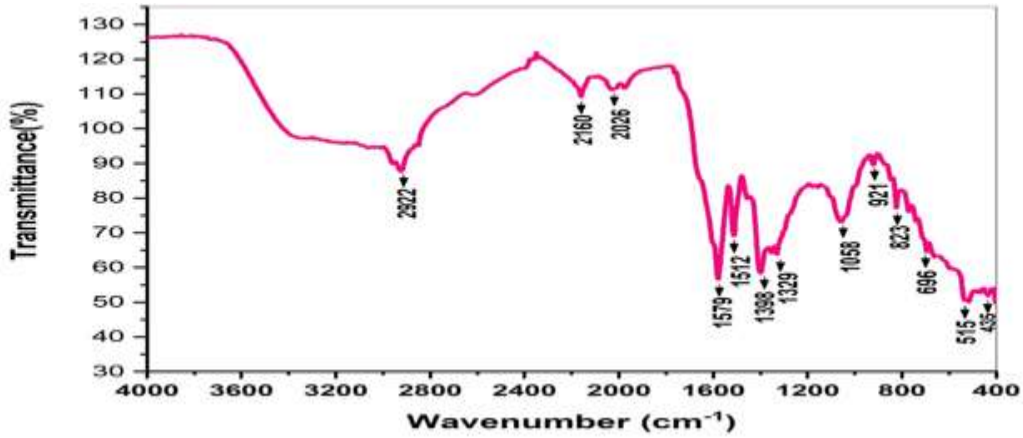


Figure 2: FTIR spectrum of *Lepidium sativum* extract

The data from GC-MS analysis support the FTIR results, as the total ion chromatogram showed several peaks with distinct retention times attributed to different bioactive compounds (Fig. 3). The essential recognised compounds included phytol, linolenic acid, benzyl isothiocyanate, stigmast-7-en-3-ol, 2-methoxy-4-vinylphenol, and n-hexadecanoic acid (Tab.1). The identified compounds were categorised among various chemical classes, including diterpenes, fatty acids, isothiocyanates, plant sterols, and phenolic compounds. Providing insights into the diversity of the extract phytochemicals, which are recognised for their biological activities as antioxidants, antimicrobials, anti-inflammatories, and cardioprotective features.

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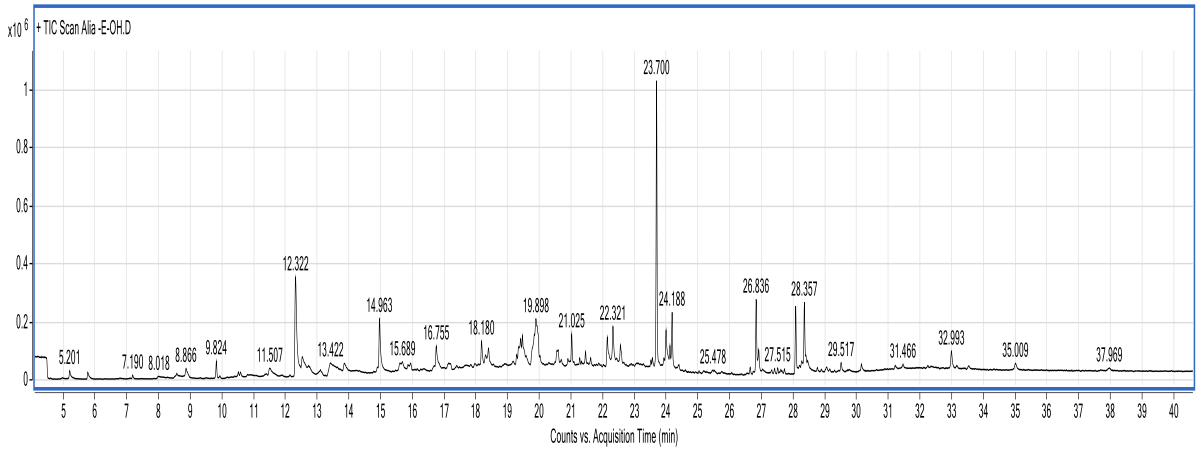


Figure 3: GC–MS chromatogram of *Lepidium sativum* extract

Table 1: Major bioactive compounds identified in *Lepidium sativum* extract by GC–MS

Compound Name	Retention Time (RT, min)	Molecular Formula	Chemical Class	Potential Biological Activity	References
Phytol	23.69	C20H40O	Diterpene alcohol	Antioxidant, antimicrobial, antibiotic-potentiating	Islam et al., 2018; de Morais et al., 2014
9,12,15-Octadecatrienoic acid (Linolenic acid)	23.99	C18H30O2	Unsaturated fatty acid	Radical scavenging, anti-inflammatory, cardioprotective	Simopoulos, 2002; Abdel-Rahman et al., 2019
Benzyl isothiocyanate	15.69	C8H7NS	Isothiocyanate	Antimicrobial, anticancer potential	Fahey et al., 2001; Dinkova-Kostova & Kostov, 2012
Stigmast-7-en-3-ol	32.99	C29H50O	Plant sterol	Antioxidant, cholesterol-regulating, antimicrobial	Kaur et al., 2011; Panda et al., 2009
2-Methoxy-4-vinylphenol	14.96	C9H10O2	Phenolic compound	Antioxidant, antimicrobial, anti-inflammatory	Kumar et al., 2015; Singh et al., 2017
n-Hexadecanoic acid	22.32	C16H32O2	Saturated fatty acid	Antioxidant, anti-inflammatory, antimicrobial	Akinmoladun et al., 2020; Kumar et al., 2010

3.2. Antioxidant Activity

DPPH assay was employed to assess the antioxidant activities of *L. sativum* extract. (Fig. 4) shows that concentrations of 12.5 to 100 µg/mL exhibited high radical-scavenging activity, ranging from 95 to 98%, significantly comparable to the control, ascorbic acid. Visual confirmation of this activity was evident in (Fig. 5), where the progressive discolouration of the DPPH solution from purple to yellow indicated effective neutralisation of free radicals. The close agreement between quantitative data and visual observations underscores the assay's robustness and confirms the antioxidant efficacy of *Lepidium sativum*

extract. These findings provide a strong experimental basis for its genome-protective potential observed in subsequent analyses. Triplicate assays yielded highly consistent values with no significant variation observed.

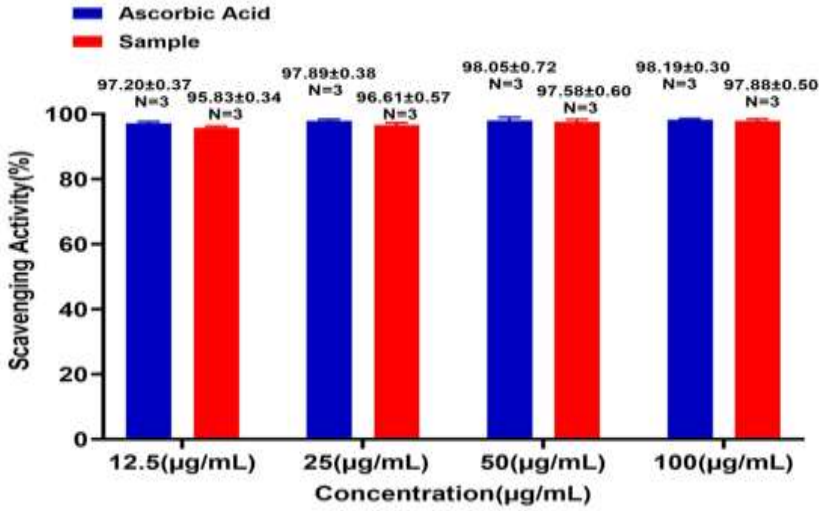


Figure 4: Radical scavenging activity of *Lepidium sativum* extract compared to ascorbic acid

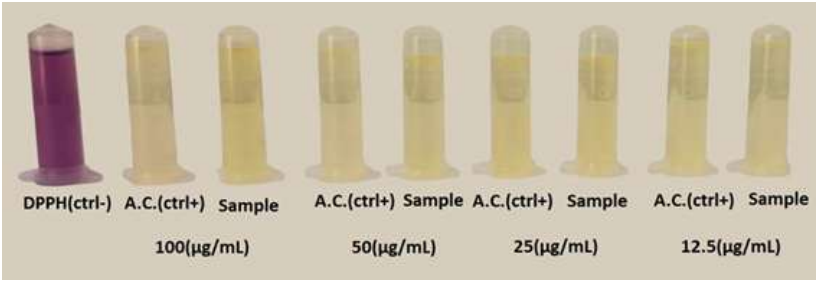


Figure 5: Visual confirmation of antioxidant activity of *Lepidium sativum* extract in the DPPH assay.

3.3. Antibacterial Activity

The antibacterial effects of Lepidium sativum extract were evaluated against S. aureus (Gram-positive) and E. coli (Gram-negative) using various concentrations from 12.5 to 100 µg/mL. (Table 2) presents the inhibition zones with obvious dose-dependent inhibition, as the larger inhibition zones were 17 and 20 mm at 100 µg/mL for E. coli and Staphylococcus aureus, respectively, while both tested bacteria displayed similar and the smallest inhibition zones of 6 mm at the minimal concentrations of 12.5 µg/mL. Visual confirmation of these results is provided in (Figs. 6 and 7), which illustrate the progressive enlargement of inhibition zones with increasing extract concentrations. The consistency between quantitative measurements and visual evidence confirms the dose-dependent antibacterial efficacy of *Lepidium sativum* extract. Triplicate assays yielded consistent inhibition zones with no significant variation among replicates.

Table 2: Antibacterial activity of *Lepidium sativum* extract against *S. aureus* and *E. coli*

Antibacterial analysis(Zone of inhibition (mm)					
Sample	**A (12.5 µg/mL)**	**B (25 µg/mL)**	**C (50 µg/mL)**	**D (100 µg/mL)**	**E**
S.aureus	**6**	**14**	**18**	**19**	**20**
E.coli	**6**	**14**	**15**	**16**	**17**

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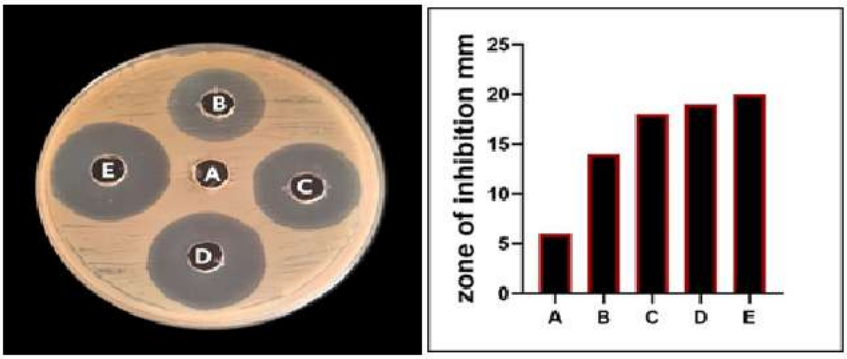


Figure 6: Antibacterial activity of *Lepidium sativum* against *S. aureus*

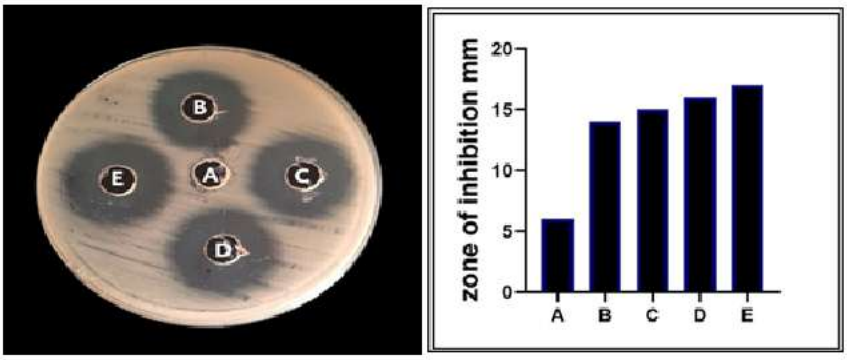


Figure 7: Antibacterial activity of *Lepidium sativum* against *E. coli*

3.4. MTT Cytotoxicity Assay

The MTT assay revealed that the extract of Lepidium sativum has a cytotoxic effect on CAL51 cells that is dependent on concentration. Cell viability decreased progressively from 95.4% at 12.5 µg/mL to 63.4% at 100 µg/mL, that correspond to values of growth inhibition ranging from 4.6% to 36.6%. The viability reduction at higher concentrations compared to the control was confirmed as significant through statistical analysis (one-way ANOVA, $p < 0.05$). (Fig. 8, Table 3). Triplicate assays produced consistent results that showed statistically significant differences when compared to the control ($p < 0.05$).

Table 3: Cytotoxicity of *Lepidium sativum* extract against CAL51 cells (MTT assay)

Concentration (µg/mL)	Mean OD ± SD	Cell Viability (%)	Growth Inhibition (%)	Significance vs. Control
Control	0.262 ± 0.027	100	–	–
12.5	0.250 ± 0.028	95.4	4.6	$p < 0.05$
25	0.210 ± 0.041	80.2	19.8	$p < 0.01$
50	0.204 ± 0.059	77.9	22.1	$p < 0.01$
100	0.166 ± 0.027	63.4	36.6	$p < 0.001$

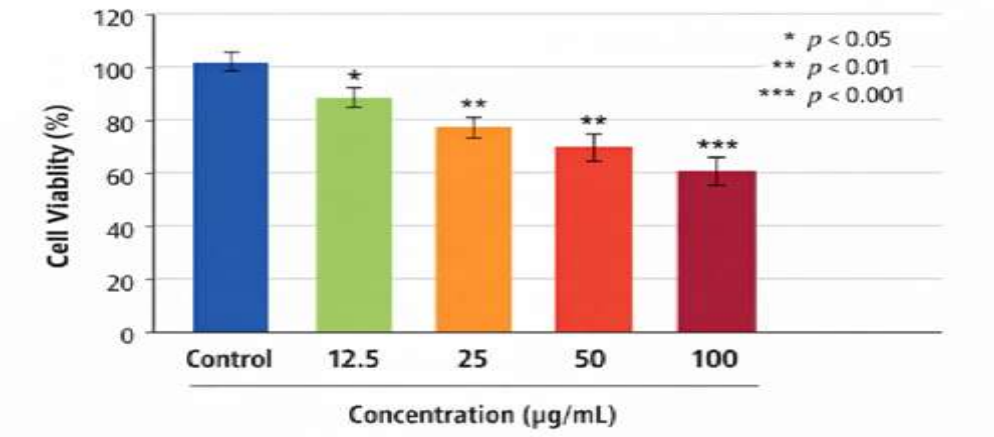


Figure 8: Effect of *Lepidium sativum* extract on CAL51 cell viability (MTT assay)

Microscopic examination of CAL51 cells following treatment with Lepidium sativum extract revealed concentration-dependent morphological alterations consistent with the viability data. Control cells that were not treated showed a normal morphology, with intact membranes and growth in a dense monolayer. In contrast, cells exposed to increasing extract concentrations showed progressive changes, including reduced cell density, rounding, shrinkage, and detachment from the culture surface. These morphological features were most pronounced at 100 µg/mL, where extensive cell loss and structural disruption were observed. The visual evidence supports the quantitative findings of the MTT assay (Fig. 9), which verifies the extract's moderate cytotoxicity in a dose-dependent fashion. Together, these quantitative and qualitative findings provide strong evidence for the dose-dependent cytotoxicity of *Lepidium sativum* extract in CAL51 cells.

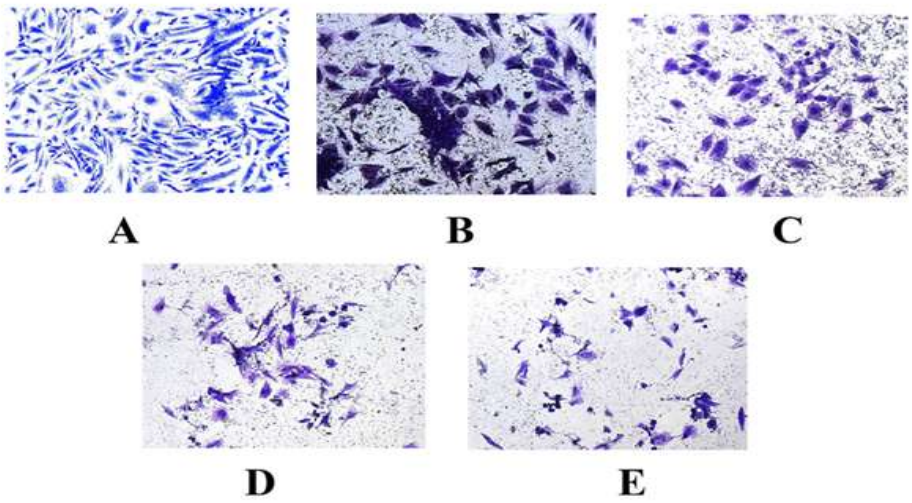


Figure 9: Representative morphological changes in CAL51 cells after treatment with *Lepidium sativum* extract. (A) Control untreated cells; (B) 12.5 µg/mL; (C) 25 µg/mL; (D) 50 µg/mL; (E) 100 µg/mL.

3.5. Gene Expression Analysis

Table 4 and Figure 10 illustrate the distinguished modulation of CAL51 cells gene expression when treated with L. sativum extract in quantitative RT-qPCR analysis in comparison to the un untreated control. TBP served as the housekeeping gene for the purpose of normalising expression levels. As can be seen in Fig. 10, the treated cells witnessed significant upregulation of TP53 expression (fold change ≈ 10.9, $p < 0.001$), proposing the p53-dependent pathways activation involving apoptosis and cell cycle arrest. While there was a notable down-regulation in NRF2 expression (fold change ≈ 0.18, $p < 0.05$), suggesting

the reduction of oxidative stress associated with transcription activity. Additionally, OGG1 displayed a moderate upregulation (fold change ≈ 2.6 < 0.01), signifying the increase in activity of DNA repair.

In general, these transcriptional adjusting consistant with the observed efficiency of antioxidants and cytotoxic results, supporting the fact that L. sativum extract is crucial to in responding to oxidative stress and the mechanisms of DNA repairing in CAL51 cells.

Table 4: Ct values, ΔCt, ΔΔCt, and relative fold change of TP53, NRF2, and OGG1 genes in CAL51 cells treated with *Lepidium sativum* extract, normalised to TBP housekeeping gene.

Gene	Ct Treated	Ct Control	ΔCt Treated	ΔCt Control	ΔΔCt	Fold Change
TP53	21.54	25.00	−3.33	5.01	−3.46	10.9 ↑
NRF2	31.82	29.33	6.95	9.34	+2.5	0.18 ↓
OGG1	21.38	22.11	−3.49	2.12	−1.4	2.6 ↑
TBP (Ref.)	24.87	19.99	—	—	—	1.0

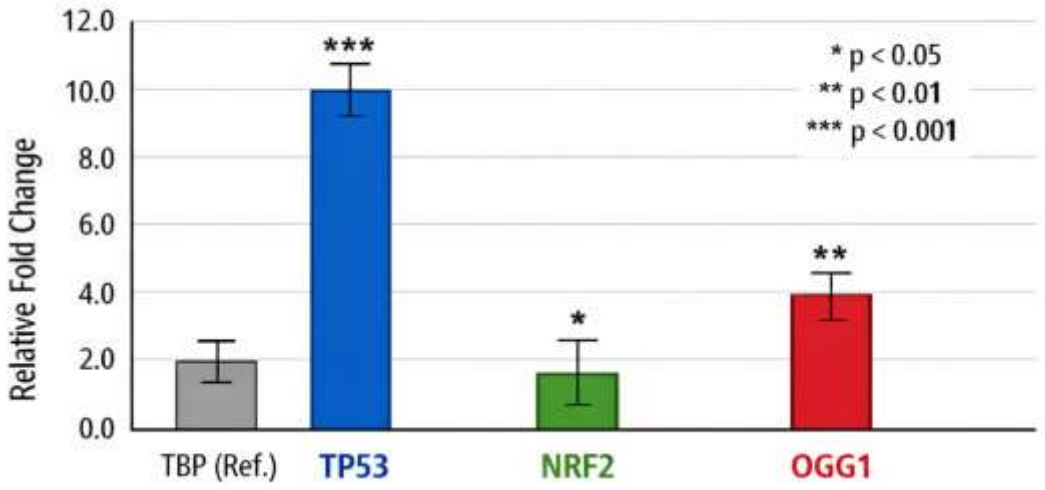


Figure 10: Relative fold change in the expression of TP53, NRF2, and OGG1 genes in CAL51 cells treated with *Lepidium sativum* extract, normalised to TBP reference gene.

4. DISCUSSION

Lepidim sativum phytochemicals analysis indicated a board range of bioactive compounds that might correlate to its antioxidant and antibacterial effects, while GC-MS retention times showed the extract chromatographic fingerprint. The biological efficiency of the identified compounds is based essentially on their structural properties and functional bioactivities rather than their elution order. There are three main compounds among the characterised ones, including phytol, linolenic acid, and benzyl isothiocyanate, suggested to be responsible for the observed strong activities of radical-scavenging and DNA protection. A previous study supports the phytol potential in antibiotic efficiency against resistant microorganisms (Almeida-Bezerra *et al.*, 2024). A different investigation conducted by Roy et al. (2022) showed the association between linolenic acid and anti-inflammatory and cardioprotective advantages in metabolic disorders. A different study reported the benzyl isothiocyanate activities as selective anticancer and antimicrobial (Paul and Basak, 2024). The DPPH assay findings and DNA-protection investigation of the current study further support the notable extract biological efficiency, which might be attributed to possible synergistic interaction among these phytochemicals. Further study on papaya leaves extract displayed richness in phytol with antimicrobial efficiency (Alhodieb *et al.*,2025). Linolenic acid is essentially involved in reducing

oxidative stress in dietary mediation (Simopoulos, 2002). Fahey *et al.* 2001 investigated benzyl isothiocyanate in cruciferous vegetables and documented their chemotherapeutic potential. By comparing these previous data with the current work results can be concluded that *L. sativum* extract is suggested as a natural source for antioxidants and antibacterial, as well as a promising agent in formulated nutraceutical and therapeutic applications. The present study proved the significant antioxidant potential of *L. sativum* extract, ranging from 95-98% at the lowest concentration, when compared to ascorbic acid. The obtained results are in agreement with previously published studies where Smolińska *et al.* (2024) explored that *L. sativum* induces enzymatic and non-enzymatic defence system mechanisms even under harsh conditions, including heavy metals that cause oxidative stress. Bin Watyan *et al.* (2025) have noticed the importance of its influence in the pathways of regulating DNA repair and sustaining genomic stability in colorectal cancer cells. Although another study by Yildirim *et al.* (2025) underlined phenolic compounds and antioxidant enzymes' contribution to *L. sativum* antioxidant activity under water stress. These findings corroborate the idea of *L. sativum* extract as a valuable natural provider of antioxidants with nutritional and therapeutic possible, and its biological capacity can be explained by the synergistic impacts of several phytochemicals instead of a singular bioactive compound. *L. sativum* extract results exhibited an observed dose-dependent antibacterial activity due to increased inhibition zone with increased concentration. *S. aureus* was more susceptible to the extract effects as the inhibition zone increased to 20 mm at the highest concentration in comparison to *E. coli,* with a 17 mm inhibition zone. The susceptibility observed as a result of the structural differences of the bacterial cell wall. The bacteria that are gram-positive are characterised by simple and more permeable membranes to the bioactive compounds, whereas the defining feature of gram-negative bacteria is external lipopolysaccharide membrane that restricts bioactive compounds' permeability (Hochvaldová *et al.*, 2024). The results of the present research align with those from other studies and indicate that antibacterial activity is dependent on concentration.

The leaf oil of L. sativum exhibited a stronger antibacterial effect against S. aureus, with Adira et al. (2022) reporting a maximum inhibition zone of 18.50 mm and both the minimum inhibitory and minimum bactericidal concentrations at 0.05 µg/ml. While Akrai *et al.* (2012) reported that extracts of both Lepidium sativum and Allium porrum had an inhibitory effect on all studied bacteria of the Gram-positive and Gram-negative types, except for Klebsiella pneumoniae. Alkahtani et al.'s study (2021) also showed that polysaccharides extracted from garden cress have high inhibitory effectiveness against E. coli and S. aureus bacteria, largely dependent on the dose. In this study, phytol, benzyl isothiocyanate, and liolenic acid where resulted in the biological activities of L. sativum extract, which corresponds to earlier research conducted by Almeida-Bezerra et al. (2024) and Fahey et al. (2001.) Generally, *L. sativum* extract is a promising natural source to be used in developing nutraceutical and therapeutic methods as a replacement for synthetic materials, which aligns with a study by Anand *et al.* (2022).

Lepidium sativum extract in this study was used as well to treat CAL51 cells. The results showed cell viability declining with increasing dose, indicating apoptotic pathways enhancement and mitochondrial disturbance. Among the bioactive compounds of L. sativum are flavonoids and glucosinolates, which are responsible for depolarisation enhancement of the mitochondrial membrane, releasing cytochrome c and inducing caspase, which all together lead to apoptosis (Khan *et al.*, 2011; Zhang *et al*, 2015). The natural compounds derived from plants are characterised by mitochondrial dysfunction potential, leading to ATP synthesis reduction and reactive oxygen species, hence causing oxidative stress and stimulating cytotoxicity (Fulda *et al.*, 2010). However, in this study, the cytotoxicity effects were moderate, which is in agreement with the earlier studies on cruciferous, which exhibited pro-apoptotic and anticancer activity against breast cancer cells (Mukherjee *et al.*, 2008; Abdel-Rahman *et al.*, 2019). The treated CAL51 cells with *L. stavium* extract demonstrated TP53 and OGG1increased expression, thus enhancing repair pathways associated with DNA damage and apoptosis. The essential TP53 gene is the tumour suppressor that is responsible for cell cycle arrest regulation and cell death programming, responding to cellular stress and DNA damage (Kastenhuber and Lowe, 2017), while OGG1 plays a key role in repairing base excision via eliminating oxidative DNA lesions, such as 8-oxoguanine (Wallace, 2014). Alongside NRF2 expression changes, these results indicate that *L. sativum* extract enhances both cytotoxicity and apoptotic efficiency, as well as activating responses of protective DNA repair. This synergistic impact is the consequence of exposing cancer cells to pro-oxidant phytochemicals and the cell responding to stabilize genomic during oxidative stress (Russo *et al.*, 2018).

According to Alkahtani et al. (2020), polysaccharides extracted from garden cress reduced the feasibility of HeLa and HepG2 cells in relation to the concentration. At the molecular level, the decrease in CAL51 viability seen in the MTT assay is consistent with transcriptional activation of pro-apoptotic and DNA repair pathways. Upregulation of TP53 suggests a

p53-mediated stress response leading to cell cycle arrest and apoptosis, while increased OGG1 expression reflects enhanced base excision repair to counteract oxidative DNA damage. Conversely, downregulation of NRF2 indicates suppression of antioxidant defences, sensitising cells to oxidative stress and facilitating cytotoxicity. Collectively, these quantitative, morphological, and molecular findings underscore the potential of *Lepidium sativum* as a provider of bioactive compounds capable of modulating mitochondrial integrity, inducing apoptosis, and engaging DNA repair pathways.

Our results align with earlier studies on air-dried fenugreek leaves and untreated, germinated seeds, which demonstrated the most significant antiproliferative and apoptotic effects on human HepG2, MCF-7, and HCT-116 cell lines in comparison to the VERO cell line (Abdelsalam *et al*., 2023). While the aqueous extract of garden cress (Lepidium sativum) seeds was observed to induce apoptosis and DNA damage in both human MCF-7 breast carcinoma cells and normal human dermal fibroblast (HFS) cells, in a manner dependent on both time and dose (Mahasni & Al-Rimi, 2013), our data suggest that garden cress (Lepidium sativum) extract may selectively enhance apoptotic pathways and DNA repair in cancer cells, highlighting the potential of phytochemicals as safer alternatives in cancer control strategies.

Our findings are consistent with previous reports on silver nanoparticles, which demonstrated growth inhibition, apoptosis, and DNA damage in both normal embryonic fibroblasts and immortalised cancer cell lines (Tawfeeq et al., 2025). While AgNPs exerted non-selective cytotoxicity, our data indicate that *Lepidium sativum* extract may selectively enhance apoptotic and DNA repair pathways in cancer cells, highlighting the potential of phytochemicals as safer alternatives to nanomaterials in anticancer strategies. Several studies have emphasised the anticancer potential of cruciferous vegetables, which share bioactive compounds with *Lepidium sativum*. Indole-3-Carbinol (I3C) with Sulforaphane (SFN) from broccoli and cabbage have been shown to activate TP53-mediated apoptosis and suppress NRF2 signalling, sensitising breast cancer cells to oxidative stress and chemotherapy (Ho et al., 2025). These findings align with our observation that *Lepidium sativum* extract upregulated TP53 and OGG1 while downregulating NRF2, suggesting a conserved mechanism among cruciferous plants in promoting apoptosis and DNA repair while limiting antioxidant defences. Beyond cruciferous species, flavonoids and phenolic compounds from non-cruciferous plants like quercetin and catechins have also been reported to modulate apoptosis and DNA repair pathways through p53 activation and oxidative stress regulation (Thakur et al., 2023; Hashim et al., 2025). Taken together, these comparative insights indicate that the molecular changes observed in CAL51 cells treated with *Lepidium sativum* extract are part of a broader pattern across both cruciferous and non-cruciferous plants. This strengthens the argument that phytochemicals can act as modulators of apoptosis and DNA repair, offering safer and potentially more selective anticancer strategies compared to synthetic agents. Importantly, this study provides one of the first integrated demonstrations linking gene expression, cytotoxicity, and phytochemical composition of *Lepidium sativum* in breast cancer cells.

CONCLUSION

The results of studying *L. stavium* extract concluded its potential for several biological activities, including antioxidants, antibacterial, cytotoxic, and genome-protective activities in CAL51 breast cancer cells. The extract caused cancer cell viability reduction depending on concentration increase, which impacts genome expression that influences apoptosis, oxidative stress, and DNA repair. Specifically, TP53 and OGG1 upregulating revealing apoptotc signaling activation and mechanisms of repairing essential excision. Thus, indicating that NRF2 downregulation caused defence capacity reduction of antioxidants, which consequently explains the cytotoxic effects. The current study introduces a comprehensive assessment of L. stavium nutrigenomic emphasizing its bioactive compounds' potential in preventing cancer and protecting genomic materials. The results of this research endorse the effectiveness of phytochemicals derived from plants regarding the genomic stability and apoptosis of cancer cells. Future clinical studies are required to prove its nutraceutical potential and complementary therapeutic procedure in controlling cancer.

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The authors declare that they have no conflicts of interest, whether actual or potential.

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