Egyptian Journal of Medical Microbiology Volume 34 / No.4 / October 2025 445-454 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Anti-Cancer, Antioxidant, and Antibacterial Activities of *Leucaena leucocephala* Seed Extract

Eman M.G. Al-Magdey*, Wejdan R. Al-Awani, Basheer H. Al-Alwani
Biology Department, College of Science, University of Babylon, Iraq

Key words:
Leucaena leucocephala seeds, Cytotoxicity, Fatty acid, antimicrobial activity, cancer

****Corresponding Author:***
Eman Muslim Ganem Al-Magdey
Biology Department, College of Science, University of Babylon
iman.hamza167@student.uobabylon.edu.iq

ABSTRACT

Background: *Cancer remains a major global health challenge. This study explores the potential of Leucaena leucocephala seed extract for its diverse medicinal properties, particularly its anticancer and antimicrobial effects, with a focus on combating antibiotic-resistant. Staphylococcus aureus* ***Objective:*** *to evaluate the therapeutic potential of Leucaena leucocephala seeds as a prospective source of anticancer, antimicrobial, and antioxidant agents.* ***Methodology:*** *Seeds of Leucaena leucocephala were collected from Babylon Governorate, Iraq, and extracted using a modified Harborne method. The alcoholic extract was tested for cytotoxicity against A431 (skin cancer) and HEK293 (normal kidney) cell lines using the Crystal Violet assay. Fatty acid composition was analyzed through Soxhlet extraction, FAME conversion, and gas chromatography. Antioxidant activity was assessed via the DPPH assay, and functional groups were identified using FTIR analysis. Antimicrobial activity against S. aureus was evaluated using spread-plate and well diffusion methods. Results: The alcoholic seed extract showed significant antioxidant, anticancer (specifically against A431 cells at high concentrations), and antibacterial activity against S.aureus, while sparing normal HEK293 cells. FTIR analysis indicated the presence of flavonoids and other key functional groups. Linoleic acid was identified as the predominant fatty acid. Notably, the crude extract exhibited a synergistic antimicrobial effect, as isolated components such as fatty acids and vitamin E showed no significant activity on their own.* ***Conclusion****: Leucaena leucocephala seed extract demonstrates promising therapeutic potential as a natural source of antioxidant, anticancer (especially against A431 skin cancer cells), and antimicrobial agents targeting S. aureus. The observed bioactivities appear to rely on a synergistic interaction among the extract's constituents.*

INTRODUCTION

Cancer constitutes a major global health challenge and remains a leading cause of mortality in both developed and developing countries[1,2]. Over the past several decades, approximately 200 new chemical compounds have been approved for cancer treatment, with nearly half derived from naturally occurring molecules that have been structurally modified to enhance their safety and efficacy[3]. The exploration of natural sources—particularly medicinal plants—for potential anticancer agents offers a promising and effective strategy for the development of novel therapeutic drugs. This has, in turn, intensified interest in the study of plants as valuable sources of medicinal compounds. One such plant of interest is L. leucocephala, a leguminous species native to tropical regions, including Central Kalimantan, Indonesia. In addition to its therapeutic potential in cancer treatment, L. leucocephala has diverse applications. Its seeds, which contain more than 5.5% fat, are used in cooking and are rich in key fatty acids such as palmitic, behenic, stearic, oleic, lignoceric, and linoleic acids. Moreover, the seeds have been traditionally used as a coffee substitute[4]. L. leucocephala has been reported to exhibit a wide range of pharmacological properties, including antimicrobial, anthelmintic, antibacterial, antiproliferative, antidiabetic, anticancer, cancer-preventive, diuretic, anti-inflammatory, antioxidant, antitumor, antihistaminic, nematicidal, pesticidal, antiandrogenic, hypocholesterolemic, and hepatoprotective activities[5]. Its anticancer and antiproliferative effects have been attributed to polysaccharides present in the seed gum[6]. It is also important to consider bacterial infections in the broader context of human health. S. aureus, though commonly found as part of the natural human flora, poses a significant threat as a persistent and opportunistic pathogen. Its ability to evade the immune system and its increasing resistance to multiple antibiotics make it particularly difficult to treat with conventional therapies[7].

Taxonomical Classification of *Leucaena leucocephala*

- **Kingdom**: Plantae
- **Order**: Fabales
- **Family**: Fabaceae
- **Subfamily**: Caesalpinioideae
- **Genus**: Leucaena
- **Species**: *Leucaena leucocephala*

A

B

Fig. 1: A) Seed of *Leucaena leucocephala*. B) Alcoholic extract of *Leucaena leucocephala* seeds

METHODOLOGY

Plant Material

Seeds of *Leucaena leucocephala* were used in this study.

Preparation of L. leucocephala Seed Extracts

Mature seeds of L. leucocephala were collected from Babylon Governorate, Iraq. The extract was prepared following a modified version of Harborne's method[8]. Briefly, 20 g of ground seeds were weighed and soaked in 200 mL of 75% ethyl alcohol in a 1-liter beaker. The mixture was kept in a cool place for 24 hours with intermittent shaking for 30 minutes. After the soaking period, the mixture was heated in a water bath at 100°C and then allowed to cool for an additional 24 hours. It was subsequently filtered through medical gauze and centrifuged to remove particulates. The resulting supernatant was collected and dried using either an oven or a thermal fan until a dry powder was obtained. The final extract was stored in plastic containers at 4°C for future use[8].

Cytotoxicity Assay

The cytotoxicity assay was conducted to evaluate the effects of L. leucocephala seed extracts and their combinations on A31 and HEK293 cell lines. The cytotoxicity of isolated compounds was assessed using a modified version of the tetrazolium-based colorimetric (MTT) assay. Cells were incubated with various concentrations of the test compounds for five days. A positive control (berberine chloride) was included alongside untreated cells and cell-free controls. At the end of the incubation period, cells were washed, and fresh Minimum Essential Medium (MEM) was added prior to the addition of MTT reagent. After incubation with MTT, the medium was removed, and dimethyl sulfoxide (DMSO) was added to solubilize the resulting formazan crystals. The plates were then incubated further to ensure complete solubilization. Absorbance was measured at 570 nm using a microplate reader to determine the extent of MTT reduction. Wells containing only medium and MTT served as blanks for baseline correction. The LC_{50} values (lethal concentration at 50%) were determined as the concentration of the extract or compound that caused a 50% reduction in absorbance relative to untreated control cells. Each treatment was performed in six replicates, and the entire experiment was repeated three times to ensure accuracy and reproducibility. It was noted that some compounds, particularly antioxidants, could react with MTT in the absence of cells, leading to color development. Therefore, cell-free wells were also included to detect and control for non-specific reactions. Additionally, microscopic examination of the cells was conducted before washing and MTT addition to assess the presence of any cytopathic effects[9].

Preparation of leuc*aena leucocephala* stock solution

A stock solution of L.leucocephala seed extract was prepared at a concentration of 1 mg/mL. This stock solution was used to prepare a series of serial dilutions for experimental use. The solution remains stable when stored at a temperature between 2–8°C.

Preparation of A31 and Hek293Cell Line for Cytotoxicity Assay

Frozen A31 and HEK293 cell lines were thawed and propagated in 25 mL culture flasks using a complete growth medium supplemented with 10% fetal bovine serum (FBS) and antibiotics. The cells were incubated at 37°C in a humidified atmosphere with 5% CO_2.

Phytochemical Analysis of L.leucocephala seed ethanolic extract

HPLC chromatograms of phytochemicals identified in the ethanolic extracts from seeds of L. leucocephala

Total fat was extracted from the samples using a Soxhlet apparatus, following the standard AOAC (1995) protocol[10]. The extracted fatty acids were then converted to fatty acid methyl esters (FAMEs) for analysis by gas chromatography (GC). This process involved reacting the extracted fat with a potassium hydroxide (KOH) methanol solution, prepared by dissolving 11.2 g of KOH in 100 mL of methanol. Specifically, 1 g of the extracted fat was mixed with 8 mL of the KOH

methanol solution and 5 mL of hexane in an appropriate container. The mixture was vigorously shaken for 30 seconds, after which the upper hexane layer containing the FAMEs was carefully separated for injection into a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector (FID). Separation was achieved using a SE-30 capillary column measuring 30 m in length and 0.25 mm in internal diameter. The chromatographic conditions were as follows: injection port temperature at 280 °C, detector temperature at 310 °C, and column temperature programmed from 120 °C to 290 °C at a rate of 10 °C per minute. The carrier gas flow rate was maintained at 100 kPa[10].

Antioxidant Activity (DPPH Assay)

Following the methodology of Brand-Williams et al. (1995), the free radical scavenging activity was evaluated using the 1,1-diphenyl-2-picryl-hydrazil (DPPH) assay[11]. The extract of *Leucaena leucocephala* and its isolated compounds were dissolved in an 85% methanol-water mixture. A volume of 0.5 mL of the sample extract was mixed with 1.0 mL of freshly prepared methanol DPPH solution at a concentration of 20 µg/mL, followed by thorough mixing. The reaction mixture was incubated for 5 minutes, after which the decrease in absorbance was measured at 517 nm. The discoloration was compared to a blank control consisting of methanol without the sample. The antioxidant activity (%) was calculated using the formula: [(control absorbance - sample absorbance) / control absorbance] × 100% [11].

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy was employed to identify the unique functional groups present in the L. leucocephala extract. This technique provides insights into the molecular structure based on characteristic absorption spectra. A small amount of the *L. leucocephala* extract was thoroughly mixed with dry potassium bromide (KBr). The mixture was then carefully ground in a mortar and compressed under a pressure of approximately six bars for two minutes to form a thin KBr pellet. The pellet was placed in a sample holder equipped with a diffuse reflectance accessory. The infrared spectrum was recorded using a Bruker Vertex 70 spectrometer (Germany). The sample was scanned over a frequency range of 4000 to 400 cm[12].

Anti-Bacterial Activity

The antimicrobial activity of the plant extract was evaluated using a combined spread-plate and well diffusion method, based on Egorov's protocol[13]. Briefly, 0.1 mL of each bacterial suspension, standardized to 1.5 × 10^8 cells/mL, was evenly spread onto Mueller-Hinton agar plates. Wells with a uniform diameter of 6 mm were aseptically created in the agar using a sterile cork borer. Next, 0.1 mL of the plant extract was carefully added to each well and gently mixed. To allow adequate diffusion of the extract into the agar, the inoculated plates were left at room temperature for four to five hours. Subsequently, the plates were incubated at 37°C for seven days. Following incubation, the diameter of the inhibition zones around each well was measured using a graduated ruler to assess the extent of bacterial growth inhibition[13].

Statistical Analysis

All data were analyzed using Microsoft Office Excel 2010 and SigmaPlot version 13 software. Analysis of variance (ANOVA) was performed to assess significant differences among group means.

RESULTS

Cytotoxicity Assay

Results of HEK 293 Normal Cell Line Viability Assay with Statistical Analysis

The results of the HEK 293 normal cell viability assay indicated that treatment with the tested substance within the concentration range of 31.25 to 1000 micrograms/ml did not significantly affect cell viability. The untreated control group exhibited high viability, averaging 100.000% (Std Dev = 7.920), and across all treated concentrations, the mean viability remained high, ranging from 95.867% to 101.212%. A One-Way ANOVA analysis ($F(6, 17) = 1.011$, $P = 0.451$) confirmed that there was no statistically significant difference in cell viability between the control group and any of the treated groups. Although slight fluctuations in the mean viability percentages were observed across different concentrations (ranging from approximately 95.867% to 101.212%), the lack of a statistically significant P-value indicates that these variations are likely due to random sampling variability rather than a true effect of the substance.

Therefore, the statistical analysis supports the conclusion that the tested substance does not exhibit a significant cytotoxic or inhibitory effect on the viability of HEK 293 normal cells under the specified experimental conditions as shown in Figure 2.

Results of A431 Skin Cancer Cell Line Viability Assay with Statistical Analysis

The A431 skin cancer cell viability assay demonstrated a concentration-dependent effect of the tested substance. One-way ANOVA confirmed a statistically significant difference between treatment groups ($P < 0.001$). Post-hoc analysis (Holm-Sidak) revealed that higher concentrations (500 and 1000 µg/ml) significantly reduced cancer cell viability ($P < 0.05$) compared to the control (≈100% viability). Lower concentrations (31.25 - 250 µg/ml) showed no significant difference from the control as shown in Figure 3.

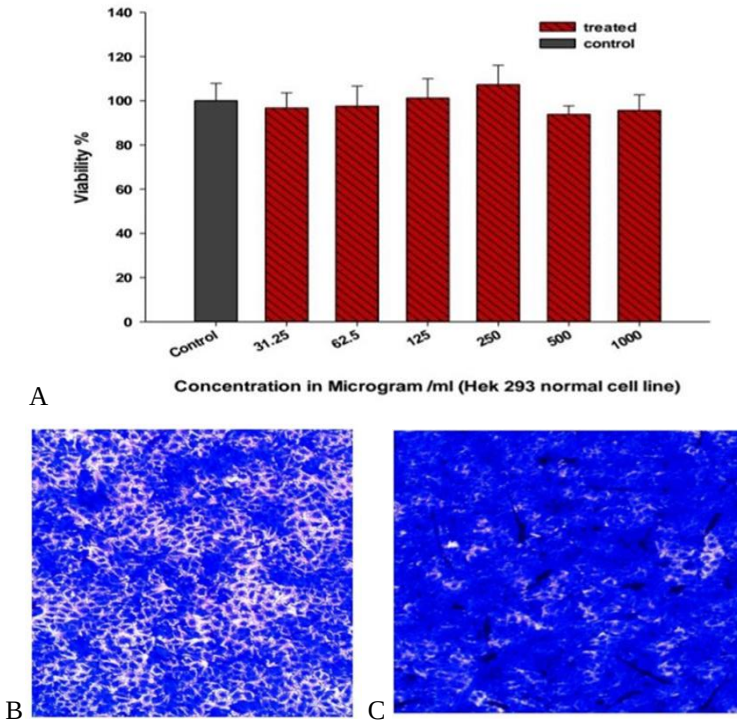

Fig. 2: Effects of ethanol extract of L.leucocephala seeds on 293 Normal cell line. A) Cell Viability Assay Results on HEK 293 Normal Cell Line. B) HEK 293 Normal Cell Treated with ethanol extract of L.leucocephala seeds. C) Control of HEK 293 Normal Cell.

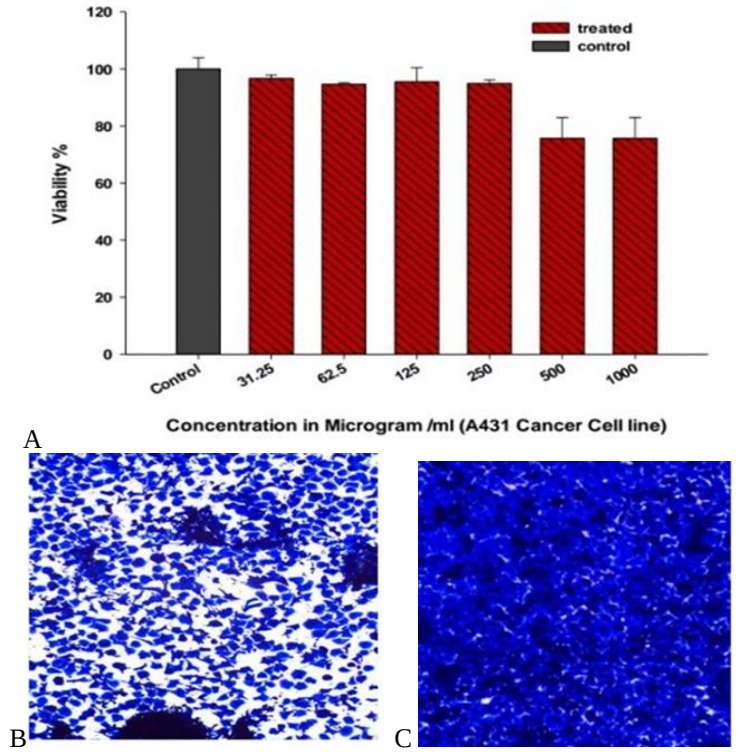

Fig. 3: Effects of ethanol extract of L.leucocephala seeds on A31 Cancer cell line. A) Cell Viability Assay Results on A31 Cancer Cell Line. B) A31 Cancer Cell Treated with ethanol extract of L.leucocephala seeds. C) Control of A31 Cancer Cell.

Phytochemical Analysis of L.leucocephala ethanol seed extract

HPLC analysis revealed the presence of five primary compounds in the ethanol extract of L. leucocephala seeds, with linoleic acid being the most abundant at 50.14%, followed by oleic acid (22.56%) and palmitic acid (12.59%). Octadecanoic acid and Vitamin E were present in lower percentages of 6.25% and 0.36%, respectively as shown in table 1 and Figure 4, 5.

Table 1: HPLC chromatograms of phytochemicals identified in ethanolic extract from seeds of *Leucaena leucocephala*

NO	NAME	CON (%)
1	Palmatic	12.59
2	Oleic	22.56
3	Lenolic	50.14
4	Octadecanoic(steraic)	6.25
5	Vitamin E	0.36

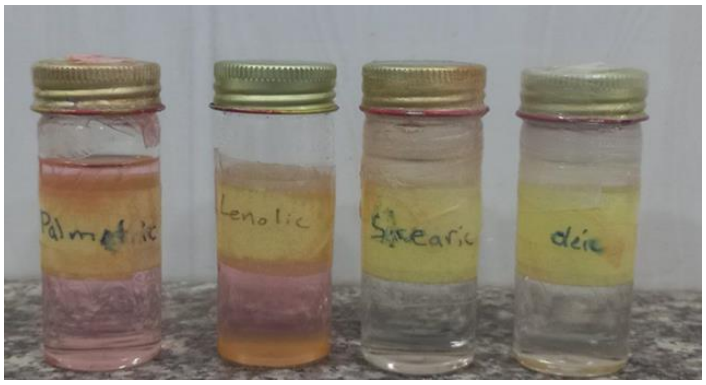

Fig. 4: Active compounds extracted by HPLC from the alcoholic extract of the seeds of L.leucocephala

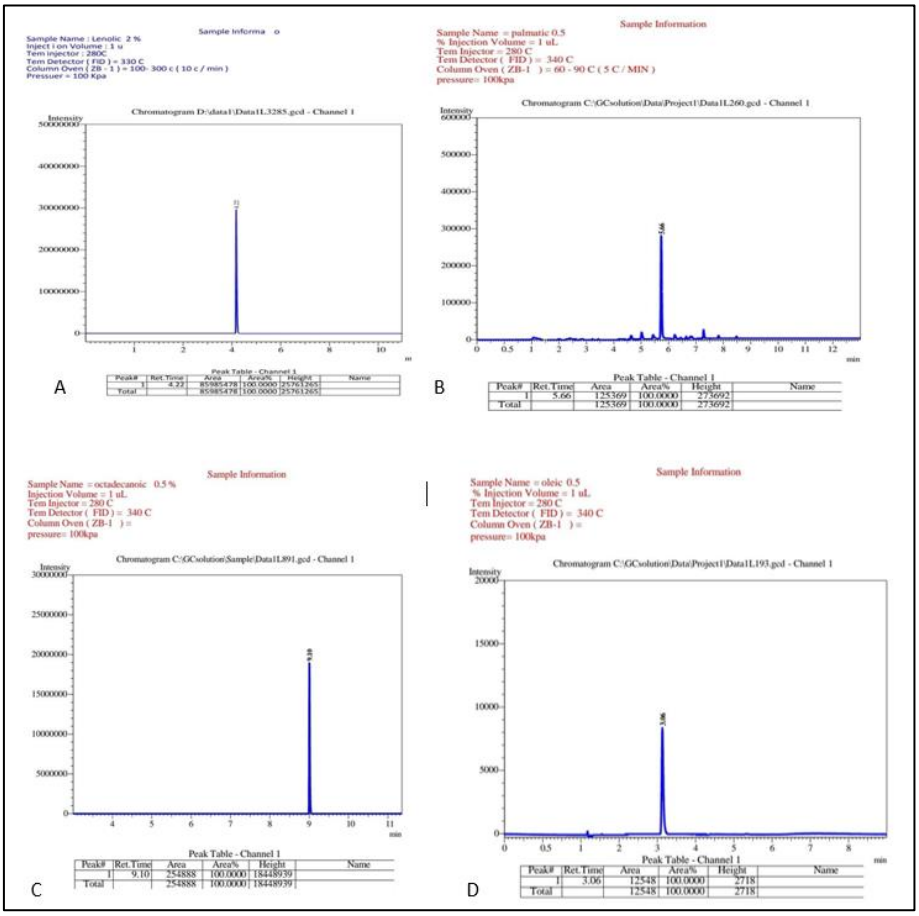

Fig. 5: Phytochemical compounds in L. leucocephala seed extracts by HPLC. A) Leonic acid, B) Palmatic acid, C) Octadecanoic acid, D) Oleic acid

Antioxidant activity of the ethanol seed extracts

As shown in table 2 The antioxidant activity assay of the alcoholic extract from *L. leucocephala* seeds yielded positive results, indicating its ability to scavenge free radicals. The extract's effectiveness in inhibiting oxidation increased progressively with higher concentrations, with the scavenging percentage rising from 54.127% at 0.12 mg/mL to 85.142% at the highest tested concentration of 1 mg/mL. This concentration-dependent increase in efficacy confirms the presence of antioxidant compounds within the alcoholic extract of *L. leucocephala* seeds. Their contribution to protecting cells from oxidative damage becomes more pronounced with greater amounts of the extract.

Table 2: anti-oxidant of extract seeds of L.lecocephala

Sample Name	Concentration (mg/ml)	Absorbency	Scavenging %
1	0.12	0.389	54.127
2	0.25	0.307	63.797
3	0.5	0.205	75.825
4	1	0.126	85.142
Control	-	0.848	-

(FTIR) Fourier Transform Infrared Spectroscopy

The FTIR spectrum of the alcoholic extract from *L. leucocephala* seeds indicates the presence of a complex mixture of organic compounds. The spectrum reveals prominent functional groups, including hydroxyl (O–H), represented by a broad peak between 3751 and 3200 cm^{-1}, suggesting the presence of phenolic compounds, alcohols, and possibly carboxylic acids or residual water. Peaks at 2958 and 2856 cm^{-1} correspond to aliphatic C–H stretching, indicative of lipids or fatty acids. A strong absorption at 1743 cm^{-1} suggests the presence of carbonyl (C=O) groups, commonly found in esters or carboxylic acids. The peak around 1637 cm^{-1} may indicate the presence of unsaturated or aromatic compounds, or amine groups. Additionally, the peak near 1066 cm^{-1} supports the presence of C–O bonds, typical of alcohols, ethers, or carbohydrates. Overall, this preliminary analysis provides valuable insight into the functional groups present in the extract, as shown in Figure 6.

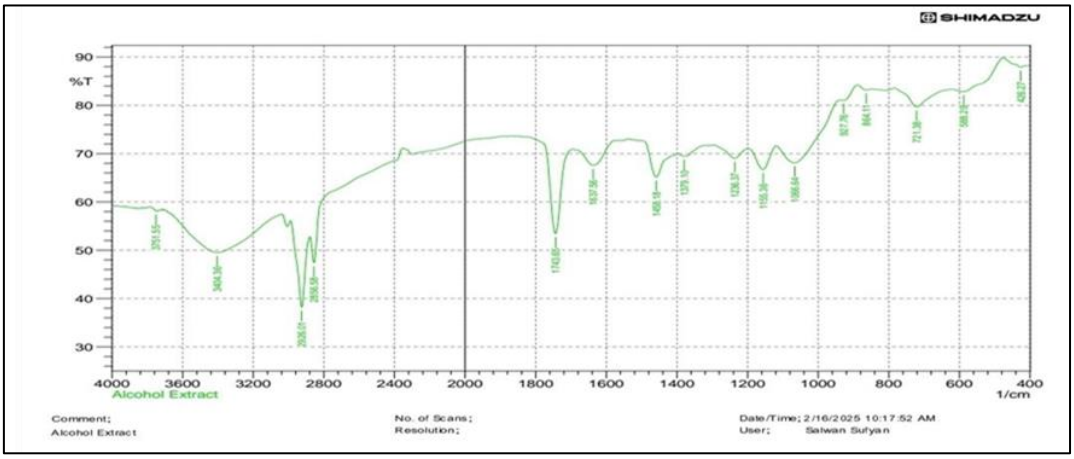

Fig. 6. Fourier transform infrared spectroscopy (FTIR) spectra of *Leucaena leucocephala* seed extract.

Anti-bacterial activity.

The antibacterial activity assay demonstrated that the crude alcoholic extract of *L. leucocephala* seeds exhibited a clear inhibitory effect on the growth of *Staphylococcus aureus*, as evidenced by a distinct zone of inhibition. In contrast, the individual compounds tested—palmitic acid, oleic acid, linoleic acid, and stearic acid—did not display significant antibacterial activity against the same *Staphylococcus* strain at the tested concentrations As shown in Figure7.

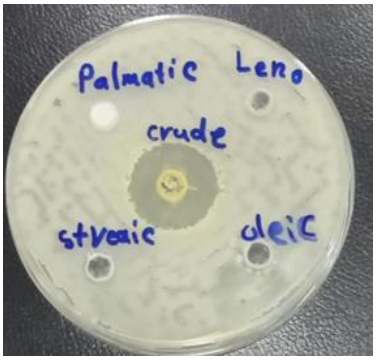

Fig. 7: Test of bioactive compounds and crude of seeds extract on S.aureus.

DISCUSSION

Under the experimental conditions employed, the tested compound did not exhibit a significant cytotoxic or inhibitory effect on the viability of HEK 293 normal cells, as indicated by statistical analysis of the cell viability assay. our results suggest that the material is well tolerated by HEK 293 cells and does not significantly impair cell growth or induce cell death within the tested dose range (31.25 to 1000 µg/mL). This finding supports the conclusion that, under the specified conditions, the compound is not harmful to HEK 293 normal cells. Such information may be valuable for future research involving this material and cell line, particularly in determining appropriate dosages for subsequent studies focused on other cellular functions or potential therapeutic applications. Our study align with previous research indicating that the active compounds present in the ethanol extract of L.leucocephala seeds, such as oleic acid, stearic acid, and phenolics, possess cytotoxic properties against cancer cells while preserving the viability of normal cells. This is consistent with the results (Roy, Wójciak-Kosior, and Khan)[25,26,27]. In our work the results indicate that the tested substance inhibits the viability of A431 skin cancer cells in a dose-dependent manner. A significant reduction in cell viability at higher concentrations suggests a cytotoxic or anti-proliferative effect, while the absence of a notable effect at lower concentrations implies the existence of a threshold for biological activity. These findings are consistent with previously reported anti-cancer properties of L. leucocephala components, supporting the potential of the tested substance as an in vitro anti-cancer agent[1].

A variety of bioactive chemicals were identified in the L. leucocephala seed extract through both qualitative and quantitative analyses. The four main fatty acids—palmitic, oleic, linoleic, and octadecanoic acids—were notably detected. These results align with previous research indicating that these fatty acids possess anionic surfactant properties in addition to their well-established antifungal and antibacterial activities[14]. Furthermore, our findings corroborate studies reporting that some plant extracts contain palmitic acid, which exhibits antioxidant activity alongside its antibacterial effects[15]. Although vitamin E was found only in trace amounts in the extract, it remains important due to its well-established role in enhancing the body's defenses against infections and strengthening the immune system[16]. Additionally, oleic acid (OA), a common monounsaturated fatty acid, participates in intracellular signaling pathways and serves as a fundamental structural component of biological membranes[17]. The extract also contains a notable amount of phenolic compounds. These findings are consistent with studies demonstrating that phenolic compounds can influence bacterial growth and metabolism[18]. Furthermore, research indicates that S. aureus is more susceptible to palmitic acid than to stearic acid[19], suggesting a potential antibacterial role for the palmitic acid present in our extract. Collectively, these results support the presence of bioactive substances with antibacterial and antioxidant properties in the seed extract of L. leucocephala.

It has been demonstrated that the active principles in *L. leucocephala* seed pod biomass include various compounds with diverse biological activities, such as dioxolane and pyridine (antioxidant, nematicide), palmitic acid (nematicidal, antioxidant, lubricant), pelargonic acid (anti-inflammatory, antimicrobial), and myristic acid (antitumor, cancer preventive)[20].

The present findings demonstrate the concentration-dependent antioxidant activity of the L.leucocephala plant extract, with a progressive increase in the inhibition percentage as the extract concentration rose. This correlation aligns with the well-established properties of plant-based antioxidants[1+21], which often comprise a mixture of secondary metabolites, including alkaloids, flavonoids, and phenols. The high inhibition percentage observed at the maximum concentration (85.1%) supports the notion that L. leucocephala seeds are a rich source of naturally occurring antioxidant compounds. Plant-based products are gaining importance in modern medicine due to their easier availability, lower cost, and perceived safety compared to synthetic alternatives. Furthermore, the antioxidant activity observed in this study is consistent with previous research identifying flavonoids with antioxidant properties in L. leucocephala. Notably, these results align closely with studies investigating the anti-cancer and anti-metastasis effects of phytochemical constituents extracted from L.leucocephala. The strong antioxidant activity present in these components supports the current findings and suggests a potential role for this property in other biological mechanisms explored[1]. An enormous library of biochemical substances that have not yet been thoroughly investigated is provided by the great diversity of plant species [21]. Overall, these results suggest that L. leucocephala is a promising source of valuable bioactive compounds, including antioxidants. This supports previous research on the plant and paves the way for further studies to explore its diverse applications. The antioxidant activity observed in the alcoholic extract of Leucaena seeds aligns with trends reported in studies of various L. leucocephala extracts. Specifically, those studies demonstrated that antioxidant activity generally increases with concentration, consistent with the marked increase in antioxidant activity seen in the alcoholic extract as concentration rose[3].In our study The spectral analysis indicates that the alcoholic extract of Leucaena seeds contains a complex mixture of organic compounds. The characteristic absorption for the O–H bond suggests the presence of alcohols, phenols, or

carboxylic acids. Absorptions corresponding to aliphatic C–H bonds point to saturated hydrocarbon components. A strong absorption band for the carbonyl (C=O) bond reflects the presence of esters or carboxylic acids. The absorption observed at 1637 cm^{-1} may indicate unsaturated or nitrogenous functional groups or possibly the presence of water. Multiple absorptions in the fingerprint region further demonstrate the chemical complexity of the extract. These findings align with previous research on L. leucocephala that investigated antioxidant compounds in various plant parts. Both studies confirm the presence of bioactive compounds with antioxidant activity, underscoring the plant's potential as a natural source of such compounds. Furthermore, the FTIR analysis in the current study is consistent with earlier findings identifying characteristic functional groups such as O–H C–H, and C=O, which are commonly associated with antioxidant molecules like flavonoids and phenolic acids—significant components highlighted in L.leucocephala by both investigations[22].

In the present work the antibacterial activity test revealed that the crude extract of L.leucocephala seeds exhibits a clear inhibitory effect on the growth of S. aureus, as evidenced by a distinct zone of inhibition surrounding the area where the crude extract was applied. In contrast, the individual compounds tested separately—palmitic acid, oleic acid, linoleic acid, and stearic acid—did not demonstrate significant antibacterial activity against the same S.aureus at the concentrations used in this assay. These preliminary results suggest that the crude extract of L. leucocephala seeds may contain compounds with antibacterial efficacy against S.aureus and that this activity could be attributed to a synergistic effect among multiple constituents within the extract, rather than solely the fatty acids and vitamin E identified in previous HPLC analysis. Regarding the synergistic effect of the crude extract align with the concept (Huang)which states that plant synergy is amplified when the interactive effect of the plant's complex chemical matrix—characterized by the plurality and diversity of chemical compounds—produces effects greater than the additive effects of individual compounds, thereby modulating biochemical processes[18,19].

Pharmacodynamic synergy arises when multiple biological pathways are targeted, potentially involving substrates, enzymes, metabolites, ion channels, ribosomes, and signaling cascades[23]. It is widely accepted that combining multiple antimicrobial agents can produce varied effects depending on the chemicals' concentrations and compositions. In particular, synergy occurs when two antimicrobial substances work together to produce antibacterial activity greater than the sum of their individual activities[24].

CONCLUSION

Based on our finding, the ethanolic extract of L. leucocephala seeds demonstrates promising biological activities. The extract exhibits selective cytotoxicity by significantly reducing the viability of A431 skin cancer cells at higher concentrations, while showing no significant toxicity toward normal HEK 293 cells across the tested range. Phytochemical analysis via HPLC identified linoleic acid, oleic acid, and palmitic acid as major constituents, alongside octadecanoic acid and vitamin E. The crude extract displayed notable antioxidant activity that increased with concentration, and FTIR analysis indicated the presence of various functional groups consistent with these compounds. Furthermore, the crude extract exhibited significant antibacterial activity against *Staphylococcus aureus*, an effect not replicated by the individual identified fatty acids, suggesting synergistic interactions among the extract's components. These results collectively highlight the potential of *Leucaena leucocephala* seed extract as a source of bioactive compounds with selective anticancer, antioxidant, and antibacterial properties.

Declarations

Ethical Approval

This study was conducted in accordance with institutional and international ethical guidelines. Ethical approval was granted by the Ethical Committee of the College of Science, University of Babylon, Iraq under approval number M240906, dated 2/9/2024. No human or animal subjects were directly involved in the study, and all laboratory procedures adhered to ethical standards for biosafety and research conduct.

Journal Publication Statement

The authors affirm that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. All authors have reviewed and approved the final version of the manuscript and consent to its submission to the journal. This study was ethically approved under approval number M240906.

Conflict of Interest

The authors declare that there are no known conflicts of interest related to this work.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

Eman Muslim Ganem: Conceptualization, experimental design, cytotoxicity and antioxidant assays, writing – original draft.

Wejdan Ridha Al-Awani: FTIR and GC analysis, methodology, data curation, writing – review & editing.

Basheer Hamza Al-Alwani: Antibacterial testing, statistical analysis, visualization, manuscript finalization.

REFERENCES

1. She LC, Liu CM, Chen CT, Li HT, Li WJ, Chen CY. The anti-cancer and anti-metastasis effects of phytochemical constituents from Leucaena leucocephala. Biomed Res. 2017;28(7):2893-2897.
2. Abu Zarin M, Wan HY, Isha A, Armania N. Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from Leucaena leucocephala hybrid-Rendang. Food Science and Human Wellness. 2016;5(2):65-75.
3. Jayanthy V, Geetha R, Rajendran R, Prabhavathi P, Karthik Sundaram S, Dinesh Kumar S, Santhanam P. Phytoremediation of dye contaminated soil by Leucaena leucocephala (subabul) seed and growth assessment of Vigna radiata in the remediated soil. Saudi Journal of Biological Sciences. 2014;21(4):324-333.
4. Agarwal G, Carcache PJB, Addo EM, Kinghorn AD. Current status and contemporary approaches to the discovery of antitumor agents from higher plants. Biotechnol. Adv. 2020;38:107337.
5. Elbanoby NE, El-Settawy AA, Mohamed AA, Salem MZ. Phytochemicals derived from Leucaena leucocephala (Lam.) de Wit (Fabaceae) biomass and their antimicrobial and antioxidant activities: HPLC analysis of extracts. Biomass Conversion and Biorefinery. 2024;14(13):14593-14609.
6. Bassey RA, Ndarake EII, Ngele BA, Iniobong EA. GC-MS Analysis and Phytochemical Constituents in Anthocleista vogelii and Leucaena leucocephala Obtained From Calabar, Cross River State, Southern Nigeria. Researchers Journal of Science and Technology. 2023;3(2):68-81.
7. Gamal-Eldeen AM, Amer H, Helmy W, Ragab H, Talaat RM. Antiproliferative and cancer-chemopreventive properties of a sulfated polysaccharide extract of Gracilaria corticata. J Basic Appl Sci. 2007;3(1):1-14.
8. Hiramatsu K, Katayama Y, Matsuo M, et al. Multi-drug-resistant Staphylococcus aureus and future chemotherapy. Journal of Infection and Chemotherapy. 2014;20(10):593-601.
9. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, England: Chapman and Hall; 1973.
10. Aderogba MA, McGaw LJ, Bezabih BT, Abegaz BM. Antioxidant activity and cytotoxicity study of Leucaena leucocephala (Lam.) de wit leaf extract constituents. Nigerian Journal of Natural Products and Medicine. 2010;13(1):65-68.
11. Zhang H, Wang Z, Liu O. Development and validation of a GC–FID method for quantitative analysis of oleic acid and related fatty acids. Journal of Pharmaceutical Analysis. 2015;5(4):223-230.
12. Mohammed RS, El Souda SS, Taie HA, Moharam ME, Shaker KH. Antioxidant, antimicrobial activities of flavonoids glycoside from Leucaena leucocephala leaves. Journal of Applied Pharmaceutical Science. 2015;5(6):138-147.
13. Jain PK, Soni A, Jain P, Bhawsar J. Phytochemical analysis of Mentha spicata plant extract using UV-VIS, FTIR and GC/MS technique. J Chem Pharm Res. 2016;8(2):1-6.
14. Egorove NS. Antibiotics Scientific Approach. Mirpublishers; 1985.
15. Septina E, Yetti RD, Rivai H. Overview of traditional use, phytochemical, and pharmacological activities of chinese petai (leucaena leucocephala). Int. J. Pharm. Sci. Med. 2020;5(12):1-10.
16. Desbois AP, Smith VJ. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl Microbiol Biotechnol. 2010;85(6):1629-1642.
17. Karimi E, Jaafar HZ, Ghasemzadeh A, Ebrahimi M. Fatty acid composition, antioxidant and antibacterial properties of the microwave aqueous extract of three varieties of Labisia pumila Benth. Biol Res. 2015;48(1):9.
18. Huang CM, Chen CH, Pornpattananangkul D, et al. Eradication of drug resistant Staphylococcus aureus by liposomal oleic acids. Biomaterials. 2011;32(1):214-221.
19. Giulitti F, Petrungaro S, Mandatori S, et al. Anti-tumor effect of oleic acid in hepatocellular carcinoma cell lines via autophagy reduction. Frontiers in Cell and Developmental Biology. 2021;9:629182.
20. Micozzi MS. Fundamentals of Complementary and Alternative Medicine. Saunders/Elsevier; 2011.
21. Vaou N, Stavropoulou E, Voidarou C, et al. Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. Antibiotics. 2022;11(8):1014.
22. Verma VK, Malik S. DETERMINATION OF TOTAL PHENOLIC, FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF LEUCAENA LEUCOCEPHALA EXTRACT. stress. 2020;5:6.

23. Wagner H, Ulrich-Merzenich G. Synergy Research: Approaching a New Generation of Phytopharmaceuticals. Phytomedicine. 2009;16:97-110.
24. Djouahri A, Saka B, Boudarene L, et al. In Vitro Synergistic/Antagonistic Antibacterial and Anti-Inflammatory Effect of Various Extracts/Essential Oil from Cones of Tetraclinis Articulata (Vahl) Masters with Antibiotic and Anti-Inflammatory Agents. Ind. Crops Prod. 2014;56:60-66.
25. Roy M, Chakraborty S, Siddiqi M, Bhattacharya RK. Induction of apoptosis in tumor cells by natural phenolic compounds. Asian Pac J Cancer Prev. 2002;3(1):61-67.
26. Wójciak-Kosior M, Paduch R, Matysik-Woźniak A, Niedziela P, Donica H. The effect of ursolic and oleanolic acids on human skin fibroblast cells. Folia Histochem Cytobiol. 2011;49(4):664-669.
27. Khan AA, Alanazi AM, Jabeen M, Chauhan A, Abdelhameed AS. Design, synthesis and in vitro anticancer evaluation of a stearic acid-based ester conjugate. Anticancer Res. 2013;33(6):2517-2524.