Investigation of the Anti-cancer, Antioxidant, and Anti-microbial Potential of Cold-Pressed *Ficus carica L. cv*. Yellow Lop Seed Oil

Adem YAVAŞ[1]

[1] *Aydın Adnan Menderes University, Çine Vocational School, Department of Food Processing, Food Quality Control and Analysis Programme, Aydın, TÜRKİYE*

Abstract: This study evaluates the bioactive properties of cold-pressed seed oil from the Turkish Yellow Lop fig (Ficus carica L. cv.), focusing on its anti-cancer, antioxidant, and antimicrobial potential for nutraceutical and pharmaceutical applications. The oil's phytochemical profile, including its fatty acid composition, tocopherol content, and total phenolic content, was characterized using chromatographic and spectrophotometric methods. Its biological activities were assessed via the DPPH radical scavenging assay, Minimum Inhibitory Concentration (MIC) determination against pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*) and MTT cytotoxicity assays on human breast (MCF-7) and prostate (PC-3) cancer cell lines. Analysis revealed the oil is a rich source of unsaturated fatty acids, dominated by linolenic acid (42.1%), linoleic acid (31.5%), and oleic acid (14.8%). It also contains high concentrations of γ-tocopherol (395 mg/100 g) and significant phenolic compounds (45.8 mg GAE/g). The oil demonstrated potent antioxidant activity (IC50=5.62 mg/mL) and broad-spectrum antimicrobial efficacy, with MIC values ranging from 0.5 to 2 mg/mL. Furthermore, it exhibited dose-dependent cytotoxic effects against both tested cancer cell lines, showing the highest selectivity towards MCF-7 breast cancer cells. These findings establish Yellow Lop fig seed oil as a valuable natural resource with significant therapeutic potential, making it a promising candidate for the development of novel functional foods, nutraceuticals, and innovative therapeutic agents.

Keywords: Anti-cancer, Antioxidant, Anti-microbial, Ficus carica, Fig seed oil

Soğuk Preslenmiş *Ficus carica L. cv*. Sarı Lop Çekirdek Yağının Anti-kanser, Antioksidan ve Anti-mikrobiyal Potansiyelinin Araştırılması

Öz *Bu çalışma, Türk Sarı Lop inciri (Ficus carica L. cv.)'nden elde edilen soğuk preslenmiş tohum yağının biyoaktif özelliklerini, nutrasötik ve farmasötik uygulamalar için antikanser, antioksidan ve anti-mikrobiyal potansiyeline odaklanarak değerlendirmektedir. Yağın yağ asidi bileşimi, tokoferol içeriği ve toplam fenolik içeriği dahil olmak üzere fitokimyasal profili, kromatografik ve spektrofotometrik yöntemler kullanılarak karakterize edildi. Biyolojik aktiviteleri, DPPH radikal temizleme testi, patojenik bakterilere (Staphylococcus aureus ve Escherichia coli) karşı Minimum İnhibisyon Konsantrasyonu (MIC) tayini ve insan meme (MCF-7) ve prostat (PC-3) kanseri hücre hatları üzerinde MTT sitotoksisite testleri ile değerlendirildi. Analizler, yağın linolenik asit (%42,1), linoleik asit (%31,5) ve oleik asit (%14,8) ağırlıklı olmak üzere doymamış yağ asitleri açısından zengin bir kaynak olduğunu ortaya koymuştur. Ayrıca yüksek konsantrasyonlarda γ-tokoferol (395 mg/100 g) ve önemli miktarda fenolik bileşikler (45,8 mg GAE/g) içermektedir. Yağ, güçlü antioksidan aktivite (IC50 = 5,62 mg/mL) ve 0,5 ila 2 mg/mL arasında değişen MIC değerleri ile geniş spektrumlu anti-mikrobiyal etkinlik göstermiştir. Ayrıca, test edilen her iki kanser hücre hattına karşı doza bağlı sitotoksik etkiler sergilemiş ve MCF-7 meme kanseri hücrelerine karşı en yüksek seçiciliği göstermiştir. Bu bulgular, Sarı Lop incir çekirdeği yağını önemli terapötik potansiyele sahip değerli bir doğal kaynak olarak konumlandırmakta ve onu yeni fonksiyonel gıdalar, nutrasötikler ve yenilikçi terapötik ajanların geliştirilmesi için umut vaat eden bir aday haline getirmektedir.*

Anahtar Kelimeler*: Keywords: Anti-kanser, Antioksidan, Anti-mikrobiyal, Ficus carica, İncir çekirdeği yağı*

INTRODUCTION

Natural products derived from medicinal plants have been the cornerstones of traditional medicine for centuries and continue to be a vital source for modern drug discovery (Chaachouay and Zidane 2024, Singh *et al.,* 2025). Indeed, a significant proportion of the fundamental pharmaceuticals in current use are of botanical origin. A case in point is paclitaxel, a drug employed in the treatment of cancer, which is isolated from the yew tree (*Taxus brevifolia*) (Devanesan 2023). Another example is morphine, a potent analgesic, which is isolated from the opium poppy (*Papaver somniferum*) (Labanca, Ovesna *et al.,* 2018). Similarly, quinine, an effective compound against malaria, is isolated from the cinchona tree (*Cinchona officinalis*) (Oran *et al.,* 2022). In this context, the fig (*Ficus carica L.*), one of the oldest cultivated plants, held an important place both culturally and medically, especially in the Mediterranean region (Crisosto *et al.,* 2011).

Various parts of the fig tree, including its fruit, leaves, and roots, have traditionally been used to treat a wide range of ailments such as gastrointestinal disorders, respiratory conditions, and inflammatory diseases (Badgujar *et al.,* 2014, Rasool, Aziz et al., 2023). While most research has focused on the fruit's pulp and leaves, the seeds within represent an

***Sorumlu Yazar:** adem.yavas@adu.edu.tr*
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underutilized resource with considerable potential (Jimenez *et al.,* 2021, Shiraishi *et al.,* 2023).

Fig seeds contain a valuable oil rich in essential nutrients and bioactive compounds (Kamiloglu and Akgun, 2023). Studies on various fig cultivars have revealed that fig seed oil is a significant source of polyunsaturated fatty acids, such as linolenic (omega-3), linoleic (omega-6), and oleic (omega-9) acids (Ergun and Bozkurt, 2020, Baygeldi *et al.,* 2021). These fatty acids are essential compounds that play a critical role in the body, such as protecting cardiovascular health, supporting brain function, and regulating inflammatory processes (Alagawany *et al.,* 2022). Additionally, this oil contains high concentrations of γ-tocopherol, a form of vitamin E with potent antioxidant properties (Güven *et al.,* 2019, Aksoz *et al.,* 2020). The role of gamma-tocopherol in the protection of cells from oxidative damage caused by free radicals is a subject of much research, with the potential to reduce the risk of chronic disease in humans (Es-Sai *et al.,* 2025). The presence of these compounds suggests that fig seed oil may offer a range of health benefits, including antioxidant, anti-inflammatory, and anti-cancer effects.

The Aydın region of Türkiye is world-renowned for its high-quality figs, with the Yellow Lop cultivar being particularly prized for its unique taste and aroma (Polat 2023). While this cultivar represents a significant portion of the world's dried fig production, its seeds are not commercially utilized. Given the specific environmental conditions and genetic makeup of the Yellow Lop fig, its seed oil may possess a unique phytochemical profile and, consequently, superior bioactive properties compared to other cultivars.

This study aims to comprehensively investigate the therapeutic potential of cold-pressed oil extracted from Yellow Lop fig seeds. The research focuses on evaluating the oil's anti-cancer, antioxidant, and antimicrobial effects. By exploring these properties, this study intends to provide scientific validation for the potential use of Yellow Lop fig seed oil as a functional food, nutraceutical supplement, and a source for the development of novel therapeutic agents. This research will contribute to the growing body of knowledge on the health benefits of fig products and promote the valorization of agricultural by-products from the Turkish fig industry.

MATERIALS AND METHODS

Plant Material and Oil Extraction

Dried Yellow Lop figs (*Ficus carica L.)* used in this study were sourced from certified organic farms in the Germencik district of Aydın province (coordinates: 37°57'46.1"N 27°35'25.4"E), Türkiye, during the 2024 harvest season. The figs were manually separated to remove the seeds from the pulp. The seeds were washed with distilled water to remove any surface sugar residues and subsequently dried in an oven at 40°C for 48 hours until a constant weight was achieved. Oil extraction was performed from the dried seeds using a laboratory-scale cold-press extractor (Koçmaksan Model KMS10, Izmir, Türkiye) under controlled conditions where the temperature did not exceed 50°C. The crude oil obtained was centrifuged at 4000 rpm for 15 minutes to remove solid particles. The final clear, golden-yellow oil was stored at 4°C under a nitrogen atmosphere in amber-colored glass bottles until analysis.

Fatty Acid Composition

The fatty acid profile was determined through a trans-esterification of the oil samples into volatile fatty acid methyl esters (FAMEs). For this purpose, 2M potassium hydroxide (Merck®, analytical grade) in methanol (Sigma-Aldrich®, HPLC grade) was employed. FAME analysis was performed on an Agilent 7890A gas chromatography system equipped with a DB-23 capillary column (60 m x 0,25 mm i.d., 0,25 µm), using helium as the carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed from an initial 130°C to a final 230°C at a ramp rate of 2°C/min. Fatty acids were identified by matching their retention times with those of a commercial FAME standard mix (Supelco 37 Component FAME Mix) (Lee *et al.,* 1998).

Tocopherol Content

Tocopherol isomers (α, β, γ, δ) were analyzed by High-Performance Liquid Chromatography (HPLC) using an Agilent 1260 Infinity system with a fluorescence detector (excitation: 295 nm, emission: 330 nm). Separation was achieved on a silica-based normal-phase column (250 mm x 4,6 mm, 5 µm) with a mobile phase of isopropanol in n-hexane (0.5:99.5, v/v) at a flow rate of 1,2 mL/min. Identification and quantification of tocopherol isomers were performed using external standards (Pyka and Sliwiok 2001).

Total Phenolic Content

The total phenolic content (TPC) of the oil was determined using the Folin-Ciocalteu spectrophotometric method (Pérez *et al.,* 2023). Before analysis, phenolic compounds were extracted by dissolving 2.5 g of oil in 5 mL of n-hexane, followed by liquid-liquid extraction with 10 mL of 60% aqueous methanol. An aliquot of the methanolic extract was mixed with Folin-Ciocalteu reagent and sodium carbonate solution. Following an incubation period in the dark at room temperature, the absorbance of the resulting blue complex was measured at 765 nm using a spectrophotometer. TPC was calculated from a calibration curve prepared using gallic acid as a standard, and the results were expressed as milligrams of gallic acid equivalent per gram of oil (mg GAE/g).

DPPH Radical Scavenging Activity

The free radical scavenging activity of the oil was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Ergun and Bozkurt 2020). For the analysis, the oil sample was first dissolved in chloroform to prepare a high-concentration stock solution (10 mg/mL). From this stock, serial dilutions were made using methanol to obtain different concentrations of the oil for testing. These samples were then reacted with a freshly prepared DPPH radical solution in methanol. The mixtures were incubated for 30 minutes at room temperature, protected from light. At the end of the incubation, the decrease in absorbance due to the reduction of the DPPH radical was measured at 517 nm using a spectrophotometer. The radical scavenging activity was calculated as the percentage of inhibition relative to the control group, and the IC_{50} value, representing the concentration that inhibits 50% of the radicals, was determined.

Antimicrobial Activity

The antimicrobial activity of the oil was evaluated against a panel consisting of Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Escherichia coli* ATCC 25922). The inhibitory effect on microorganisms was determined by establishing the Minimum Inhibitory Concentration (MIC) values using the broth microdilution method (Duman *et al.,* 2018). To ensure homogeneous dispersion of the oil in the broth, a stock emulsion was prepared using Tween 80 (0,5%, v/v). From this stock, serial dilutions were made in 96-well microplates containing Mueller-Hinton Broth (MHB). Each well was then inoculated with microbial suspensions standardized to the 0,5 McFarland standard to achieve a final concentration of approximately $5x10^5$ CFU/mL. The plates were incubated at 37°C for 24 hours. After the incubation period, the MIC value was recorded as the lowest oil concentration at which visible bacterial growth was completely inhibited.

Anti-cancer Activity

Human cancer cell lines (breast: MCF-7; prostate: PC-3) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cell lines were cultured under sterile conditions in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA), supplemented with 10% (v/v) fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, MA, USA) and 1% (v/v) penicillin-streptomycin (100 U/mL penicillin, 100 µg/mL streptomycin) (Sigma-Aldrich, St. Louis, MO, USA). Cells were passaged in a humidified atmosphere containing 5% CO_2 at 37°C (Alqurashi *et al.,* 2022). The cytotoxic effect of the oil on cancer cells was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay. Cells were seeded into 96-well microplates at a density of $5x10^3$ to $1x10^4$ cells/well and incubated for 24 hours to allow for adhesion. Following incubation, the cells were treated with various concentrations (10-1000 µg/mL) of fig seed oil, which was prepared by dissolving it in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA). Care was taken to ensure that the final DMSO concentration did not exceed 0,5% (v/v) to prevent cell toxicity. The treated cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 and 48 hours. At the end of the treatment period, the medium in each well was aspirated, and 0.5 mg/mL MTT solution (Sigma-Aldrich, St. Louis, MO, USA) was added. The cells were further incubated for 4 hours at 37°C to form formazan crystals. Subsequently, the MTT solution was removed, and the formed formazan crystals were dissolved in 100 µL of DMSO. The absorbance of the resulting-colored solution was measured at 570 nm using a microplate reader (Thermo Scientific, USA). The percentage of cell viability was calculated relative to the absorbance values of the untreated control group using the following formula:

Cell Viability (%) = (Absorbance of Treated Group / Absorbance of Control Group) x 100

The concentration of the oil that caused 50% inhibition of cell viability (IC_{50} value) was determined from the resulting dose-response curves using non-linear regression analysis.

Statistical Analysis

All experiments were performed in triplicate, and the data are presented as mean ± standard deviation (SD). Statistical analysis of the data was performed using a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Results with $P<0.05$ were considered statistically significant.

RESULTS

Fatty Acid Profile and Tocopherol/Phenolic Content

The yield of oil obtained from Yellow Lop fig seeds by cold pressing was determined to be 15,5% (w/w) on a dry weight basis. The phytochemical characterization of the obtained oil revealed a rich profile of bioactive compounds. The detailed fatty acid profile of the fig seed oil is presented in Table 1.

Table 1: Fatty acid composition of Yellow Lop fig seed oil

Fatty Acid	Abbreviation	Percentage (%)
Palmitic Acid	C16:0	7.2 ± 0.3
Stearic Acid	C18:0	4.4 ± 0.2
Oleic Acid	C18:1	14.8 ± 0.5
Linoleic Acid	C18:2	31.5 ± 1.1
α-Linolenic Acid	C18:3	42.1 ± 1.5

The oil was found to be composed of a high proportion of unsaturated fatty acids, at 88,4%, with polyunsaturated fatty acids (PUFAs) constituting a major part of this fraction. The dominant fatty acids were identified as α-linolenic acid (C18:3, omega-3; 42,1%), linoleic acid (C18:2, omega-6; 31,5%), and oleic acid (C18:1, omega-9; 14,8%), respectively. The total saturated fatty acid content was measured at 11,6%, with palmitic acid (C16:0) and stearic acid (C18:0) being the main components in this category (Figure 1).

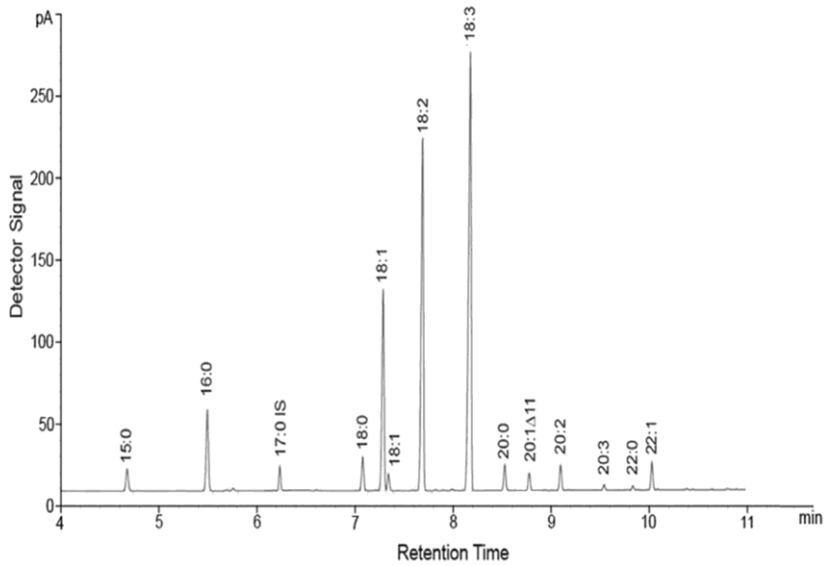

Figure 1. Gas Chromatography (GC) Chromatogram of Fatty Acid Methyl Esters (FAMEs) in Yellow Lop fig seed oil

The chromatogram illustrates the fatty acid profile of the oil sample. The y-axis represents the detector signal intensity in picoamperes (pA), and the x-axis shows the retention time in minutes. Each peak corresponds to a specific fatty acid, identified by the notation C:D, where 'C' is the number of carbon atoms and 'D' is the number of double bonds. The major peaks identified are: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and α-linolenic acid (18:3). The profile is dominated by unsaturated fatty acids, particularly α-linolenic acid (18:3), which is the most abundant fatty acid in the sample. HPLC analysis revealed that γ-tocopherol was the most dominant isomer with a concentration of 395 mg/100 g, accounting for more than 90% of the total tocopherol content (Figure 2). The total phenolic content of the oil was determined by the Folin-Ciocalteu method to be 45.8 ± 2.1 mg GAE/g

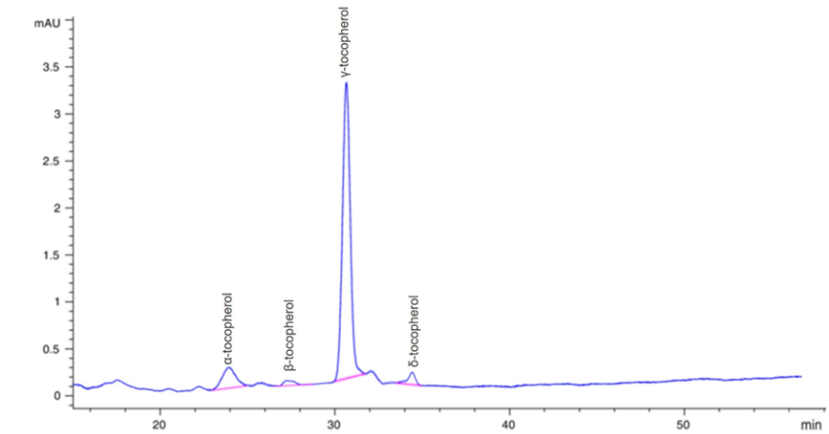

Figure 2. High-Performance Liquid Chromatography (HPLC) Chromatogram of Tocopherol Isomers in Yellow Lop fig seed oil.

Antioxidant Activity

The fig seed oil exhibited strong antioxidant activity. In the DPPH test, the oil showed a dose-dependent radical scavenging effect, and its IC50 value was calculated to be 5.62 ± 0.24 mg/mL.

Antimicrobial Activity

Antimicrobial analyses revealed that oil has a broad spectrum of activity (Table 2). The oil exhibited the most pronounced inhibitory effect against the Gram-positive bacterium S. aureus, displaying lower MIC values for this strain compared to those against Gram-negative bacteria.

Table 2: Anti-microbial activity of Yellow Lop fig seed oil

Microorganism	MIC (mg/mL)
Staphylococcus aureus	0.5
Escherichia coli	2.0

Anti-cancer Activity

The results of the MTT assay showed that the oil exhibited dose-dependent cytotoxic effects on all tested cancer cell lines after 48 hours of incubation (Figure 3). The highest sensitivity was observed in the MCF-7 breast cancer cell line, with an IC50 value of 250 ± 1.2 µg/mL. The PC-3 prostate cancer cell line showed an IC50 value of 300 ± 2.5 µg/mL.

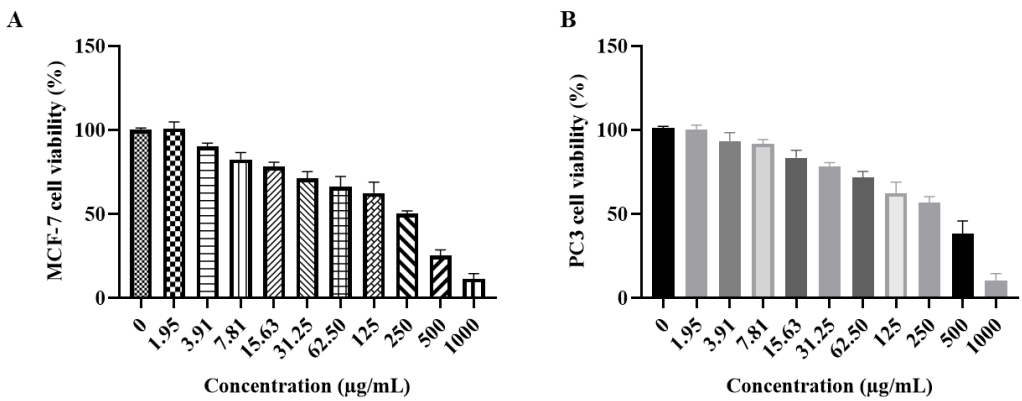

Figure 3. Cytotoxic Effects of Yellow Lop Fig Seed Oil on Cancer Cell Lines. The dose-dependent effects of the oil on the viability of (A) MCF-7 human breast cancer cells and (B) PC3 human prostate cancer cells after 48 hours of treatment, as determined by the MTT assay. The bar charts represent the percentage of cell viability relative to untreated control cells (set to 100%). The results demonstrate a clear and significant decrease in cell viability with increasing oil concentration for both cell lines. The values are expressed as mean ± standard deviation (SD) from three independent experiments.

DISCUSSION

This study presents a comprehensive investigation into the phytochemical profile and biological activities (antioxidant, antimicrobial, and anti-cancer) of oil obtained by cold pressing the seeds of *Ficus carica L. cv*. Yellow Lop, a cultivar of economic importance in Türkiye. The findings strongly support the potential of this material, traditionally considered an agricultural by-product, as a valuable source of bioactive compounds for nutraceutical and pharmaceutical applications.

The therapeutic potential of a natural oil is directly related to its chemical composition. Our analysis revealed that Yellow Lop fig seed oil has a unique profile that distinguishes it from other fig cultivars reported in the literature on several critical points.

While the oil's high content of unsaturated fatty acids (88.4%) is consistent with studies on other cultivars like 'Bursa Siyahı', the most distinctive finding of this study is the predominance of α-linolenic acid (ALA), an omega-3 fatty acid, at a high concentration of 42.1% (Ishnaiwer 2023, level, contributing meaningfully to the overall bioactivity of the oil. It is believed that the choice of the cold-press Kamiloglu and Akgun 2023, Uslu *et al.,* 2024). This represents a significant deviation from many reports that typically indicate linoleic acid (omega-6) as the dominant component (Hssaini *et al.,* 2020, Hssaini *et al.,* 2021). The observed high ALA content places Yellow Lop fig seed oil in a similar category to flaxseed oil, one of the richest plant-based sources of omega-3. It is thought that this unique profile is a result of the cultivar's genetic background and the specific terroir conditions of the Aydın region. A high omega-3/omega-6 ratio is highly desirable for human health, as it is associated with anti-inflammatory, cardioprotective, and neuroprotective benefits (Gutierres *et al.,* 2025).

Furthermore, the high concentration of γ-tocopherol (395 mg/100 g) in the oil is another significant finding. This result is consistent with literature reporting γ-tocopherol as the dominant form of vitamin E in fig seeds (Rajendran 2023). γ-Tocopherol is known to have superior antioxidant activity compared to α-tocopherol, particularly in its ability to detoxify nitrogen radicals (Es-Sai *et al.,* 2025). The measured total phenolic content (45.8 mg GAE/g) is also at a significant

extraction method, as opposed to solvent or heat-based methods, played a critical role in preserving these thermally sensitive compounds (Di Giacomo and Di Giacomo 2002).

The antioxidant activity demonstrated by the low IC_{50} value is a direct functional reflection of the oil's rich phytochemical profile. The primary driving forces behind this activity are high levels of γ-tocopherol and phenolic compounds. 283 molecules are powerful free radical scavengers that neutralize reactive oxygen species (ROS), protecting cellular macromolecules such as lipids, proteins, and DNA from oxidative damage. The synergistic interaction between different antioxidant molecules (tocopherols, phenolics, and unsaturated fatty acids) is thought to provide stronger protection than the isolated effect of a single compound (Chen *et al.,* 2022).

Our findings indicate that the oil exhibits remarkable antimicrobial properties against a range of pathogens. The higher susceptibility of Gram-positive bacteria compared to Gram-negative bacteria can be explained by the barrier formed by their lipopolysaccharide (LPS)-containing outer membrane against the penetration of hydrophobic compounds (Vaara 2020). The antimicrobial mechanism of the oil is thought to be based on a multifactorial interaction, including the disruption of the bacterial cell membrane integrity by fatty acids and the inhibition of essential enzymatic activities by phenolic compounds. These findings support the potential use of the oil as a natural preservative in the food and cosmetic industries or as a topical agent for treating skin infections.

Another important finding of the study is the selective cytotoxicity of the oil on cancer cell lines. The fact that the oil causes dose-dependent cell death in the tested cancer lines indicates therapeutic potential. This selective activity is thought to be largely due to the oil's high α-linolenic acid (ALA) content. Omega-3 fatty acids have been extensively reported to exert anti-cancer effects through multiple mechanisms, such as inducing apoptosis, modulating inflammation, and inhibiting angiogenesis (Wendel and Heller 2009, Ma *et al.,* 2021). The pronounced effect on breast cancer (MCF-7) cells, in particular, parallels studies with ALA-rich flaxseed oil that reported similar pro-apoptotic results (Truan *et al.,* 2010, Hu *et al.,* 2019). It is likely that the synergistic interaction of ALA with other bioactive molecules, such as γ-tocopherol and phenolic compounds, further enhances this potent observed activity.

Although this study provides important findings on fig seed oil, the findings were obtained under in vitro conditions and need to be examined in the complex biological system of a living organism. Therefore, the next logical step is to conduct in vivo studies in appropriate animal models to validate the oil's efficacy and safety. Furthermore, elucidating the molecular mechanisms underlying the observed anti-cancer effects, such as apoptosis, cell cycle, and inflammatory signaling pathways, should be a primary goal for future research.

CONCLUSION

This study provides significant evidence that cold-pressed oil derived from Yellow Lop fig seeds is not merely a byproduct but a high-value functional oil. Its unique chemical profile, characterized by a high content of α-linolenic acid and γ-tocopherol, translates into potent antioxidant, broad-spectrum antimicrobial, and most importantly, selective anti-cancer activities under in vitro conditions. These findings place Yellow Lop fig seed oil in a special position among other vegetable oils and strongly support its development both as a nutraceutical for disease prevention and as a potential source for new therapeutic strategies against diseases such as cancer.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are not publicly available. However, the data can be requested from the corresponding author upon reasonable request, where necessary.

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