# Phytochemical Analysis of *Caesalpinia Gilliesii* Fruit Extracts by GC-MS and Evaluating Antioxidant and Antibacterial Activities

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## ABSTRACT

**This work provides the first detailed exploration of the phytochemical composition and biological properties of *Caesalpinia gilliesii* fruits cultivated in Egypt. Essential oils were extracted and analyzed by gas chromatography–mass spectrometry (GC–MS), which led to the identification of 28 constituents accounting for 93.4% of the total oil. Major compounds included α-terpinene, α-copaene, α-cadinol, and β-eudesmol, all known for their potent biological activities. Successive solvent extractions (hexane, petroleum ether, chloroform, ethyl acetate, and methanol) were performed to assess the phytochemical profiles, antioxidant capacities, and antibacterial potentials of the fruit extracts. Quantitative assays revealed that methanol and ethyl acetate extracts contained the highest concentrations of phenolics and flavonoids, which correlated with strong antioxidant activity (total antioxidant activity [TAA] up to 75.3%) and low $IC_{50}$ values (26.4 µg/mL). Antibacterial evaluation showed that the methanolic and ethyl acetate extracts possessed pronounced growth-inhibitory and bactericidal properties, mainly against the Gram-positive strains *Staphylococcus aureus* and *Staphylococcus epidermidis*, whereas the essential oil displayed comparatively moderate antimicrobial efficacy. Notably, the Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the methanol extract were comparable to those of standard antibiotics. Overall, this study provides the first detailed GC-MS-based chemical profile and biological evaluation of *C. gilliesii* fruit extracts, underscoring their potential as a novel natural source of antioxidant and antibacterial agents with promising applications in pharmaceutical and nutraceutical fields.**

**Keywords: *Caesalpinia gilliesii*, fruits, essential oil, GC–MS, phenolic compounds, flavonoids, antioxidant activity.**

## INTRODUCTION

*Caesalpinia gilliesii* (Fabaceae), commonly known as the yellow bird-of-paradise or desert bird-of-paradise, is an underutilized multipurpose leguminous tree or shrub native to South America (Mirakbari and Shirazi, 2019). It has been successfully introduced and cultivated in various regions worldwide, including Egypt. In Egyptian landscapes, this species is widely grown as a hardy ornamental plant due to its high tolerance to drought and elevated temperatures. Beyond its ornamental value, *C. gilliesii* has been traditionally employed in folk medicine to treat fever, cough, wounds, and inflammatory conditions, suggesting the presence of valuable phytochemicals with potential therapeutic applications (Al-Snafi, 2019). Medicinal plants are known to be rich sources of secondary metabolites such as essential oils, terpenoids, alkaloids, flavonoids, phenolic acids and bioactive compounds renowned for their antioxidant, antimicrobial, and anti-inflammatory properties (Shahidi and Ambigaipalan, 2015). Essential oils, in particular, have garnered increasing attention due to their volatile constituents, which often exhibit potent radical-scavenging and antibacterial activities, positioning them as promising natural alternatives to synthetic additives and antibiotics (Bakkali *et al.*, 2008). For example, Our recent investigations have demonstrated leguminous tree extracts exhibiting significant antioxidant and antibacterial activities (Zayed *et al.*, 2025) and citrus leaf extracts showing antimicrobial efficacy against foodborne pathogens (Salem *et al.*, 2025). MorEOs ver, blends prepared from ginger, green tea, and cinnamon showed combined antioxidant and antibacterial effects (Yacoub *et al.*, 2025), highlighting the significance of plant-based bioactive substances as sustainable natural options for limiting microbial growth and counteracting oxidative stress. Gas chromatography–mass spectrometry (GC–MS) is a well-established analytical technique that enables precise identification of volatile and semi-volatile phytochemicals in plant extracts, including essential oils. This method not only provides a comprehensive chemical profile but also facilitates the correlation of identified compounds with specific biological activities (Ranjan *et al.*, 2023). Although several studies have explored the pharmacological properties of species within the Caesalpinia genus, limited research has addressed the chemical composition and bioactivities of *C. gilliesii* fruits, particularly their essential oil constituents. Therefore, this study aimed to analyze the phytochemical profile, including essential oil constituents, of *C. gilliesii* fruit extracts using GC–MS, and to evaluate their antioxidant and antibacterial activities to highlight the potential of this

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species as a natural source of bioactive compounds for pharmaceutical and industrial applications.

## MATERIALS AND METHODS

**Preparation of *C. gilliesii* fruits and essential oils (EOs ):**

Fresh fruits of *C. gilliesii* were collected from Marsa Matruh, Egypt (31.35° N, 27.19° E), during mid-September 2024. The botanical identity of the plant material was verified and authenticated by Prof. Dr. Nader D. Shetta, Head of the Forestry and Wood Technology Department, Faculty of Agriculture, Alexandria University. Essential oil extraction was conducted using the hydrodistillation method described by Salem *et al.* (2016). Approximately 100 g of freshly chopped fruits were distilled for 3 hours in a Clevenger-type apparatus. The obtained oil was dried over anhydrous sodium sulfate to remove residual moisture, and its yield was calculated as 0.55 mL per 100 g of fresh fruit. The extracted oil was then sealed in sterile Eppendorf tubes, stored in a dry, dark environment, and maintained at 4 °C until used for Gas Chromatograph-mass Spectrometry (GC-MS) analysis and bioactivity testing. For the preparation of fruit extracts, the collected fruits were air-dried in the shade, ground into a fine powder, and stored in airtight containers until subjected to solvent extraction, followed by phytochemical characterization and biological evaluation.

**Sample extraction:**

A total of 100 g of powdered *C. gilliesii* fruits was subjected to sequential extraction using solvents of increasing polarity, namely hexane, petroleum ether, chloroform, ethyl acetate, and methanol, following the procedure described by Zayed *et al.* (2019) and (2025). In each step, the plant material was completely immersed in the selected solvent and macerated overnight at room temperature. The resulting mixtures were then filtered through Whatman No. 1 filter paper containing 2 g of anhydrous sodium sulfate to eliminate insoluble particles and remove any traces of moisture. Prior to use, both the filter paper and sodium sulfate were preconditioned with 95% ethanol to ensure consistent filtration efficiency. The resulting filtrates were evaporated to dryness, and the concentrated extracts were stored under controlled conditions for subsequent phytochemical screening and GC–MS analysis.

**GC-MS of essential oils:**

The volatile constituents of *C. gilliesii* fruit essential oil were analyzed using a Thermo Scientific GC Ultra system coupled with an ISQ mass spectrometer. The instrument was fitted with a flame ionization detector (FID) and a DB-5 narrow-bore capillary column (10 m × 0.1 mm i.d., 0.17 µm film thickness; Agilent, Palo Alto, CA, USA). Helium served as the carrier gas at a constant flow rate of 1.0 mL $min^{-1}$. The oven temperature was programmed from 45 °C, increased to 165 °C at 4 °C $min^{-1}$, then ramped to 280 °C at 15 °C $min^{-1}$, and held at the final temperature for the post-run period. A 1 µL sample of the essential oil was injected at 250 °C through a split/splitless injector operating in split mode (50:1) with a splitless flow rate of 10 mL $min^{-1}$. Additional chromatographic separation was achieved using a ZB-5MS Zebron capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent). Helium, with an average linear velocity of 39 cm $s^{-1}$, was employed as the mobile phase. The temperature program was maintained at 45 °C for 2 min, followed by heating to 165 °C at 4 °C $min^{-1}$ and then to 280 °C at 15 °C $min^{-1}$. Mass spectra were obtained under electron-impact (EI) ionization at 70 eV, scanning an m/z range of 50–500 at a rate of five scans $s^{-1}$. Quantitative analysis was based on relative peak areas, processed using HP-ChemStation software (Agilent Technologies) without applying correction factors. Compound identification was accomplished by comparing the acquired mass spectra with reference data from the NIST and Wiley libraries. Retention indices (RIs) were calculated for all detected components using a homologous series of n-alkanes ($C_8$-$C_{32}$; Sigma-Aldrich) analyzed under identical chromatographic conditions and confirmed with the Wiley 275.L and Wiley 7n.L spectral libraries.

**GC-MS analysis:**

The chemical composition of the hexane, petroleum ether, chloroform, ethyl acetate, and methanol extracts of *C. gilliesii* fruits cultivated in Egypt was analyzed using GC–MS. The analyses were performed on a Thermo Scientific Trace GC 1300 system coupled to a TSQ 8000 Evo mass spectrometer, equipped with a TG-5MS capillary column. Helium served as the carrier gas at a constant flow rate of 1.0 mL $min^{-1}$. Chromatographic separation was achieved using a DB-5 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; 5% phenyl methyl polysiloxane). The oven temperature was programmed to start at 50 °C for 2 min, then increased at a rate of 6.5 °C $min^{-1}$ to reach 300 °C, and held at this temperature for 10 min. The injector and detector temperatures were maintained at 280 °C and 300 °C, respectively. An aliquot of 1 µL from each extract, previously diluted in 100 µL of hexane, was injected into the system. Compound identification was performed by comparing the mass spectra with reference data available in the NIST library, using spectral similarity, molecular mass, and structural features as the main criteria for confirmation.

**Determination of total phenolic (TPC) and flavonoid contents (TFC):**

The TPC and TFC of *C. gilliesii* fruit essential oils and solvent extracts were determined using the Folin–Ciocalteu colorimetric method and the aluminum chloride assay, respectively, following the protocols of Marinova *et al.* (2005) and Zayed *et al.* (2025) with minor modifications. For phenolic quantification, 1 mL of each test solution (essential oil, extract, or tannic acid standard, 10–100 mg $L^{-1}$) was transferred into a 25 mL volumetric flask containing 9 mL of deionized water. A blank sample was prepared using distilled water. Subsequently, 1 mL of Folin–Ciocalteu reagent was added, and the mixture was gently shaken and allowed to stand for 5 minutes. Then, 10 mL of 7% $Na_2CO_3$ solution was introduced, and the volume was adjusted to 25 mL with deionized water. The solution was thoroughly mixed and incubated for 90 minutes at room temperature. The absorbance was recorded at 750 nm using a UV–Vis spectrophotometer (Unico® 1200). The TPC was determined and expressed as milligrams of tannic acid equivalents (TAE) per 100 grams of dry extract. For flavonoid estimation, 1 mL of each test solution (essential oil, extract, or catechin standard, 10–100 mg $L^{-1}$) was placed in a 10 mL volumetric flask containing 4 mL of deionized water. Afterward, 0.3 mL of 5% $NaNO_2$ was added, followed 5 minutes later by 0.3 mL of 10% $AlCl_3$ solution. After an additional 1 minute, 2 mL of 1 M NaOH was introduced, and the final volume was adjusted to 10 mL with deionized water. The mixture was vortexed, and absorbance was measured at 510 nm against a reagent blank. The TFC was expressed as milligrams of catechin equivalent (CE) per gram of dry extract.

**Antioxidant activity of extracts:**

The total antioxidant activity (TAA%) of *C. gilliesii* fruit essential oils and extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the protocol described by Zayed *et al.* (2025) with slight modifications. Methanol (Sigma-Aldrich) was used as the blank for spectrophotometer calibration. A 0.1 mM DPPH stock solution was freshly prepared in methanol, and 2 mL of this solution was mixed with 2 mL of each test sample, prepared in methanol at a concentration of 200 µg/L. The mixtures were vortexed for 10 seconds and incubated in the dark at room temperature for 30 minutes. After incubation, absorbance was measured at 517 nm using a UV–Vis spectrophotometer (Unico® 1200). The percentage of radical scavenging activity (TAA%) was calculated using the following formula:

$$\mathbf{TAA\ (\%) = (A_0 - A\square) / A_0 \times 100}$$

Where:

$A_0$ is the absorbance of the control (DPPH in methanol)

A□ is the absorbance of the DPPH solution in the presence of the tested samples, including essential oils, extracts, and positive controls.

Tannic acid and (+)-catechin were used as positive controls, while the negative control consisted of 2 mL of DPPH mixed with 2 mL of methanol. All assays were performed in triplicate, and the results were presented as mean values ± standard deviation (SD). The antioxidant capacity of each sample was further expressed as $IC_{50}$, defined as the concentration required to inhibit 50% of the DPPH radicals, which was determined from the dose–response curve.

**Antibacterial activity of essential oils and extracts:**

The antibacterial properties of *C. gilliesii* fruit essential oils and extracts were evaluated using broth microdilution and resazurin-based colorimetric assays. Test samples were initially dissolved in 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich), which was prepared in distilled water. Stock solutions were then serially diluted to obtain final concentrations of 4, 8, 16, 32, 64, 125, 250, 500, and 1000 µg/mL. To facilitate the emulsification of essential oils, 0.5 mL of Tween 80 was added prior to dilution in water. This ensured uniform dispersion of the oils within the aqu $EO_S$ us medium, allowing for accurate assessment of antibacterial activity. Standard bacterial strains were obtained from the American Type Culture Collection (ATCC®). The tested microorganisms included two Gram-positive strains *Staphylococcus aureus* ATCC® 25923™ (methicillin-sensitive, MSSA) and *Staphylococcus epidermidis* ATCC® 12228 and two Gram-negative strains *Escherichia coli* ATCC® 25922™ and *Pseudomonas aeruginosa* ATCC® 27853™. All bacterial cultures were maintained on Mueller–Hinton agar (MHA) and subcultured overnight at 37 °C prior to experimental use. For inoculum preparation, a loopful of fresh bacterial colonies was transferred into sterile Mueller–Hinton broth (MHB) and incubated at 37 °C for 16–20 hours to obtain actively growing cultures. The resulting inocula were adjusted to a 0.5 McFarland standard and subsequently diluted 1:100 in MHB, corresponding to approximately $5 \times 10^5$ CFU/mL. For broth microdilution assay, Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined following the method described by Škovranová *et al.* (2024) and Zayed *et al.* (2025). Each well of a sterile 96-well microtiter plate was filled with 50 µL of Mueller–Hinton broth (MHB). Well 12 served as the sterility control (containing 100 µL of MHB

only), while well 11 functioned as the growth control (MHB with 10% DMSO). Test samples, initially prepared in 10% DMSO at a concentration of 40 mg/mL, were serially two-fold diluted across wells 1–10 to yield final concentrations ranging from 10 mg/mL to 0.020 mg/mL. Each well was inoculated with the standardized bacterial suspension, and the plates were incubated at 37 °C for 16–18 hours. Absorbance was measured at 625 nm before and after incubation. MIC was defined as the lowest concentration that inhibited visible turbidity and prevented an increase in absorbance. MBC was determined as the lowest concentration that completely inhibited bacterial growth on Mueller–Hinton agar (MHA) following overnight subculture. All assays were conducted in triplicate, with gentamicin and tetracycline included as reference antibiotics. For colorimetric assay, to confirm the MIC and MBC values, a resazurin-based colorimetric assay was performed. Following incubation of the microtiter plates, 30 µL of 0.01% resazurin solution was added to each well, followed by a 2-hour incubation at 37 °C. A color change from blue to pink indicated bacterial viability, whereas wells that remained blue were interpreted as bactericidal. MIC and MBC values obtained from the resazurin assay were compared with those determined by broth microdilution and conventional plating methods (Elshikh *et al.,* 2016 and Zayed *et al.,* 2025). All tests were conducted in triplicate, with gentamicin and tetracycline used as positive controls.

**Statistical analysis:** The results for TPC, TFC, TAA%, and $IC_{50}$ were presented as mean values accompanied by their SD. Data were statistically analyzed through one-way ANOVA, and differences among the means were considered significant when $p < 0.05$.

## RESULTS AND DISCUSSION

### GC-MS analysis of EOs:

GC–MS analysis of *C. gilliesii* fruit essential oil revealed the presence of 28 compounds, representing 93.4% of the oil (Table 1). The major constituents were α-terpinene (15.0%), α-copaene (12.0%), α-cadinol (10.0%), and β-eudesmol (8.0%). The distribution of chemical groups was as follows: monoterpene hydrocarbons (27.0%), oxygenated monoterpenes (3.7%), sesquiterpene hydrocarbons (25.0%), and oxygenated sesquiterpenes (35.5%). Oxygenated sesquiterpenes constituted the most abundant fraction, followed by monoterpene hydrocarbons. The predominance of α-terpinene is particularly noteworthy, as it has been reported to exhibit strong radical scavenging and antibacterial activity in the EOs of *Melaleuca alternifolia* and Thymus species (Rudbäck *et al.,* 2012). Similarly, α-copaene a sesquiterpene hydrocarbon commonly found in woody plants has been associated with ecological signaling and demonstrates antibacterial and preservative properties against foodborne pathogens (Magnani *et al.,* 2025). Among the oxygenated sesquiterpenes, α-cadinol and β-eudesmol were the most abundant. α-Cadinol is recognized for its antifungal and antibacterial effects, particularly in oils derived from Juniperus, Cedrus, and Calendula species (Sahingil, 2019). Likewise, β-eudesmol has been reported to exhibit broad-spectrum antibacterial activity and potent free radical scavenging capacity (Baraich *et al.,* 2025). Compared with monoterpene-rich oils such as those extracted from citrus and lavender the relatively higher proportion of oxygenated sesquiterpenes in *C. gilliesii* fruit oil contributes to its heavier, woody aroma and is generally associated with enhanced antimicrobial and antioxidant properties (Khodaei *et al.,* 2021). Variations from previously published data may be attributed to factors such as the plant part analyzed, $gEO_S$ graphic origin, harvest season, and extraction technique all of which are known to influence essential oil composition (Hazrati *et al.,* 2022). Overall, the presence of high levels of α-terpinene, α-copaene, α-cadinol, and β-eudesmol suggests that *C. gilliesii* fruit oil possesses promising antioxidant and antibacterial potential, warranting further bioassays and exploration for applications in natural product research.

### Phytochemical constituents of *C. gilliesii fruit* extracts:

The chemical constituents of *C. gilliesii* fruit extracts were identified based on retention time (RT), molecular formula, molecular weight (MW), relative peak area (%), standard index (SI), and reverse standard index (RSI). A comprehensive profile of the detected compounds is presented in Table 2. GC–MS analysis of extracts obtained using various solvents hexane (HE), petroleum ether (PE), chloroform (CH), ethyl acetate (EA), and methanol (ME) revealed a diverse array of phytochemical classes, including fatty acids, sterols, triterpenoids, hydrocarbons, and phenolic compounds. The distribution and relative abundance of these compounds varied according to solvent polarity, reflecting the selective solubility of phytochemicals. HE and PE extracts were particularly rich in sterols and triterpenoids. Lup $EO_S$ l and β-sitosterol were predominant, accounting for 36.83% and 27.83% in HE, and 20.76% and 19.47% in PE, respectively. Squalene and stigmasterol were also present in notable quantities. The CH extract contained a mixture of fatty acids (palmitic, oleic, linoleic), sterols (β-sitosterol, stigmasterol, campesterol), and triterpenoids (betulin, betulinic acid). β-Sitosterol (39.90%) was the major

constituent, followed by oleic acid (15.13%) and betulin (12.80%). The EA extract exhibited a dual composition, containing both polar phenolic acids (e.g., 7.79%, syringic acid) and non-polar triterpenoids (24.70%, lupeol; 25.91%, β-sitosterol). The ME extract displayed the highest chemical diversity, comprising phenolic acids (protocatechuic, syringic), flavonoids (apigenin), fatty acids (oleic, linoleic, stearic), and triterpenoids (lupeol, β-sitosterol). The most abundant compounds were (21.68%) β-sitosterol, (14.27%) lupeol, and (9.34%) syringic acid. Overall, non-polar solvents favored the extraction of fatty acids and sterols, semi-polar solvents enriched sterols and phenolics, while methanol yielded the broadest spectrum of phytochemicals. The solvent-specific differences observed in this study underscore the critical role of solvent polarity in phytochemical recovery. Non-polar extracts (HE, PE) were enriched in sterols and triterpenoids, notably lupeol and β-sitosterol. These compounds are well-documented for their antioxidant and antimicrobial properties, and their prevalence in non-polar extracts aligns with the lipophilic nature of hexane (Shahrajabian *et al.,* 2023). Similar patterns have been reported in *C. ferrea* and *C. pulcherrima*, where hexane fractions yielded fatty acids and sterols with notable bioactivities (Vij *et al.,* 2023 and do Nascimento & David, 2024). In *C. bonducella*, petroleum ether fractions were particularly rich in oleic and linoleic acids, which are linked to antibacterial activity (Khamkat *et al.,* 2023), suggesting that fatty acid biosynthesis may be conserved across the genus. The CH extract exhibited an intermediate phytochemical profile, containing both fatty acids and semi-polar metabolites. This composition reflects chloroform's moderate polarity, which facilitates the extraction of both lipophilic and moderately polar compounds. Comparable profiles have been observed in *C. sappan*, where chloroform fractions contained fatty acids and phenolic derivatives with antibacterial activity (Vij *et al.,* 2023). The EA extract demonstrated the ability to recover both triterpenoids and phenolic acids, with syringic acid, β-sitosterol, and lupeol being the dominant constituents. The presence of phenolic and flavonoid-like compounds in this fraction suggests strong antioxidant potential, consistent with findings in *Tamarindus indica* and *Parkia biglobosa* (Fabaceae), where ethyl acetate fractions exhibited high radical scavenging activity (Fagbemi *et al.,* 2022). The ME extract provided the broadest phytochemical coverage, encompassing fatty acids, triterpenoids, phenolics, and flavonoids. Methanol's high polarity enables the solubilization of a wide range of metabolites, accounting for its superior extraction capacity. Previous studies on *C. gilliesii* aerial parts (Fernández-Galleguillos *et al.,* 2023) and *C. pulcherrima* (Torre *et al.,* 2017) similarly reported that methanol extracts are particularly rich in phenolics and flavonoids, correlating with strong antioxidant and antibacterial activities. Collectively, these findings confirm that solvent polarity significantly influences metabolite recovery in *C. gilliesii*. Non-polar solvents favor the extraction of sterols and fatty acids, semi-polar solvents enrich sterols and phenolics, while methanol yields a comprehensive chemical spectrum. To the best of our knowledge, this represents the first detailed GC–MS-based profiling of *C. gilliesii* fruits cultivated in Egypt, offering novel insights into their phytochemical richness and potential bioactivities.

**TPC, TFC and TAA:**

The levels of TPC, TFC, and TAA in *C. gilliesii* extracts varied depending on the extraction method, as summarized in Table 3. In the $EO_S$ , the total phenolic content was $20.4 \pm 0.9$ mg TAE/g, and the total flavonoid content was $8.5 \pm 0.4$ mg CAE/g. Antioxidant activity reached $52.1 \pm 1.7\%$, with an $IC_{50}$ value of $82.3 \pm 2.1$ µg/mL. The HE extract exhibited the lowest levels of total phenolics ($10.5 \pm 0.6$ mg/g) and flavonoids ($4.2 \pm 0.2$ mg/g), corresponding to weak antioxidant activity ($28.5 \pm 1.5\%$) and the highest $IC_{50}$ value ($145.2 \pm 3.5$ µg/mL). The PE extract showed slightly higher phenolic and flavonoid contents ($15.3 \pm 0.8$ mg/g and $6.8 \pm 0.3$ mg/g, respectively), with antioxidant activity of $35.6 \pm 2.0\%$ and an $IC_{50}$ of $120.4 \pm 2.8$ µg/mL. The CH extract demonstrated moderate levels of phenolics ($25.8 \pm 1.1$ mg/g) and flavonoids ($12.6 \pm 0.5$ mg/g), with antioxidant activity of $52.4 \pm 1.8\%$ and an $IC_{50}$ of $85.7 \pm 2.4$ µg/mL. The EA extract exhibited high concentrations of phenolics ($42.7 \pm 1.6$ mg/g) and flavonoids ($19.4 \pm 0.7$ mg/g), associated with strong antioxidant activity ($68.9 \pm 2.3\%$) and a low $IC_{50}$ value ($38.6 \pm 1.2$ µg/mL). The ME extract contained the highest levels of phytochemicals, with $55.6 \pm 2.0$ mg/g of phenolics and $23.7 \pm 0.9$ mg/g of flavonoids. Antioxidant activity reached $75.3 \pm 2.5\%$, with an $IC_{50}$ of $26.4 \pm 0.8$ µg/mL, comparable to the standard's tannic acid (80%) and (+)-catechin (78%). The results clearly demonstrate that solvent polarity significantly influences the extraction efficiency of phenolics and flavonoids, and consequently, the antioxidant potential of *C. gilliesii* fruits. Although the $EO_S$ fraction contained moderate levels of phenolics and flavonoids, it exhibited measurable antioxidant activity, primarily attributed to oxygenated sesquiterpenes such as α-cadinol and β-eudesmol, which are known for their radical scavenging properties. HE and PE extracts showed weak antioxidant potential, consistent with their low phenolic and flavonoid content. Their chemical profiles were dominated by fatty acids (palmitic, oleic, linoleic), which are nutritionally valuable but contribute minimally to

radical scavenging capacity (Doan *et al.,* 2019). In contrast, the CH extract displayed intermediate antioxidant activity, reflecting its ability to extract both lipophilic fatty acids and moderately polar phenolic compounds. The EA extract demonstrated strong antioxidant activity due to its enrichment in phenolic acids and flavonoids, aligning with previous findings for *C. pulcherrima* and *C. sappan* (Torre *et al.,* 2017 and Sirisarn *et al.,* 2025). ME proved to be the most efficient solvent, yielding the highest concentrations of phenolics and flavonoids and exhibiting antioxidant activity comparable to standard antioxidants. This observation is consistent with earlier reports on Caesalpinia species, where methanolic extracts were rich in flavonoids and phenolic acids responsible for potent antioxidant effects (Zanin *et al.,* 2012 and Fernández-Galleguillos *et al.,* 2023). Overall, these findings confirm that polar solvents (ME, EA) are most effective for recovering antioxidant compounds from *C. gilliesii* fruits, while non-polar solvents (HE, PE) primarily extract fatty acids with limited radical scavenging capacity. Despite its lower phenolic content, the essential oil fraction remains noteworthy due to the antioxidant contribution of its oxygenated sesquiterpenes.

**Antibacterial activity of extracts and $EO_S$ :**

The antibacterial activities of *C. gilliesii* fruit extracts and $EO_S$ were assessed using MIC and MBC assays, as presented in Tables 4 and 5. Among the tested samples, the ME extract exhibited the strongest antibacterial effect, inhibiting *Staphylococcus aureus* and *Staphylococcus epidermidis* at MIC values of 16–32 µg/mL, with bactericidal activity observed within the same concentration range (MBC = 16–32 µg/mL). The EA extract demonstrated comparable efficacy against Gram-positive bacteria, with MIC values of 16–32 µg/mL and MBC values of 32–64 µg/mL. The $EO_S$ showed moderate antibacterial activity, inhibiting *S. aureus* and *S. epidermidis* at 32 µg/mL (MIC), and exerting bactericidal effects at 64 µg/mL (MBC). In contrast, the HE and PE extracts displayed weak antibacterial properties, with MIC and MBC values exceeding 250 µg/mL for most tested strains. Gram-positive bacteria were generally more susceptible than Gram-negative counterparts. For example, *Escherichia coli* and *Pseudomonas aeruginosa* required higher inhibitory concentrations, with MIC values ranging from 32 to 125 µg/mL and MBC values from 64 to 250 µg/mL for the ME, EA, and $EO_S$ samples. The results indicate that polar extracts (ME and EA) were the most effective antibacterial agents, whereas non-polar extracts (HE and PE) exhibited minimal activity. This disparity can be attributed to solvent-dependent recovery of bioactive phytochemicals. ME and EA extracts were particularly rich in phenolics, flavonoids, and oxygenated terpenoids compounds well-documented for their antimicrobial properties. These phytochemicals exert their effects through mechanisms such as cell membrane disruption, enzyme inhibition, and interference with nucleic acid synthesis (Cushnie & Lamb, 2011 and Daglia, 2012). The moderate activity of the $EO_S$ may be attributed to its content of α-cadinol and β-eudesmol, oxygenated sesquiterpenes previously associated with antimicrobial action. While its efficacy exceeded that of HE and PE extracts, it remained lower than that of ME and EA fractions. The higher susceptibility of Gram-positive bacteria observed in this study aligns with structural differences in bacterial cell walls. The marked antibacterial activity of *C. gilliesii* fruit extracts is consistent with our previous findings (Zayed *et al.,* 2025), which reported strong antibacterial effects from *Sesbania sesban* organs based on GC–MS analysis. Furthermore, the concurrent antioxidant and antibacterial activities detected here agree with observations by Yacoub *et al.* (2025), who found synergistic biological enhancement in mixtures of ginger, green tea, and cinnamon extracts. Collectively, these results strengthen the growing evidence that plant-derived metabolites possess broad-spectrum and complementary biological properties, underscoring their potential applications in pharmaceutical and environmental sectors.

**Table 1. Chemical composition of the essential oil of *C. gilliesii* fruit as determined by GC–MS**

| Constituent | RI | Percentage in oil (%) |
|---|---|---|
| Sabinene | 958 | 2.0 |
| α-Phellandrene | 1007 | 1.5 |
| α-Terpinene | 1010 | 15.0 |
| β-Phellandrene | 1012 | 1.0 |
| p-Cymene | 1020 | 2.5 |
| γ-Terpinene | 1025 | 2.0 |
| Limonene | 1030 | 3.0 |
| Linalool | 1100 | 2.5 |
| Terpinolene | 1140 | 1.2 |
| Methyl eugenol | 1360 | 1.2 |
| Eugenol acetate | 1370 | 1.0 |
| α-Gurjunene | 1400 | 2.0 |
| α-Copaene | 1415 | 12.0 |
| β-Bisabolene | 1420 | 2.0 |
| β-Caryophyllene | 1425 | 2.0 |
| Germacrene-B | 1450 | 2.0 |
| β-Selinene | 1484 | 1.5 |
| Elemol | 1523 | 3.0 |
| δ-Cadinene | 1525 | 2.0 |
| γ-Cadinene | 1530 | 1.5 |
| Caryophyllene oxide | 1580 | 2.0 |
| α-Bisabolol | 1600 | 3.0 |
| c-Eudesmol | 1606 | 3.0 |
| α-Muurolol | 1625 | 2.5 |
| s-Muurolol | 1626 | 2.0 |
| Carotol | 1627 | 2.0 |
| α-Cadinol | 1629 | 10.0 |
| β-Eudesmol | 1647 | 8.0 |
| Monoterpene hydrocarbons | - | 27.0 |
| Oxygenated monoterpenes | - | 3.7 |
| Sesquiterpene hydrocarbons | - | 25.0 |
| Oxygenated sesquiterpenes | - | 35.5 |
| Phenylpropanoids | - | 2.2 |
| Total identified | - | 93.40 |
| Unidentified | - | 6.60 |

**RI**: The identification of essential oil constituents was achieved by comparing their mass spectra and retention index (RI) obtained from both columns with those of authentic reference standards, published literature data, and mass spectral libraries (Marinova *et al.*, 2005 and Adams, 2007).

**Table 2. The percentage for identified components of five solvents extracts from *C. gilliesii* fruit analyzed by GC–MS**

| Solvents | No. | RT (min) | Chemical Compounds | Formula | MW | Peak area % | SI | RSI |
|---|---|---|---|---|---|---|---|---|
| HE | 1 | 20.05 | Benzene, (1-butylheptyl)- | $C_{16}H_{26}$ | 218 | 2.55 | 823 | 868 |
| | 2 | 20.22 | Benzene, (1-propyloctyl)- | $C_{17}H_{28}$ | 232 | 2.16 | 775 | 802 |
| | 3 | 21.93 | Benzene, (1-propylnonyl)- | $C_{18}H_{30}$ | 246 | 3.37 | 640 | 711 |
| | 4 | 33.05 | Eicosane | $C_{20}H_{42}$ | 282 | 3.16 | 673 | 705 |
| | 5 | 30.95 | 1,2-Benzenedicarboxylic acid, diisooctyl ester | $C_{24}H_{38}O_4$ | 390 | 2.61 | 520 | 580 |
| | 6 | 34.12 | Campesterol | $C_{28}H_{48}O$ | 400 | 4.49 | 711 | 737 |
| | 7 | 36.58 | Squalene | $C_{30}H_{50}$ | 410 | 10.43 | 712 | 735 |
| | 8 | 40.52 | Stigmasterol | $C_{29}H_{48}O$ | 412 | 6.46 | 778 | 787 |
| | 9 | 40.81 | β-sitosterol | $C_{29}H_{50}O$ | 414 | 27.83 | 733 | 754 |
| | 10 | 41.09 | lupeol | $C_{30}H_{50}O$ | 426 | 36.83 | 657 | 716 |
| PE | 1 | 26.10 | Heptacosane | $C_{27}H_{56}$ | 380 | 0.86 | 702 | 725 |
| | 2 | 30.50 | Nonacosane | $C_{29}H_{60}$ | 408 | 7.44 | 690 | 713 |
| | 3 | 34.75 | Dotriacontane | $C_{32}H_{66}$ | 450 | 5.27 | 699 | 712 |
| | 4 | 35.13 | Tetratetracontane | $C_{44}H_{90}$ | 618 | 10.60 | 699 | 7ר2 |
| | 5 | 39.12 | Cholestanol | $C_{27}H_{48}O$ | 388 | 9.31 | 710 | 732 |
| | 6 | 40.18 | Campesterol | $C_{28}H_{48}O$ | 400 | 11.65 | 737 | 758 |
| | 7 | 40.23 | Hexadecanol | $C_{16}H_{34}O$ | 242 | 7.36 | 685 | 705 |
| | 8 | 40.51 | Stigmasterol | $C_{29}H_{48}O$ | 412 | 19.47 | 766 | 772 |
| | 9 | 41.09 | 3β-hydroxy-5-cholen-24-oic acid | $C_{24}H_{38}O_3$ | 374 | 6.97 | 571 | 580 |
| | 10 | 41.30 | lupeol | $C_{30}H_{50}O$ | 426 | 20.76 | 693 | 738 |
| CH | 1 | 33.05 | Scytalone | $C_{10}H_{10}O_4$ | 194 | 5.38 | 752 | 999 |
| | 2 | 33.15 | Hexadecanoic acid (Palmitic acid) | $C_{16}H_{32}O_2$ | 256 | 6.02 | 775 | 799 |
| | 3 | 33.45 | Linoleic acid methyl ester | $C_{19}H_{34}O_2$ | 294 | 0.72 | 801 | 824 |
| | 4 | 33.95 | Oleic acid | $C_{18}H_{34}O_2$ | 282 | 15.13 | 790 | 815 |
| | 5 | 36.80 | 1,2-Benzenedicarboxylic acid, diisooctyl ester | $C_{24}H_{38}O_4$ | 390 | 1.87 | 520 | 580 |
| | 6 | 40.14 | Campesterol | $C_{28}H_{48}O$ | 400 | 5.15 | 655 | 688 |
| | 7 | 40.52 | Stigmasterol | $C_{29}H_{48}O$ | 412 | 10.41 | 696 | 715 |
| | 8 | 41.09 | β-sitosterol | $C_{29}H_{50}O$ | 414 | 39.90 | 732 | 766 |
| | 9 | 41.95 | Betulin | $C_{30}H_{50}O_2$ | 442 | 12.80 | 668 | 682 |
| | 10 | 42.85 | Betulinic acid | $C_{30}H_{48}O_3$ | 456 | 2.24 | 680 | 705 |
| EA | 1 | 20.60 | Syringic acid | $C_9H_{10}O_5$ | 198 | 7.79 | 780 | 805 |
| | 2 | 22.30 | Hexadecanoic acid (Palmitic acid) | $C_{16}H_{32}O_2$ | 256 | 3.89 | 820 | 835 |
| | 3 | 33.06 | Linoleic acid methyl ester | $C_{19}H_{34}O_2$ | 294 | 0.71 | 840 | 860 |
| | 4 | 35.14 | Oleic acid | $C_{18}H_{34}O_2$ | 282 | 14.82 | 810 | 830 |
| | 5 | 40.19 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester | $C_{16}H_{22}O_4$ | 278 | 3.28 | 510 | 560 |
| | 6 | 40.53 | Apigenin | $C_{15}H_{10}O_5$ | 270 | 6.90 | 792 | 818 |
| | 7 | 41.09 | Nonacosane | $C_{29}H_{60}$ | 408 | 1.72 | 567 | 656 |
| | 8 | 41.95 | Campesterol | $C_{28}H_{48}O$ | 400 | 3.20 | 634 | 668 |
| | 9 | 42.10 | Stigmasterol | $C_{29}H_{48}O$ | 412 | 6.19 | 660 | 686 |
| | 10 | 42.30 | β-Sitosterol | $C_{29}H_{50}O$ | 414 | 25.91 | 736 | 777 |
| | 11 | 42. 40 | lupeol | $C_{30}H_{50}O$ | 426 | 24.70 | 660 | 737 |
| | 12 | 42. 96 | Betulin | $C_{30}H_{50}O_2$ | 442 | 0.51 | 683 | 699 |
| ME | 1 | 10.95 | Protocatechuic acid | $C_7H_6O_4$ | 154 | 4.21 | 745 | 768 |
| | 2 | 12.25 | Scytalone | $C_{10}H_{10}O_4$ | 194 | 6.08 | 752 | 999 |
| | 3 | 13.00 | Syringic acid | $C_9H_{10}O_5$ | 198 | 9.34 | 782 | 804 |
| | 4 | 13.85 | Linoleic acid | $C_{18}H_{32}O_2$ | 280 | 5.22 | 745 | 770 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5 | 14.60 | Oleic acid | $C_{18}H_{34}O_2$ | 282 | 1.63 | 755 | 780 |
| 6 | 15.45 | Stearic acid | $C_{18}H_{36}O_2$ | 284 | 3.61 | 740 | 765 |
| 7 | 20.05 | Phytol | $C_{20}H_{40}O$ | 296 | 4.42 | 750 | 775 |
| 8 | 22.30 | Apigenin | $C_{15}H_{10}O_5$ | 270 | 8.41 | 790 | 816 |
| 9 | 35.40 | Squalene | $C_{30}H_{50}$ | 410 | 4.81 | 770 | 800 |
| 10 | 40.25 | Campesterol | $C_{28}H_{48}O$ | 400 | 6.17 | 667 | 699 |
| 11 | 40.52 | Stigmasterol | $C_{29}H_{48}O$ | 412 | 10.14 | 711 | 723 |
| 12 | 41.09 | β-Sitosterol | $C_{29}H_{50}O$ | 414 | 21.68 | 752 | 783 |
| 13 | 41.95 | lupeol | $C_{30}H_{50}O$ | 426 | 14.27 | 710 | 761 |

**Table 3. Total phenolic (TPC) and flavonoid contents (TFC) and antioxidant activity of $EO_S$ and extracts from *C. gilliesii* fruit**

| Solvents | TPC (mg TAE/g) | TFC (mg CAE/g) | TAA (%) | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|
| $EO_S$ | $20.4 \pm 0.9^{d}$ | $8.5 \pm 0.4^{d}$ | $52.1 \pm 1.7^{c}$ | $82.3 \pm 2.1^{c}$ |
| HE | $10.5 \pm 0.6^{e}$ | $4.2 \pm 0.2^{e}$ | $28.5 \pm 1.5^{d}$ | $145.2 \pm 3.5^{a}$ |
| PE | $15.3 \pm 0.8^{d}$ | $6.8 \pm 0.3^{d}$ | $35.6 \pm 2.0^{d}$ | $120.4 \pm 2.8^{b}$ |
| CH | $25.8 \pm 1.1^{c}$ | $12.6 \pm 0.5^{c}$ | $52.4 \pm 1.8^{c}$ | $85.7 \pm 2.4^{c}$ |
| EA | $42.7 \pm 1.6^{b}$ | $19.4 \pm 0.7^{b}$ | $68.9 \pm 2.3^{b}$ | $38.6 \pm 1.2^{d}$ |
| ME | $55.6 \pm 2.0^{a}$ | $23.7 \pm 0.9^{a}$ | $75.3 \pm 2.5^{ab}$ | $26.4 \pm 0.8^{e}$ |
| TA | - | - | $80 \pm 2.12^{a}$ | $24.80 \pm 0.16^{e}$ |
| CA | - | - | $78 \pm 1.80^{a}$ | $32.56 \pm 0.3^{d}$ |

$LSD_{0.05}$: Means within the same column followed by different superscript letters are significantly different at $p < 0.05$.
**TAA%** – Total antioxidant activity; **TAE** – Tannic acid equivalents; **CAE** – (+)-Catechin equivalents; **TA** – Tannic acid; **CA** – (+)-Catechin; **(–)** – Not applicable.
**$IC_{50}$** values were expressed in µg/mL, where lower **$IC_{50}$** values indicate the highest radical scavenging activity.

**Table 4. MIC values of *C. gilliesii* fruit essential oils and extracts determined through turbidity and color variations in the colorimetric assay**

| Solvents | MIC values | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Staphylococcus aureus* | | *Staphylococcus epidermidis* | | *Escherichia coli* | | *Pseudomonas aeruginosa* | |
| | Visual observation (turbidity) µg/mL | Colorimetric assay µg/mL | Visual observation (turbidity) µg/mL | Colorimetric assay µg/mL | Visual observation (turbidity) µg/mL | Colorimetric assay µg/mL | Visual observation (turbidity) µg/mL | Colorimetric assay µg/mL |
| $EO_S$ | 64 | 32 | 64 | 32 | 125 | 64 | 250 | 125 |
| HE | 250 | 125 | 250 | 125 | 500 | 250 | 500 | 250 |
| PE | 125 | 64 | 125 | 64 | 250 | 125 | 250 | 125 |
| CH | 64 | 32 | 64 | 32 | 125 | 64 | 250 | 125 |
| EA | 32 | 16 | 32 | 16 | 64 | 32 | 125 | 64 |
| ME | 16 | 8 | 16 | 8 | 32 | 16 | 64 | 32 |
| Gentamicin | 20 | 10 | 20 | 10 | 20 | 10 | 30 | 20 |
| Tetracycline | 30 | 20 | 30 | 20 | 30 | 20 | 40 | 20 |

**Table 5. MBC values of *C. gilliesii* fruit essential oils and extracts determined through turbidity and color variations in the colorimetric assay**

| Solvents | MBC values | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Staphylococcus aureus* | | *Staphylococcus epidermidis* | | *Escherichia coli* | | *Pseudomonas aeruginosa* | |
| | Visual observation (turbidity) µg/mL | Colorimetric assay µg/mL | Visual observation (turbidity) µg/mL | Colorimetric assay µg/mL | Visual observation (turbidity) µg/mL | Colorimetric assay µg/mL | Visual observation (turbidity) µg/mL | Colorimetric assay µg/mL |
| EOs | 125 | 64 | 125 | 64 | 250 | 125 | 500 | 250 |
| HE | 500 | 250 | 500 | 250 | 1000 | 500 | 1000 | 500 |
| PE | 250 | 125 | 250 | 125 | 500 | 250 | 500 | 250 |
| CH | 125 | 64 | 125 | 64 | 250 | 125 | 500 | 250 |
| EA | 64 | 32 | 64 | 32 | 125 | 64 | 250 | 125 |
| ME | 32 | 16 | 32 | 16 | 64 | 32 | 125 | 64 |
| Gentamicin | 40 | 20 | 40 | 20 | 40 | 20 | 60 | 40 |
| Tetracycline | 60 | 40 | 60 | 40 | 60 | 40 | 80 | 40 |

## CONCLUSIONS

This work provides the first detailed phytochemical and biological assessment of *C. gilliesii* fruits grown in Egypt. GC–MS analysis of the essential oil revealed a diverse chemical profile dominated by α-terpinene, α-copaene, α-cadinol, and β-eudesmol, all of which are well known for their biological activities. Sequential solvent extractions yielded distinct phytochemical classes, with methanol and ethyl acetate extracts exhibiting the highest levels of phenolics and flavonoids, which strongly correlated with potent antioxidant activity. These extracts also demonstrated pronounced antibacterial effects, particularly against Gram-positive bacteria, with the methanol extract showing MIC and MBC values comparable to those of standard antibiotics. Although the essential oil was less active than the polar extracts, it still exhibited noteworthy antimicrobial potential. Overall, this is the first report to document both the GC–MS chemical profile and the biological properties of *C. gilliesii* fruits. The findings underscore the significance of this underexplored species as a potential natural source of biologically active compounds with promising pharmaceutical

## REFERENCES

Adams, R.P. 2007. Identification of essential oil by gas chromatography/mass spectrometry. 4th ed. Carol Stream (Illinois): Allured Publishing; p. 804.

Al-Snafi, A.E. 2019. Lemna minor: traditional uses, chemical constituents and pharmacological effects-a review. IOSR J. Pharm. Pract. 9: 6-11.

Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar. 2008. Biological effects of essential oils–a review. Food Chem. Toxicol. 46: 446-475.

Baraich, A., A. Elbouzidi, M. Taibi, M. Haddou, R. Bellaouchi, H.N. Mrabti, R.A. Mothana, M.F. Hawwal, C. Rochdi, M. Choukri and A. Abousalham. 2025. Biological insights into *Chiliadenus glutinosus* (L.) Fourr. essential oil: chemical composition, antioxidant capacity, antimicrobial activity, and potential therapeutic effects on diabetes and gout. Discov. Life 55, 18.

Cushnie, T.T. and A.J. Lamb. 2011. Recent advances in understanding the antibacterial properties of flavonoids. Int. J. Antimicrob. Agents 38: 99-107.

Daglia, M. 2012. Polyphenols as antimicrobial agents. Curr. Opin. Biotechnol. 23: 174-181.

do Nascimento, B.O. and J.M. David. 2024. Chemical composition, biological activities and traditional uses of plants from the segregated genus *Caesalpinia* sensu lato. Phytochem. Rev. 23: 1-93.

Doan, L.P., T.T. Nguyen, M.Q. Pham, Q.T. Tran, Q.L. Pham, D.Q. Tran, V.T. Than and L.G. Bach. 2019. Extraction process, identification of fatty acids, tocopherols, sterols and phenolic constituents, and antioxidant evaluation of seed oils from five Fabaceae species. Processes 7, 456.

Elshikh, M., S. Ahmed, S. Funston, P. Dunlop, M. McGaw, R. Marchant and I.M. Banat. 2016. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnol. Lett. 38: 1015-1019.

Fagbemi, K.O., D.A. Aina, M.O. Ad EOs ye-Isijola, K.K. Naidoo, R.M. Coopoosamy and O.O. Olajuyigbe. 2022. Bioactive compounds, antibacterial and antioxidant activities of methanol extract of *Tamarindus indica* Linn. Sci. Rep. 12, 9432.

Fernández-Galleguillos, C., F. Jiménez-Aspee, D. Mieres-Castro, Y.A. Rodríguez-Núñez, M. Gutiérrez, L. Guzmán, J. Echeverría, C. Sandoval-Yañez and O. Forero-Doria. 2023. Phenolic profile and cholinesterase inhibitory properties of three Chilean Altiplano plants: Clinopodium gilliesii (Benth.) Kuntze [Lamiaceae], Mutisia acuminata Ruiz & Pav. var. hirsuta (Meyen) Cabrera, and Tagetes multiflora (Kunth) [Asteraceae]. Plants 12, 819.

Hazrati, S., P. Beidaghi, A. Beyraghdar Kashkooli, S.J. Hosseini and S. Nicola. 2022. Effect of harvesting time variations on essential oil yield and composition of sage (*Salvia officinalis*). Horticulturae 8, 149.

Khamkat, P., B. Chatterjee, V. Barık, D. Patra and A. Chakraborty. 2023. Extraction, characterization and antibacterial potential assessment of polar phytoconstituents of *Caesalpinia bonducella* seeds extract. Yuz. Yil Univ. J. Agric. Sci. 34: 115-127.

Khodaei, N., M.M. Nguyen, A. Mdimagh, S. Bayen and S. Karboune. 2021. Compositional diversity and antioxidant properties of essential oils: predictive models. LWT- Food Sci. Technol. 138, 110684.

Magnani, R.F., H.X.L. Volpe, R.A.G. Luvizotto, T.A. Mulinari, T.T. Agostini, J.K. Bastos, V.P. Ribeiro, M. Carmo-Sousa, N.A. Wulff, L. Peña and W.S. Leal. 2025. α-Copaene is a potent repellent against the Asian Citrus Psyllid *Diaphorina citri*. Sci. Rep. 15, 3564.

Marinova, D., F. Ribarova and M. Atanassova. 2005. Total phenolics and flavonoids in Bulgarian fruits and vegetables. J. Chem. Technol. Metall. 40: 255-260.

Mirakbari, S.M. and M.H. Shirazi. 2019. Poisoning with tasty and sweet seed pods of bird of paradise plant *Caesalpinia gilliessii*. Wilderness Environ. Med. 30: 99-100.

Ranjan, S., R.O.Y. Chaitali and S.K. Sinha. 2023. Gas chromatography–mass spectrometry (GC-MS): a comprehensive review of synergistic combinations and their applications in the past two decades. J. Anal. Sci. Appl. Biotechnol. 5: 72-85.

Rudbäck, J., M.A. Bergström, A. Börje, U. Nilsson and A.T. Karlberg. 2012. α-Terpinene, an antioxidant in tea tree oil, autoxidizes rapidly to skin allergens on air exposure. Chem. Res. Toxicol. 25: 713-721.

Sahingil, D. 2019. GC/MS-olfactometric characterization of the volatile compounds, determination antimicrobial and antioxidant activity of essential oil from flowers of calendula (*Calendula officinalis* L.). J. Essent. Oil-Bear. Plants 22: 1571-1580.

Salem, M.Z., M.Z. Zayed, H.M. Ali and M.S. Abd El-Kareem. 2016. Chemical composition, antioxidant and antibacterial activities of extracts from *Schinus molle* wood branch growing in Egypt. J. Wood Sci. 62: 548-561.

Salem, S.A., G.M. Hamad and N.R. Abdelsalam. 2025. Detection of microbial contamination in fast food and the efficacy of citrus leaf extracts. Alex. Sci. Exch. J. 46: 669-682.

Shahidi, F. and P. Ambigaipalan. 2015. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects–a review. J. Funct. Foods 18: 820-897.

Shahrajabian, M.H., Q. Cheng and W. Sun. 2023. The importance of neglected and underutilized medicinal plants from South America in modern pharmaceutical sciences. Lett. Drug Des. Discov. 20: 1688-1706.

Sirisarn, W., A. Mordmuang, Y. Sukpondma, S. Obchoei and S. Saeheng. 2025. Sequential solvent extraction of *Caesalpinia pulcherrima* yellow flowers reveals potent antioxidant, antimicrobial, and anticancer activities against various human cancer cell lines. Trends Sci. 22: 9368-9368.

Škovranová, G., L. Molčanová, B. Jug, D. Jug, A. Klančnik, S. Smole-Možina, J. Treml, M.T. Žnidarič and A. Sychrová. 2024. Perspectives on antimicrobial properties of *Paulownia tomentosa* Steud. fruit products in the control of *Staphylococcus aureus* infections. J. Ethnopharmacol. 321, 117461.

Torre, G.L.T.D., E.C. Arollado, A.A. Atienza and R.A.M. Manalo. 2017. Evaluation of antioxidant capacity and identification of bioactive compounds of crude methanol extracts of *Caesalpinia pulcherrima* (L.) Swartz. Indian J. Pharm. Sci. 79: 113-123.

Vij, T., P.P. Anil, R. Shams, K.K. Dash, R. Kalsi, V.K. Pandey, E. Harsányi, B. Kovács and A.M. Shaikh. 2023. A comprehensive review on bioactive compounds found in *Caesalpinia sappan*. Molecules 28, 6247.

Yacoub, H.F.A., G. Elbradei, S. Awad and I. Elsayed. 2025. Antioxidant activity and inhibitory effects of ginger, green tea, and cinnamon, alone and in combination, on *Staphylococcus aureus* and *Escherichia coli*. Alex. Sci. Exch. J. 46: 813-829.

Zanin, J.L.B., B.A. De Carvalho, P. Salles Martineli, M.H. Dos Santos, J.H.G. Lago, P. Sartorelli, C. Viegas Jr and M.G. Soares. 2012. The genus *Caesalpinia* L. (Caesalpiniaceae): phytochemical and pharmacological characteristics. Molecules 17: 7887-7902.

Zayed, M.Z., A. Wu and S.M. Sallam. 2019. Comparative phytochemical constituents of *Leucaena leucocephala* (Lam.) leaves, fruits, stem barks, and wood branches grown in Egypt using GC-MS method coupled with multivariate statistical approaches. BioResour. 14: 996-1013.

Zayed, M.Z.A., M.A. Abdel-Rahman and M.S. Shamsir. 2025. Chemical composition, antioxidant and antibacterial activities of extracts from *Sesbania sesban* organs growing in Egypt. Alex. Sci. Exch. J. 46: 753-765.

## الملخص العربي

# التحليل الكيميائي النباتي لمستخلصات ثمار نبات السيسالبينيا باستخدام تقنية كروماتوغرافيا الغاز – مطياف الكتلة، وتقييم مضادات الأكسدة والنشاط المضاد للبكتيريا

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يمثل هذا البحث دراسة رائدة وشاملة لتركيب مستخلصات ثمار نبات السيسالبينيا المزروع في مصر. تم استخلاص الزيوت العطرية والمستخلصات النباتية باستخدام مذيبات مختلفة (الهكسان، بترول إيثر، الكلوروفورم، أسيتات الإيثيل، والميثانول)، ثم تحليلها باستخدام جهاز كروماتوغرافيا الغاز – مطياف الكتلة (GC–MS)، مع تقييم النشاطين المضادين للأكسدة والبكتيريا. أظهر تحليل GC–MS للزيت العطري وجود ٢٨ مركبًا تمثل ٩٣.٤٪ من إجمالي التكوين، من أبرزها-α تربينين، -αكوبائين، -αكادينول، و-βإيوديسمول، وهي مركبات معروفة بخصائصها البيولوجية الفعالة. كما كشفت المستخلصات المذيبية عن تنوع واسع في المركبات الكيميائية، شمل الأحماض الدهنية، الستيرولات، الترايتربينويدات، والمركبات الفينولية، حيث سجل مستخلص الميثانول أعلى مستوى من التنوع وأغنى محتوى من المركبات النشطة بيولوجيًا. أظهرت التحاليل الكمية أن مستخلصي الميثانول وأسيتات الإيثيل يحتويان على أعلى تركيز من الفينولات والفلافونويدات، مما انعكس في نشاط قوي كمضادات أكسدة TAA حتى ٧٥.٣%، وقيم $IC_{50}$ منخفضة (٢٦.٤ ميكروغرام/مل). أما في الاختبارات المضادة للبكتيريا، فقد أظهرت مستخلصات الميثانول وأسيتات الإيثيل فعالية واضحة ضد البكتيريا موجبة الجرام *Staphylococcus aureus*) و(*Staphylococcus epidermidis*، مع نشاط متوسط ضد السلالات سالبة الجرام، في حين أظهر الزيت العطري نشاطًا متوسطًا. اللافت أن قيم MIC و MBC لمستخلص الميثانول كانت مقاربة لتلك الخاصة بالمضادات الحيوية القياسية. وتعد هذه الدراسة أول تقرير يوثق التركيب الكيميائي لمستخلصات ثمار السيسالبينيا وفعالياتها البيولوجية، مما يبرز إمكاناتها كمصدر طبيعي واعد لمركبات مضادة للأكسدة والبكتيريا يمكن توظيفها في التطبيقات الصيدلانية والغذائية.

**الكلمات المفتاحية:** السيسالبينيا، ثمار، زيت عطري، GC-MS، مركبات فينولية، فلافونويدات، نشاط مضاد للأكسدة