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Comparative Chemical and Bioactivity Profiling of Polar and Resinous Fractions of Yemeni *Commiphora myrrha.*

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Abstract

The oleo-gum resin of *Commiphora myrrha* (Nees) Engl. has been a cornerstone of traditional Yemeni medicine for centuries. This study provides a comparative chemical and bioactivity evaluation of two distinct fractions: a polar fraction (E1) obtained via aqueous maceration and a lipophilic fraction (E5) obtained via methanolic Soxhlet extraction. The crude resin exhibited moisture content (10.28 ± 0.14%), ash content (7.44 ± 0.11%), pH (4.31 ± 0.16), viscosity (0.932 mPa·s), and protein content (17.06 ± 0.61%). Antimicrobial assays demonstrated that E5 exhibited significant inhibitory activity against gram-positive bacteria and *Candida albicans*, whereas E1 remained inactive. Gas chromatography–Mass spectrometry (GC-MS) analysis revealed that E5 is rich in bioactive oxygenated sesquiterpenes and sesquiterpene lactones, whereas E1 is dominated by fatty acid esters with minimal bioactivity. The superior antioxidant capacity of E5 ($IC_{50} \approx 170 g/mL$) exceeded E1 ($IC_{50} \approx 1480 \mu g/mL$) strongly correlated with higher phytochemical yields: total phenolics (46.04 vs. 6.35 mg gallic acid equivalents per gram of dry extract (mg GAE/g)), flavonoids (6.55 vs. 0.69 mg quercetin equivalents per gram (mg QE/g)), and tannins (19.49 vs. 4.48 mg tannic acid equivalents per gram (mg TAE/g)). These results confirm that the primary bioactivity of *C. myrrha* is sequestered within the resinous terpenoid fraction. This study establishes critical quality control parameters for Yemeni *C. myrrha* and supports a dual-use strategy: E5 for pharmaceutical development and E1 for industrial applications as a natural hydrocolloid.

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Keywords:
Commiphora myrrha, Yemeni myrrh, Polar gum fraction, Resinous bioactive fraction, GC-MS, Antimicrobial activity.

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1. INTRODUCTION

Natural oleo-gum resins have been pivotal in ethnomedicine and industry for millennia, owing to their unique physicochemical and biological properties [1, 2]. Among the most significant are resins from the genus *Commiphora* (Burseraceae), particularly myrrh (*Commiphora myrrha* (Nees) Engl.), which is renowned for its pharmacological potential [3, 4]. In Yemen, *C. myrrha* is distributed across several governorates and is traditionally used to treat inflammatory conditions, wounds, gastrointestinal disorders, and infections [5–8]. Despite these applications, comprehensive scientific evaluations of Yemeni-sourced *C. myrrha* are still sparse.

The raw oleo-gum resin of *C. myrrha* is a complex matrix of water-soluble polysaccharides (gum) and lipophilic terpenoids (resin). Isolating these specific functional fractions is critical for fully exploring their respective potentials [8]. While the polar gummy matrix is conventionally valued for its physical properties, the lipophilic resinous constituents harbor the majority of the active secondary metabolites [9, 10]. A dual approach targeting both distinct fractions facilitates the comprehensive valorization of raw materials for both industrial (gum-based) and pharmaceutical (resin-based) applications.

The physicochemical profile of natural gums determines their stability, quality, and industrial utility [11]. Key parameters, such as moisture content (shelf-life stability), ash content (mineral purity), pH (formulation compatibility), viscosity (rheological processing), and protein content (emulsifying capacity), are essential for characterizing these materials [12–14]. From a pharmaceutical perspective, the escalating global threat of antimicrobial resistance has revitalized interest in plant-derived antimicrobial agents [15, 16]. Previous studies have attributed the antimicrobial and antioxidant efficacy of myrrh to its rich terpenoid and phenolic compound profiles [17, 18].

This study aimed to establish a comprehensive profile of Yemeni *C. myrrha* by comparing a traditional cold-water maceration method (yielding a polar gum fraction) with hot methanolic Soxhlet extraction (yielding a bioactive resinous fraction), which has not been performed previously. Following the determination of the fundamental physicochemical parameters of the raw resin, both distinct functional extracts were characterized. Beyond assessing their antimicrobial potential and antioxidant capacity via DPPH assays, the investigation utilized UV-Vis, FT-IR, and GC-MS to elucidate spectroscopic profiles, functional groups, and volatile chemical compositions. Furthermore, rigorous phytochemical screening was performed to quantify the total phenolic, flavonoid, and tannin contents, providing a robust chemical basis for the observed biological activities and supporting the potential industrial and pharmaceutical valorization of this traditional natural resource.

2. MATERIALS AND METHODS

2.1. Plant Material Collection

Fresh oleo-gum resin of *C. myrrha* (Nees) Engl. was collected in September 2023 from mature trees in the Al-Zahra District, Al-Hudaydah Governorate, western Yemen (15°47'N, 43°14'E). Prof. Dr. Hassan Ibrahim from the Department of Biology at Sana'a University confirmed the plant species identification. The faculty herbarium received a voucher specimen with accession number CMR-2023-1629. The resin was air-dried at 20-25 °C for 14 days, then ground into a coarse powder with a size range of 1-2 mm, which was stored in amber glass containers at room temperature [8, 19].

2.2. Chemicals and Reagents

Methanol ($\geq$ 99.9%), quercetin ($\geq$ 95%), tannic acid, gallic acid, Folin-Ciocalteu reagent, aluminum chloride, ferric chloride, DPPH (2,2-diphenyl-1-picrylhydrazyl), and ascorbic acid were purchased from Sigma-Aldrich (USA) and Merck (Germany). All reagents were of analytical or HPLC grade.

2.3. Preparation of Fractions

To functionally characterize the different components of the oleo-gum resin, two distinct fractions were prepared from 30 g of powdered resin using 300 mL of solvent (10:1, w/v). The distinct polarity indices of water (10.2) and methanol (5.1) were selectively exploited to target and isolate different bioactive groups [20, 21].

Polar Gum Fraction (E1 - Water Maceration): Water was specifically chosen as the solvent to target the water-soluble gum and polysaccharide portion of the resin. Maceration at room temperature (22 ± 2 °C) was selected as the extraction technique to prevent the thermal degradation of these heat-sensitive polysaccharides. Thirty grams of resin powder was extracted with 300 mL of distilled water with occasional shaking for 24 h. The fraction was filtered, then concentrated using a rotary evaporator (40 °C), and dried to constant weight. Yield: 56.57% w/w.

Resinous Bioactive Fraction (E5 - Methanol Soxhlet): Methanol and continuous heat (reflux) were used in tandem to effectively liberate medium-polarity to lipophilic bioactive constituents (such as terpenoids) that are tightly trapped within the complex gummy matrix of the resin. Thirty grams of resin powder was placed in a cellulose thimble and extracted with methanol (300 mL) using a Soxhlet apparatus for 6 h. The fractions were concentrated and dried as previously described. Yield: 8.93% w/w [8].

2.4. Physicochemical Characterization

2.4.1. Moisture Content

The moisture content was determined by oven-drying at 105 °C to a constant weight. About 1-2 g of resin was placed in an oven for 3 h, cooled in a desiccator, and then weighed. The determination was performed in triplicate [12].

2.4.2. Total Ash Content

The ash content of the resin was determined by igniting 1-2 g of the sample at 550-600 °C for 4-6 hours until a constant white or grey ash remained [12, 22].

2.4.3. pH Determination

A 1% (w/v) solution of the resin was prepared using distilled water and stirred for 30 min at room temperature, and the pH was determined using a calibrated pH meter [13].

2.4.4. Viscosity Measurement

The viscosity of the 1% (w/v) solution was determined at room temperature (25 °C) using an Ostwald viscometer. The flow times of the sample and distilled water were measured, and the viscosity was calculated from the

known viscosity of water (0.89 mPa·s) [13].

2.4.5. Nitrogen and Protein Content (kjeldahl Method)

The nitrogen content of the resin was determined using the Kjeldahl method. One gram of resin was digested with concentrated H_2SO_4 and a catalyst mixture. The released ammonia was distilled into 4% boric acid solution and titrated with 0.080 N HCl. The protein content was computed by multiplying the nitrogen content by 6.25. Standard sample urea (43.7% N recovery) was used to determine the accuracy of the results [23].

2.5. Qualitative Phytochemical Screening

Qualitative phytochemical tests were performed for both the water maceration fraction (E1) and methanol Soxhlet fraction (E5), including terpenoids (Salkowski test), steroids (Liebermann-Burchard test), alkaloids (Dragendorff's and picric acid tests), phenols (ferric chloride test), flavonoids (Shinoda test), tannins (gelatin test), saponins (foam test), and glycosides (Keller-Killiani test), according to standard protocols [24–26].

2.6. UV-Vis Spectroscopy

Fraction samples (E1 and E5) were dissolved in water (E1) or methanol (E5) and the UV-Vis absorption spectra were recorded from 300 to 900 nm using a UV-Vis spectrophotometer (Lasany LI-722), with pure solvent as a blank [27].

2.7. FT-IR Spectroscopy

The spectra of the fraction samples (E1 and E5) were recorded on a Jasco FT/IR-4100 spectrophotometer in the range of 400-4000cm^{-1} with a resolution of 4 cm^{-1} and 32 scans per spectrum using the KBr pellet technique [28].

2.8. Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was performed at the Public Authority for Standardization and Metrology, Sana'a, Yemen, using a Shimadzu GC-MS-QP2020 NX system equipped with a DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.69 mL/min. The injection mode was splitless, with an injection volume of 1.0 μL. The injector temperature was maintained at 250 °C. The oven temperature program was: initial temperature 50 °C held for 4 min, increased to 280 °C at a rate of 15 °C/min, and held at 280 °C for 16 min. The transfer line temperature was maintained at 285 °C. Electron impact (EI) ionization was performed at 70 eV, and the mass spectra were recorded in full scan mode (m/z 50-500). The compounds were identified by comparing the mass spectral fragmentation patterns with the NIST17 and Wiley 9 mass spectral libraries. Identification was accepted when the similarity index exceeded 80% [29].

2.9. Antimicrobial Activity

The fractions were tested against five selected microbes using the agar well diffusion technique [16]. Test microorganisms included *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and *Candida albicans* ATCC 10231.

Fraction solutions were prepared at a concentration of 100 mg/mL in 10% DMSO. Using a sterile cork borer, wells with a diameter of 6 mm were made in Mueller-Hinton agar plates (for bacteria) and Sabouraud dextrose agar plates (for *Candida albicans*). An aliquot of 100 μL of each fraction solution was introduced into the respective wells.

For the positive control, a solution of amoxicillin was prepared at a concentration of 300 μg/mL in 10% DMSO (equivalent to 30 μg in 100 μL). An aliquot of 100 μL of this solution was placed into a well with a 6 mm diameter under the same conditions as the test fractions. The negative control consisted of 100 μL of 10% DMSO (without fraction or antibiotic) placed in a separate well [30].

2.10. DPPH Radical Scavenging Assay

The antioxidant activity of the fractions was determined using the DPPH radical scavenging assay as described by Blois [31] with minor modifications. A fresh solution of DPPH (0.1 mM in methanol) was then prepared. Different concentrations of the fraction were prepared, ranging from 10 to 1500 μg/mL. The fraction (2mL) was mixed with DPPH solution (2.0 mL of the DPPH solution and left in the dark at room temperature for 30 min. Ascorbic acid was used as the positive control. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The DPPH radical scavenging activity (%) was calculated as follows:

$$Scavengingactivity(\%) = [(A_0 - A_1)/A_0]100,$$

where A_0 is the absorbance of the control (DPPH + methanol) and A_1 is the absorbance of the sample (DPPH + the fraction). IC_{50} values (concentration required to scavenge 50% of DPPH radicals) were obtained by plotting the percentage inhibition activity versus concentration of fraction, and non-linear regression analysis was carried out using GraphPad Prism 8.0 software [32].

2.11. Total Phenolic Content (TPC)

The TPC was determined using the Folin-Ciocalteu method [33, 34]. Gallic acid was used as the standard (1-10 μg/mL, R^2 = 0.987). Five milligrams of the fraction was dissolved in 3 mL of solvent, and the absorbance was measured at 765 nm. The results were calculated as mg gallic acid equivalents per gram of dry fraction (mg GAE/g).

2.12. Total Flavonoid Content (TFC)

TFC was measured using the aluminum chloride colorimetric method [35]. Quercetin was used as a standard (20-200 μg/mL, R^2 = 0.994). Fraction solutions (1 mg/mL) were analyzed, and the absorbance was measured at 415 nm. The results were reported as mg quercetin equivalents per gram of dry fraction (mg QE/g).

2.13. Tannin Content

Tannin content was measured using the ferric chloride method [36]. Tannic acid was used as the standard (20-200 μg/mL, R^2 = 0.999). Fraction solutions (0.1 g in 100 mL of solvent) were analyzed, and the absorbance was measured at 550 nm. The results were reported as mg tannic acid equivalents per gram of dry fraction (mg TAE/g).

2.14. Statistical Analysis

All experiments were performed in triplicate and expressed as mean $\pm$ standard deviation (SD). IC_{50} values were calculated using non-linear regression analysis in GraphPad Prism 8.0 software (GraphPad Software, USA). Comparisons between E1 and E5 were performed using an unpaired, two-tailed Student's t-test. Differences were considered statistically significant at $p < 0.05$. All p-values were calculated using GraphPad Prism 8.0 software [37].

3. RESULTS AND DISCUSSION

3.1. Physicochemical Characterization of the Crude Resin

The fundamental physicochemical properties of crude *C. myrrha* resin are summarized in Table 11. The moisture content was determined to be 10.28 ± 0.14%, aligning with the established range for stable natural gums (8–14%) [12, 13]. This low moisture level is a critical quality indicator, as it suggests high storage stability and inherent resistance to hydrolytic degradation and microbial proliferation [38]. The total ash content (7.44 ± 0.11%) reflects a moderate mineral profile, remaining within the standardized purity limits ($\leq$ 8%) typically required for pharmaceutical-grade raw materials [22].

Table 1. Physicochemical properties of *C. myrrha* crude resin

Parameter	Value (Mean ± SD)
Moisture content (%)	10.28 ± 0.14
Total ash content (%)	7.44 ± 0.11
pH (1% aqueous solution)	4.31 ± 0.16
Viscosity (1% solution, 25 °C, mPa·s)	0.932 ± 0.008
Nitrogen content (%)	2.73 ± 0.10
Protein content (%)	17.06 ± 0.61

The aqueous solution of the crude resin exhibited a slightly acidic pH (4.31 ± 0.16), a characteristic frequently attributed to the presence of uronic acid moieties within the polysaccharide backbone, and the presence of acidic phenolic constituents [3, 13]. Interestingly, the viscosity of the 1% aqueous solution was notably low (0.932 ± 0.008 mPa·s), suggesting favorable rheological properties for industrial processing and ease of formulation in liquid dosage forms [14].

A significant finding was the high protein content (17.06 ± 0.61%), which substantially exceeds that of common commercial hydrocolloids, such as *Acacia senegal* gum (2–3%), but remains consistent with specialized literature on *Commiphora* species (15–20%) [3, 38]. This proteinaceous fraction is of particular interest because it likely serves as a natural emulsifier, potentially stabilizing oil-in-water emulsions. Furthermore, this high nitrogenous content may contribute to the complex biological activities observed in the resin, as protein-polysaccharide conjugates often influence both the bioavailability and immunological response of natural gums [39].

Data are presented as mean ± standard deviation (SD) of triplicate determinations

3.2. Extraction Yields

Water maceration yielded a high percentage of 56.57 ± 1.22% w/w, owing to the presence of water-soluble polysaccharides (gum fraction) [3, 40]. In contrast, the methanol Soxhlet resin fraction (E5) yielded 8.93 ± 0.35% w/w. The difference in the yields was statistically significant ($p < 0.001$, unpaired t-test). The remarkable difference in yields reflects the distinct chemical nature of the targeted components: water selectively isolates polar gums and polysaccharides, whereas methanol extracts medium-polarity substances such as phenolics, flavonoids, and terpenoids [3, 9].

3.3. Qualitative Phytochemical Screening

Qualitative phytochemical screening revealed considerable differences between the two fractions, E1 and E5 (Table 22). E5 (methanol Soxhlet fraction) demonstrated strong signals (+++) for terpenoids, steroids, phenols, and flavonoids, whereas the water maceration fraction (E1) exhibited only a slight presence (+), traces (±), or absence **(-)** of these compound classes. As expected,

Table 2. Qualitative phytochemical screening of E1 and E5 fractions

Phytochemical Class	Test Method	E1 (Water Maceration)	E5 (Methanol Soxhlet)
Terpenoids	Salkowski test	+	+++
Steroids	Liebermann-Burchard test	+	+++
Alkaloids	Dragendorff's test	+	++
Phenols	Ferric chloride test	±	+++
Flavonoids	Shinoda test	±	+++
Tannins	Gelatin test	+	++
Saponins	Foam test	+++	-
Glycosides	Keller-Killiani test	+	+

Soxhlet extraction under continuous heating recovered greater amounts of medium-polarity compounds. However, saponins were present (+++) in E1 but were completely absent (-) in E5. This is mainly due to the presence of strongly hydrophilic functional groups in the saponin structure, which require a polar solvent for effective extraction [25, 26].

Thus, this fraction (E5) was specifically targeted for the recovery of bioactive constituents (flavonoids and terpenoids), which exhibit excellent synergistic effects on biological activities (e.g., antioxidant and antibacterial actions) [6, 17]. Conversely, the absence of these compounds in E1 is an expected outcome of the water maceration process, which selectively isolates polar, non-bioactive gum [3, 40].

(+++) strong presence; (++) moderate; (+) slight; (±) trace; (-) absent.

3.4. UV-Vis Spectroscopy

UV-Visible spectra of the water maceration (E1) and methanol Soxhlet fractions (E5) showed different patterns (Fig. 11). E1 exhibited a weak absorption spectrum with a maximum at approximately 340 nm (absorbance 0.05-0.10), indicating a very small amount of UV-Visible active compounds such as flavonoids and phenolics [20, 41]. The E5 fraction demonstrated robust absorption with a strong maximum at 350 nm and two minor peaks at 330 and 370 nm. These peaks correspond to the ($\pi \rightarrow \pi*$) transitions of the conjugated system, which are typical of flavones, flavanols, and chalcones [42]. The significant increase in the absorbance of E5 compared to that of E1 reflects the higher mobilization of chromophores by methanol [20, 42].

The extended absorption up to 600 nm suggests the presence of extended chromophores such as chalcones and aurones, which give the typical dark amber color of the Soxhlet fraction [3, 20]. These findings confirm that the methanol Soxhlet fraction (E5) is highly enriched with a broad spectrum of UV-VIS active phenolic and flavonoid compounds compared to the polar gum fraction (E1), reflecting the intended functional separation of the resin's components.

3.5. FT-IR Spectroscopy

The FT-IR spectra of the E1 and E5 fractions are shown in Table 3 and Fig. 2. A broad and strong O-H stretching vibration peak centered at approximately 3400 cm^{-1} indicated the presence of alcohols and polysaccharides of gum, as well as phenolic components. The intensity of this band was more significant for E5, suggesting a higher abundance of phenolic/terpenoid compounds in the sample compared to E1, as confirmed by UV-Vis and phytochemical data [27, 43].

The C-H stretching of aliphatic methyl and methylene groups at approximately 2920-2850 cm^{-1} confirmed the presence of carbon chains in E5 with greater intensity than in E1 [44]. A sharp C=O stretching peak appeared exclusively at approximately 1710 cm^{-1} in the E5 sample. This band corresponds to carbonyl-containing compounds, such as ketones, aldehydes, and esters, which are characteristic of sesquiterpenoids and resin acids. It is important to note that this carbonyl band is not present in non-oxygenated hydrocarbon sesquiterpenes (e.g., β-elemene and γ-elemene), which lack carbonyl functional groups [45]. The absence of this peak in E1 reflects the selective recovery of medium-polarity compounds using organic solvents and heating. The conjugated C=C stretching at 1600-1615 cm^{-1} indicated aromatic rings or conjugated alkenes that were present in both samples. The C-O stretching within the range of 1250-1030 cm^{-1} indicated ether or ester functional groups, which might exist as oxygenated terpenoids or the glycosidic portion of phenols [46]. The strong functional peaks and the presence of additional functional peaks in the E5 spectrum demonstrated that the bioactive constituents were effectively extracted from the raw gum matrix. This targeted extraction is attributed to the combined effect of methanol' polarity and thermal energy, which effectively penetrates the gum-resin matrix of *C. myrrha* [8, 9].

3.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was conducted to determine the volatile and semi-volatile compounds in the E1 and E5 fractions. The Total Ion Chromatogram (TIC) profiles showed markedly different chemical compositions between E5 and E1, demonstrating the different selectivity of the

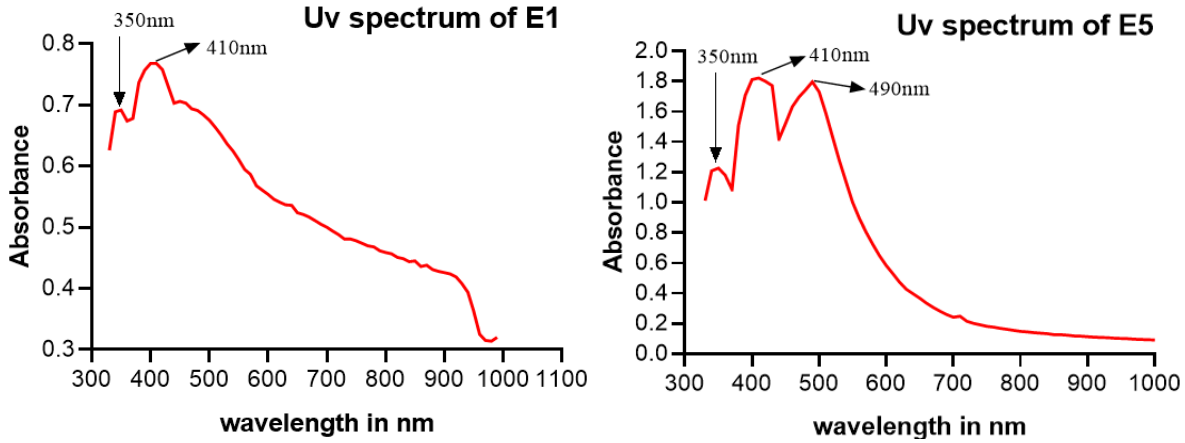

Figure 1. UV-Vis spectra of water maceration fraction (E1) and methanol Soxhlet fraction (E5)

Table 3. FT-IR absorption bands and functional group assignments for E1 and E5

Wavenumber (cm^{-1})	Functional Group	Compound Class	E1 Intensity	E5 Intensity
~3400	O-H stretching	Alcohols, phenolics	Medium, broad	Strong, broad
~2925, 2850	C-H stretching	Aliphatic compounds	Medium	Strong
~1710	C=O stretching	Carbonyl compounds	Not detected	Sharp
~1605, 1515	C=C stretching	Aromatic compounds	Weak	Medium
~1450, 1375	C-H bending	Aliphatic compounds	Weak	Medium
1250-1050	C-O stretching	Alcohols, esters, ethers	Medium	Very strong

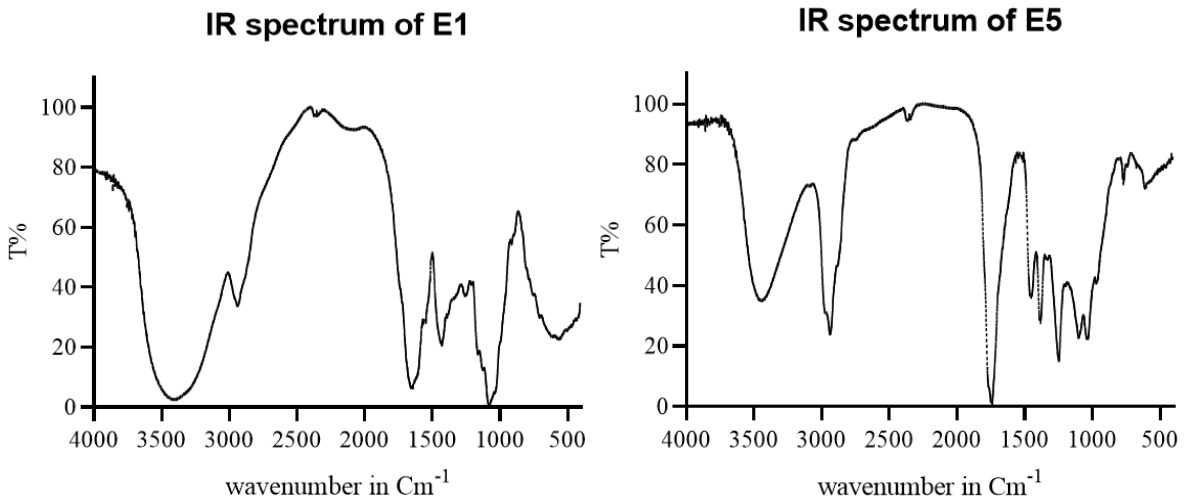

Figure 2. FT-IR spectra of water maceration fraction (E1) and methanol Soxhlet fraction (E5)

chosen extraction method and solvent.

3.6.1. GC-MS Profile of E5 (Methanol Soxhlet fraction)

E5 (methanol Soxhlet fraction)exhibited a highly complex chemical profile. A total of 138 compounds were successfully integrated by the GC-MS software, accounting for 100% of the integrated chromatographic peak area, as shown in Fig. 3. Table 4 selectively presents the 12 major bioactive constituents (comprising 21.64% of the total profile) that characteristically define the pharmacological properties of this fraction. The remaining 78.36% of the profile was distributed across a highly diverse matrix of 126 minor and trace compounds, each occurring in negligible individual quantities. This substantial trace fraction is predominantly composed of low-abundance monoterpene hydrocarbons, oxygenated monoterpenes, minor sesquiterpene isomers, and long-chain fatty acid derivatives. This high concentration of diverse trace constituents is an inherent chemotaxonomic feature of *C. myrrha* resin extracts, where a wide array of minor components work synergistically to support the overall biological efficacy without dominating the quantitative profile. To provide a clearer chemotaxonomic understanding, the major bioactive constituents were classified into three distinct sub-classes based on their structural frameworks and their oxygenation states.

3.6.1.1. Sesquiterpene Hydrocarbons

Sesquiterpene hydrocarbons are pure lipophilic cyclic compounds composed of three isoprene units without

oxygen functional groups, typically sharing the molecular formula $C_{15}H_{24}$. Within this class, our analysis identified β-elemene (0.62%), γ-elemene (0.67%), α-selinene (0.11%), and germacrene B (1.23%). It is crucial to clarify that the elements (β and γ isomers) belong strictly to this non-oxygenated hydrocarbon class. These compounds are renowned for suppressing tumor proliferation, overcoming multi-drug resistance, and inducing apoptosis by modulating key intracellular signaling cascades, such as the PI3K/AKT/mTOR pathway [47, 48].

3.6.1.2. Oxygenated Sesquiterpenes (Sesquiterpenoids)

Oxygenated sesquiterpenes (commonly referred to as sesquiterpenoids) are structural derivatives that undergo biochemical modifications, introducing functional groups such as hydroxyls, epoxides, ethers, or ketones. This structural functionalization often drastically alters their physical properties and biological activities. In this study, calarene epoxide (2.45%) is an oxygenated derivative with a highly reactive epoxide ring that enhances its electrophilic interaction with biological targets [49]. Additionally, 1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol (3.51%) was identified at a notably high level in *C. myrrha* fractions, which has not been commonly reported in previous studies, showcasing the efficiency of the thermal methanolic reflux in liberating matrix-bound polar terpenoids.

3.6.1.3. Sesquiterpene Lactones

Sesquiterpene lactones are a highly specialized and pharmacologically potent subgroup of oxygenated sesquiterpenes, characterized by the integration of a cyclic ester (lactone) ring, usually a γ-lactone fused to the main carbocyclic framework. The fraction was exceptionally rich in these architectures, represented mainly by telekin (2.78%), reynosin (1.36%), and 6-hydroxy-5a,9-dimethyl-3-methylidene-4,5,6,7,9a,9b-hexahydro-3aH-benzo[g][1]benzofuran-2-one (2.19%). Telekin and associated lactones are historically recognized for their formidable anti-inflammatory, anticancer, and antioxidant profiles, primarily mediated by the inhibition of the NF-B transcription factor and structural disruption of fungal or bacterial cell walls [50, 51].

3.6.1.4. Other Compounds

Apart from sesquiterpenoids, the GC-MS profile of E5 revealed other notable phytochemicals, including a steroidal derivative, estr-4-en-3-one, 17-(acetyloxy)-, (17-beta.) (2.27%), and a macrocyclic diterpene, 1,3,6,10-cyclotetradecatetraene derivative (2.62%). Additionally, a long-chain fatty acid ester, hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester (1.83%), was identified.

The abundance of oxygenated sesquiterpenoids in E5 accounts for its potent antimicrobial and antioxidant activities, with its lipophilic character mediating cell membrane interactions with microorganisms and its hydroxyl and carbonyl groups scavenging free radicals [52, 53].

3.6.2. GC-MS Profile of E1 (Water Maceration fraction)

E1 (water maceration fraction) displayed an entirely different chemical profile from E5 and predominantly contained fatty acid esters and alkanes. In total, 103 compounds were identified, accounting for 100.00% of the total integrated peak area, as shown in Fig. 44. Table 55 selectively presents the 10 major constituents (comprising 50.46% of the profile), while the remaining 49.54% is distributed across 93 minor and trace compounds occurring in negligible individual quantities. The two main compounds were ethyl palmitate (hexadecanoic acid ethyl ester) and ethyl stearate (octadecanoic acid ethyl ester), totaling 14.20% and 12.31%, respectively. Hexadecanoic acid, hexadecyl ester (cetyl palmitate) were identified at a concentration of 6.72%. These compounds are derived from the resin gum and do not exhibit antimicrobial or antioxidant activity [54, 55].

Notably, 1H-Indole-3-ethanamine (tryptamine) was detected at 4.78%, an amine compound with potential biological activities. Several alkanes and siloxanes were also identified, likely originating from the resin matrix or as artifacts from GC column bleed during the analysis process [56]. The near absence of sesquiterpenoids in E1 explains its weak antimicrobial and antioxidant activities.

3.6.3. GC-MS Profiling Insights

GC-MS analysis revealed a striking difference in the composition between E5 and E1. E5 is rich in sesquiterpenoids and sesquiterpene lactones (telekin, calarene epoxide, and reynosin), which exhibit broad antimicrobial, anti-inflammatory, and antioxidant properties [50, 51]. In stark contrast, E1 predominantly comprises fatty acid esters (ethyl palmitate and ethyl stearate) with no reported biological activity [54, 55]. This difference in composition can explain the potent antimicrobial and antioxidant effects of E5 compared to E1 and is consistent with the nature of extraction, with methanol generally extracting medium-polarity compounds such as terpenes and phenolics, and water extracting polar compounds such as polysaccharides and gums [9].

3.7. Antimicrobial Activity

Both E1 and E5 fractions were evaluated for their ability to inhibit the growth of various clinical microorganisms, as shown in Table 6 and Fig. 5. E5 (methanol Soxhlet fraction) exhibited a potent effect against both gram-positive bacteria, *Staphylococcus aureus* (16 mm) and *Bacillus subtilis* (15 mm), as well as a strong effect against *Candida albicans* (20 mm). However, E1 (water maceration fraction) did not exhibit any activity against any of the microorganisms at the same concentration (100 mg/mL). The lack of activity of E1 is largely due to the presence of polysaccharides and fatty acid esters within the fraction,

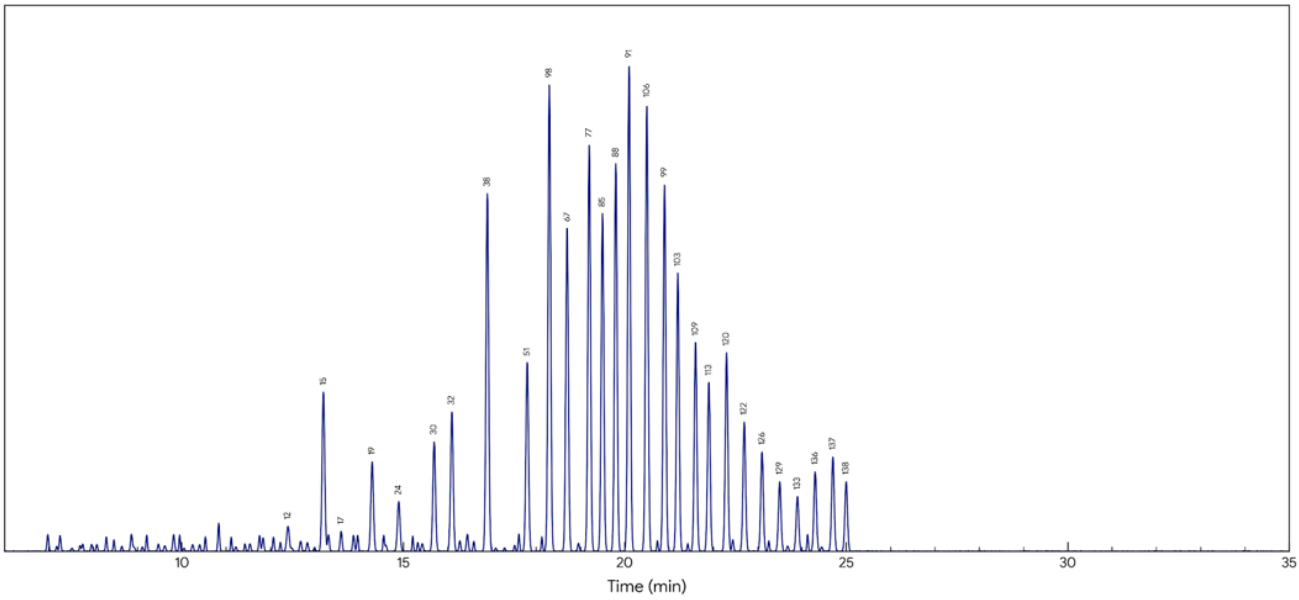

Figure 3. Total ion chromatogram (TIC) of the resinous bioactive fraction (E5) obtained by GC-MS analysis.

Table 4. Major compounds identified in E5 (Methanol Soxhlet fraction) by GC-MS

Peak #	Compound Name	Chemical molecular formula	Retention Time (min)	Area (%)
Sesquiterpene hydrocarbons				
1	β-elemene	$C_{15}H_{24}$	12.893	0.62
2	α-selinene	$C_{15}H_{24}$	14.021	0.11
3	γ-elemene	$C_{15}H_{24}$	17.357	0.67
4	Germacrene B	$C_{15}H_{24}$	19.127	1.23
Oxygenated Sesquiterpenes				
5	Calarene epoxide	$C_{15}H_{24}O$	19.009	2.45
6	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	$C_{15}H_{26}O_2$	20.339	3.51
Sesquiterpene Lactones				
7	Reynosin	$C_{15}H_{20}O_3$	18.592	1.36
8	6-Hydroxy-5a,9-dimethyl-3-methylidene-4,5,6,7,9a,9b-hexahydro-3aH-benzo[g][1]benzofuran-2-one	$C_{15}H_{20}O_3$	19.822	2.19
9	Telekin	$C_{15}H_{20}O_3$	20.060	2.78
Other Compounds				
10	Estr-4-en-3-one, 17-(acetyloxy)-, (17.beta.)-	$C_{20}H_{28}O_3$	17.113	2.27
11	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	$C_{20}H_{32}$	17.620	2.62
12	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	21.537	1.83

Table 5. Major compounds identified in E1 (Water Maceration fraction) by GC-MS

Peak #	Compound Name	Chemical molecular formula	Retention Time (min)	Area (%)
1	Eicosane	$C_{20}H_{42}$	13.538	1.32
2	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	14.040	1.15
3	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	$C_{18}H_{36}O_2$	17.663	14.20
4	Ethyl Oleate	$C_{20}H_{38}O_2$	18.848	1.70
5	Octadecanoic acid, ethyl ester (Ethyl stearate)	$C_{20}H_{40}O_2$	18.982	12.31
6	Hexadecanoic acid, hexadecyl ester	$C_{32}H_{64}O_2$	20.396	6.72
7	1,3-Dibutoxy-2-propanol, TMS	$C_{14}H_{32}O_3Si$	21.057	5.84
8	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	21.514	2.14
9	1H-Indole-3-ethanamine (Tryptamine)	$C_{10}H_{12}N_2$	22.713	4.78
10	Squalene	$C_{30}H_{50}$	24.342	0.30

which have no known antimicrobial or antioxidant effects [3, 40]. E5 exhibited strong inhibitory activity, which is consistent with the sesquiterpenoid and terpenoid compositions previously demonstrated using phytochemical screening, GC-MS, and UV-Vis/FT-IR analyses [17, 52, 57].

None of the fractions exhibited any activity against gram-negative bacteria, *Escherichia coli*, and *Pseudomonas aeruginosa*. This may be attributed to the low permeability of the outer membrane containing

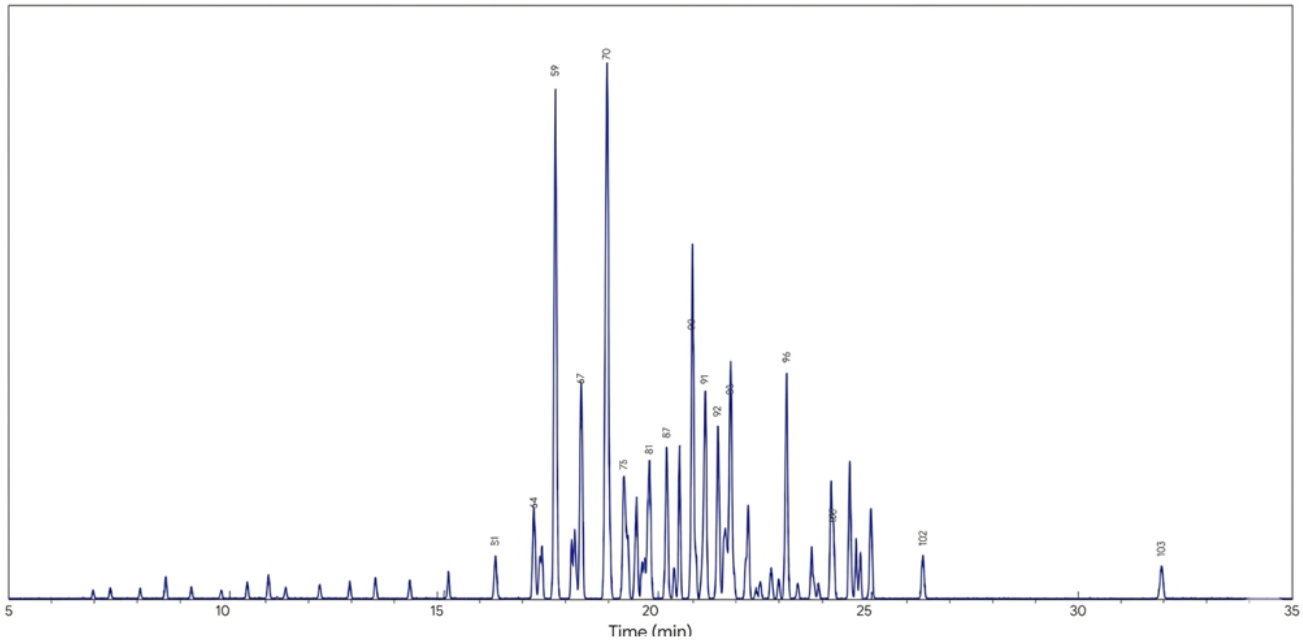

Figure 4. Total ion chromatogram (TIC) of the polar gum fraction (E1) obtained by GC-MS analysis.

lipopolysaccharides (LPS), which limits the penetration of lipophilic compounds, such as terpenoids. This selective activity against gram-positive bacteria and fungi is characteristic of many plant-derived antimicrobials, supporting the traditional use of myrrh in treating wounds and fungal infections [7, 58].

Nonetheless, the lack of gram-negative activity of *C. myrrha* should be carefully interpreted, as previous studies have reported inconsistent results for its gram-negative activity, which could be related to the extraction technique, polarity of the solvent used, and even the specific microorganisms employed [29].

While clinical antibiotics such as colistin directly disrupt LPS architecture [59]. The lipophilic sesquiterpenoids within our E5 fraction likely exert alternative mechanisms, such as anti-virulence activity, rather than direct outer-membrane penetration.

Furthermore, the lack of activity against gram-negative bacteria can be attributed to diffusion limitations, where the thick, asymmetric lipopolysaccharide (LPS) outer membrane establishes a formidable hydrophobic barrier that inherently restricts the passage of lipophilic sesquiterpenoids, even at high extract concentrations [60]. Additionally, the concentration of individual active compounds in the crude E5 extract may fall below the threshold required to effectively disrupt the Gram-negative envelope. However, the total extract exhibited clear activity against the more permeable gram-positive strains [61, 62]. Finally, although the sesquiterpenoids in E5 likely act synergistically against gram-positive bacteria and fungi, this synergy may be insufficient to overcome the more robust permeability barrier of gram-negative pathogens [61, 62]. Therefore, future checkerboard assays are needed to evaluate the potential synergy among isolated compounds against resistant Gram-negative strains.

The notable activity of E5 against *C. albicans* (20 mm inhibition zone) is significant because of the resistance it poses to current antifungal drugs [15], which may be partly due to the presence of telekin and sesquiterpene lactones, both of which are reported to interact with fungal cell membranes and prevent ergosterol synthesis [51, 52]. The single-concentration (100 mg/mL) assay was designed as a preliminary comparative screening to track the partitioning of active principles between polar and non-polar fractions. As bioactivity was localized exclusively in E5, MIC determination was prioritized for future studies on isolated pure sesquiterpenoids from this fraction.

Table 6. Antimicrobial activity of E1 and E5 fractions (inhibition zone diameter, mm) at 100 mg/mL

Microorganism	E1 (Water Maceration)	E5 (Methanol Soxhlet)	Amoxicillin
S. aureus	-	16	32.5
B. subtilis	-	15	26.0
E. coli	-	-	-
P. aeruginosa	-	-	-
C. albicans	-	20	-

(-) = no inhibition zone

3.8. DPPH Radical Scavenging Activity (Antioxidant Activity)

Both E1 and E5 fractions were subjected to DPPH radical scavenging assay [31]. The DPPH radical scavenging results are presented in Tables 7 and 8 and Fig. 6

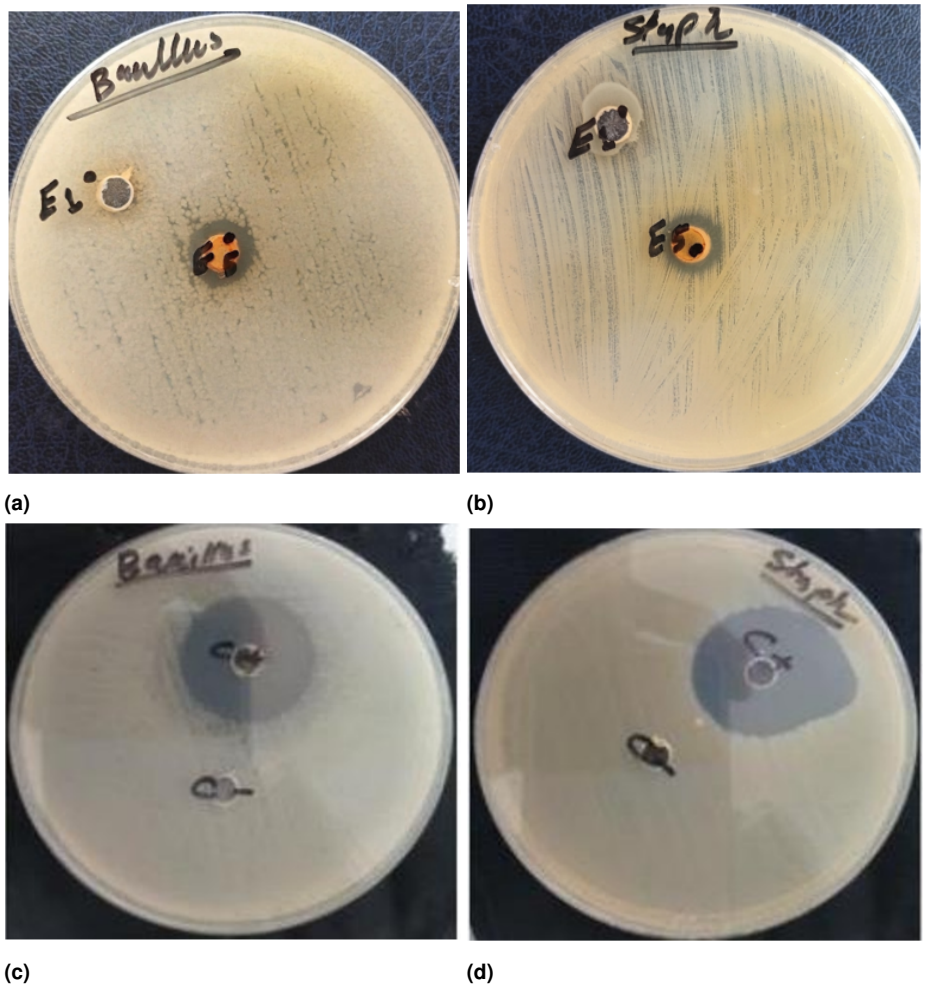

Figure 5. Antimicrobial activity of *Commiphora myrrha* resin fractions. (a) Fractions E1 and E5 against *Bacillus subtilis*; (b) fractions E1 and E5 against *Staphylococcus aureus*; (c) and (d) positive control (Amoxicillin) and negative control (DMSO) corresponding to each bacterial strain.

(a) and (b). E5 (Methanol Soxhlet fraction) displayed a concentration-dependent DPPH radical scavenging activity, where the inhibition at 10 μg/mL was 7.7%, which increased progressively to 80.6% at 1000 μg/mL, indicating good antioxidant capacity. From these values, the IC_{50} value of E5 could be estimated to be approximately 170 μg/mL (it falls between 150 μg/mL showing 46.6% and 350 μg/mL showing 56.6% inhibition), indicating potent antioxidant activity, similar to many known active medicinal plant fractions [63].

The weak antioxidant activity of E1 is shown in Table 8. Even at 1000 µg/mL, the antioxidant activity recorded was only 30.15%, and at 1500 μg/mL, it was only 50.37%, yielding an IC_{50} value of approximately 1480 μg/mL. The resinous bioactive fraction (E5) exhibited substantially higher antioxidant capacity than the polar gum fraction (E1). E5 has strong antioxidant activity due to the presence of sesquiterpenoids, mainly telekin (2.78%), calarene epoxide (2.45%), and 1,1,4,7-Tetramethyldecahydro-1H cyclopropa[e]azulene-4,7-diol (3.51%), as determined using GC-MS. All of these are hydroxyl and carbonyl-containing compounds and are capable of transferring electrons and hydrogen atoms, which can neutralize free radicals [64, 65]. E5 also contains phenolic compounds, as confirmed by phytochemical screening and TPC, which contribute to its potent antioxidant activity [66].

In contrast, E1 primarily comprises fatty acid esters and alkanes, which do not have a hydroxyl group and thus are not as effective in donating electrons to scavenge free radicals [54, 55].

Table 7. DPPH radical scavenging activity of E5 (Methanol Soxhlet fraction)

Concentration (µg/mL)	Inhibition (%)
10	7.7
25	9.8
50	20.1
100	39.1
150	46.6
350	56.6
500	64.5
750	71.1
1000	80.6

$IC_{50} \approx 170 \mu g/mL$

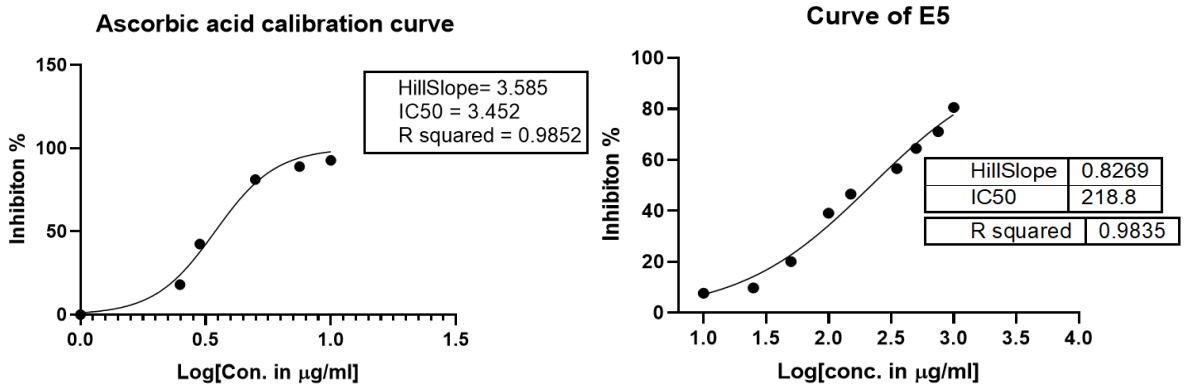

Figure 6. DPPH radical scavenging activity curves of (a) ascorbic acid standard and (b) methanolic resinous fraction (E5).

Table 8. DPPH radical scavenging activity of E1 (Water Maceration fraction)

Concentration (µg/mL)	Inhibition (%)
1000	30.15
1500	50.37

$IC_{50} \approx 1480 \mu g/mL$

3.9. Total Phenolic, Flavonoid, and Tannin Contents

The total phenolic, flavonoid, and tannin contents of E1 and E5 are presented in Table 9. Fraction E5 was significantly enriched in TPC, TFC, and tannins relative to E1; there were 46.04 mg GAE/g of TPC in E5 versus 6.35 mg GAE/g in E1 (7.3 fold). The TFC in E5 was 6.55 mg QE/g versus 0.69 mg QE/g in E1 (9.5 fold), and tannin contents were 19.49 mg TAE/g in E5 versus 4.48 mg TAE/g in E1 (4.4 fold).

These results are consistent with the solubility of the phenolic compounds. Methanol (polarity index 5.1) has been identified as an efficient solvent for the extraction of moderately polar phenolic compounds, flavonoids, and tannins because of its effectiveness in disrupting hydrogen bonding and dissolving polar substances bearing hydroxyl groups [20, 41]. Although the polarity index is higher for water, the solubility of phenolic compounds is lower because water cannot' disrupt hydrophobic interactions in the resinous matrix [9].

The TPC in E5 (46.04 mg GAE/g) was higher than previously reported values for *Commiphora molmol* (a synonym of *C.myrrha*) (39.00 mg GAE/g) [53]. This variation may be due to the different locations and extraction methods. The TFC of E5 (6.55 mg QE/g) is within the range reported for *Commiphora* species [67]. The Tannin content in E5 (19.49 mg TAE/g) indicated that the methanol Soxhlet fraction recovered well, as they have proven to be good candidates for antibacterial, antioxidant, and wound healing agents [68]. As shown in Table 9, these differences were statistically significant ($p < 0.001$ for each comparison, unpaired t-test).

Table 9. Total phenolic, flavonoid, and tannin contents of E1 and E5 fractions

Fraction and method	TPC (mg GAE/g)	TFC (mg QE/g)	Tannin (mg TAE/g)
E1 (Water maceration)	6.35 ± 0.22	0.69± 0.03	4.48 ± 0.10
E5 (Methanol Soxhlet)	46.04 ± 1.38	6.55 ± 0.20	19.49 ± 0.45
p-value (unpaired t-test)	< 0.001	< 0.001	< 0.001

3.10. Correlation Between Phytochemical

Content and Biological Activities

The correlation between phytochemical content and biological activity was demonstrated in the results. E5 exhibited significantly higher TPC, TFC, and tannin content, along with a rich sesquiterpenoid profile, including telekin, calarene epoxide, and reynosin. This fraction also demonstrated potent antimicrobial and antioxidant activities, whereas E1, which contains only negligible phenolics and the majority of fatty acid esters, demonstrated weak antioxidant and no antimicrobial activities.

The strong antioxidant activity of E5 ($IC_{50} \approx 170 \mu g/mL$) correlates well with the highest TPC (46.04 mg GAE/g), as phenolic compounds are widely recognized for their contribution to radical scavenging activity via hydrogen atom donation and electron transfer mechanisms [66, 69]. The contribution of sesquiterpenes, such as telekin, which contains an α,β-unsaturated carbonyl functional group, to the antioxidant activity of E5 may also be significant through potential electron-donating and radical-stabilizing mechanisms [50, 51].

The antimicrobial activity of E5 against *S. aureus*, *B. subtilis*, and *C. albicans* can be attributed to the synergistic effect of the various compounds in the fraction, with sesquiterpenoids disrupting microbial membranes, and phenolics chelating metal ions and inhibiting enzymatic activity [52, 70].

The remarkable activity against *C. albicans* (20 mm)

indicates that E5 is a potentially promising antifungal candidate.

4. CONCLUSION

This study established a comprehensive physicochemical and phytochemical baseline for *Commiphora myrrha* resin from Al-Hudaydah, Yemen. Using a sequential valorization framework, this study delineates the distinct characteristics of two resin fractions: a lipophilic bioactive fraction (E5) and a polar gum fraction (E1).

Phytochemical quantification revealed that E5 contained significantly higher levels of total phenolics (7.3-fold), flavonoids (9.5-fold), and tannins (4.4-fold) than E1. GC-MS profiling confirmed that E5 is rich in bioactive sesquiterpenoids, including the first reported detection of 1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol (3.51%) in Yemeni specimens, whereas E1 is dominated by fatty acid esters. Consequently, E5 exhibited potent antimicrobial activity against *S. aureus*, *B. subtilis*, and *C. albicans*, along with a strong antioxidant capacity ($IC_{50} \approx 170 \mu g/mL$). In contrast, E1 exhibited negligible bioactivity, confirming that the therapeutic potential resides in the lipophilic terpenoid fraction.

These findings support a dual-use strategy: E5 as a promising candidate for pharmaceutical and nutraceutical research and E1 as a potential natural industrial hydrocolloid. Future studies should focus on isolating key sesquiterpenoids and evaluating their *in vivo* safety and mechanisms of action.

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Ethical Compliance

Not applicable. This study does not involve human or animal subjects.

Conflict of Interest

The authors declare no competing financial interests.

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Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Author Contributions

E.A.A.: Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – original draft, Supervision, and Project administration. S.H.A.: Methodology, Formal analysis, Investigation, Data curation, Writing original draft, and Visualization. Q.Y.M.A.: Methodology, Validation, Formal analysis, Data curation, and Visualization. B.A.: Formal analysis and Visualization. K.M.N.: Conceptualization, Investigation, Project administration, and Writing review & editing. All authors contributed to the review and editing of the manuscript and have read and agreed to publish the manuscript.

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