

Chemical Composition Characterization and Potential Medicinal Utilization of Extracts and Pyrolysis Products from *Carpinus cordata* Wood

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Woody plants represent a valuable source of bioactive compounds for medicine. In this study, *Carpinus cordata* wood was extracted with different solvents. The extracts were analyzed using Fourier transform infrared (FTIR), thermogravimetric analysis (TGA), gas chromatography-mass spectrometry (GC-MS), pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), and thermal desorption - gas chromatography - mass spectrometry (TD-GC-MS). The results revealed a diverse array of compounds, including aromatic and aliphatic hydrocarbons, alkanes, aldehydes, ketones, carboxylic acids, and esters. These chemical constituents demonstrate significant potential as feedstocks for bio-oils and novel biomaterials in industrial and agricultural sectors. Furthermore, specific bioactive molecules with potential anti-inflammatory, anticancer, and anti-HIV properties were identified, underlining their promise for pharmaceutical and biomedical agents. Live/dead staining results of ethanol extracts of *C. cordata* wood exhibited significant cytotoxicity against the human chronic myeloid leukemia cell line K-562, indicating the presence of potent anti-cancer constituents. Overall, the multifaceted potential of *C. cordata* wood was shown to be a source of both bioactive extracts for medicine and valuable chemicals through thermochemical conversion.

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INTRODUCTION

Biorefining represents a pivotal strategy for the sustainable utilization of resources, aiming to maximize the extraction of value-added components from renewable biomass while minimizing environmental impact (Long *et al.* 2025). As global interest shifts toward eco-friendly alternatives to fossil-based products, lignocellulosic materials have emerged as critical feedstocks for producing biofuels, biochemicals, and bioactive compounds (Cespi *et al.* 2023; Cherwoo *et al.* 2023). Wood, a primary renewable resource composed of lignin, cellulose, and hemicellulose, holds significant potential beyond traditional applications in construction and energy (Cherwoo *et al.* 2023; Wang *et al.* 2024a; Liu *et al.* 2025). Increasingly, research is focusing on extracting specialized compounds from underutilized wood species for pharmaceutical and functional applications, thereby enhancing both the economic viability and ecological sustainability of forest resources (Bhadange *et al.* 2024).

Carpinus cordata, a deciduous tree native to East Asia, has been historically valued for its dense wood in tool manufacturing and carpentry (Park *et al.* 2020). Despite its wide distribution, scientific investigation into its phytochemical profile and potential medicinal utility has remained remarkably limited. Although some *Carpinus* species have been documented in ethnobotanical contexts for their anti-inflammatory and antimicrobial properties, there has been a need for a comprehensive chemical characterization of *C. cordata* wood, with particular emphasis on its extractable metabolites and thermochemical conversion products (Rzepka *et al.* 2022). This knowledge gap impedes the development of high-value applications and prevents the species from reaching its full biorefinery potential within a circular bioeconomy framework.

Extraction techniques remain fundamental for elucidating the chemical diversity of biomass (Antoun *et al.* 2024). Solvent-based methods, including both polar and non-polar systems, have been successfully used to identify bioactive constituents—such as polyphenols, terpenoids, and fatty acids—in numerous woody species (Budzaki *et al.* 2022; Li *et al.* 2023; Lin *et al.* 2025). For instance, sequential extraction of *Acacia nilotica* leaves revealed tannins and flavonoids with therapeutic relevance, while extracts from *Pterocarpus santalinus* wood yielded over 50 bioactive compounds (Verma *et al.* 2023; Ibrahim *et al.* 2025).

Complementarily, analytical pyrolysis (Py) coupled with gas chromatography-mass spectrometry (Py-GC-MS) offers a rapid and efficient thermochemical approach for deconstructing biomass (Nair *et al.* 2023). This technique serves two key purposes in this context: (a) as an analytical tool to probe the inherent composition and thermal degradation pathways of the wood polymer matrix, thereby complementing solvent extraction data; and (b) to identify and quantify the volatile pyrolyzate compounds which are themselves potential valuable products in a biorefinery setting, either as bio-oil components or as sources of specific chemicals. When combined with thermogravimetric analysis (TGA), this technique provides insights into thermal degradation behavior and identifies key pyrolytic intermediates, such as phenols, furans, and oxygenated hydrocarbons—compounds frequently associated with antioxidant, antimicrobial, and pharmaceutical activities (Neiva *et al.* 2018). Studies on diverse feedstocks, including water hyacinth and agricultural residues, have demonstrated the utility of Py in mapping compound distributions and evaluating valorization pathways for bioresources (Huang *et al.* 2020; Calixto *et al.* 2022).

In this study, the critical research gap concerning *Carpinus cordata* wood was addressed by integrating extraction and pyrolysis methodologies. Soluble constituents were isolated using methanol, ethanol, and a benzene/ethanol mixture (1:1, v/v), while Py-GC-MS and TGA tests were employed to systematically profile the thermal decomposition products and kinetics of the raw wood. The chemical composition was further elucidated using Fourier-transform infrared spectroscopy (FTIR) and GC-MS. The objectives of this work are threefold: (1) to characterize the bioactive constituents in the solvent extracts; (2) to identify and quantify the major pyrolytic compounds; and (3) to evaluate the potential medicinal applications of these fractions based on their bioactive properties. This multidisciplinary approach not only advances the fundamental understanding of *C. cordata* chemistry, but it also provides actionable data for its utilization in the development of pharmaceutical, nutraceutical, or biocidal products—aligning with the commitment for sustainable biomass exploitation.

While this study primarily focused on the fundamental chemical characterization of *C. cordata* wood, the findings can provide critical data for informing future biorefinery strategies. The identification of specific high-value bioactive compounds supports a ‘cascading utilization’ approach within a biorefinery concept. In such a scheme, initial extraction steps could target these medicinally relevant metabolites for nutraceutical or pharmaceutical isolation. Subsequently, the extracted residue, enriched in lignocellulose, remains a valuable feedstock for thermochemical conversion to produce bio-oils or platform chemicals. This work suggests that *C. cordata*, as a single species, possesses a unique chemical portfolio justifying its consideration in dedicated or mixed-feedstock biorefining operations aimed at maximizing co-product diversity and value. Scale-up considerations would involve optimizing sequential extraction parameters and integrating pyrolysis units, guided by the composition data presented.

EXPERIMENTAL

Materials

The *C. cordata* tree was collected from the Laojun Mountain Nature Reserve in Luanchuan County, Luoyang City, Henan Province (sample collection area: longitude 33° 43.04' E, latitude 111° 37.32' N). Wood samples (heartwood) were collected from the same *C. cordata* tree and designated as *Cc*. The samples were ground into powder and stored under vacuum at -3 °C prior to use. Chromatographic-grade methanol, benzene, and ethanol were purchased from Henan Huihong Reagent Co., Ltd. (Henan, China). Anhydrous sodium sulfate was obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). For extraction, aliquots of the wood powder were enclosed in pre-extracted cotton sachets and subjected to sequential 12-hour solvent treatments using pure ethanol and a benzene/ethanol mixture, respectively.

Extraction

Two different solvent systems were employed sequentially: ethanol and ethanol/benzene (1:1, v/v). Approximately 40 g (accurately weighed to 0.1 mg) of *Carpinus cordata* wood powder (60 to 80 mesh) was weighed in triplicate. Each portion was sealed in a cotton bag, secured with cotton thread, and labeled. Extraction was performed using a Soxhlet apparatus with 300 mL of solvent for 5 h at 60 °C. Following extraction, approximately 90% of solvent was removed from each extract by rotary evaporation. The evaporation was conducted under reduced pressure with a water bath temperature maintained at 40 °C for ethanol extracts, and at 35 °C for the benzene/ethanol extract to prevent excessive heating of benzene. The process typically required 45 to 60 min per extract until the volume was reduced to a viscous concentrate. The resulting concentrates were stored at -3 °C for subsequent analysis.

Characterizations

GC/MS analysis

The GC conditions were as follows: Separation was performed on a quartz capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed as follows: initial temperature of 50 °C, increased at 8 °C/min to 250 °C, and then raised at 5 °C/min to 300 °C (0 min hold). The injector temperature was set at 250 °C

with a split ratio of 20:1. High-purity helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The MS conditions were as follows: Electron ionization (EI) was employed at 70 eV. The ion source and quadrupole temperatures were maintained at 230 °C and 150 °C, respectively. Mass spectra were acquired in full-scan mode over a mass range of 30 to 600 m/z. Compounds were identified by comparing the acquired spectra with the Wiley7n.1 library database (Xie *et al.* 2023).

TD/GC/MS analysis

The chromatographic analysis was performed using a thermal desorption (TD) system coupled with gas chromatography (Agilent 8890 GC, Agilent Technologies, USA) and mass spectrometry (Agilent 5977B GC/MSD, Agilent Technologies, USA). Mass selective detection was operated in selected-ion monitoring (SIM) mode.

Py/GC/MS analysis

For *in-situ* analysis of bio-oil components, fast pyrolysis was performed using a CDS Pyroprobe 5000 coupled with an Agilent 7890B/5977A GC/MS system. Samples were rapidly heated to 850 °C at 250 °C/s under helium flow and held at the final temperature for 15 s. The helium served both as an inert atmosphere and as a carrier gas to transfer the volatilized products into the GC/MS. To avoid condensation, the transfer line and injection valve were maintained at 300 °C. Separation was carried out on a TR-5MS capillary column in split mode (split ratio 50:1, total flow 50 mL/min). The GC oven temperature program was as follows: held at 40 °C for 2 min, increased to 120 °C at 5 °C/min, then raised to 200 °C at 10 °C/min and held for 15 min. MS detection employed electron ionization at 230 °C, with a mass scanning range of 28 to 500 amu.

FTIR analysis

Fourier-transform infrared (FTIR) spectroscopy was performed directly on the obtained *Carpinus cordata* extracts. Spectra were acquired using an IR100 FTIR spectrometer with samples prepared as KBr pellets containing 1.00% (w/w) of the finely ground extractive (Li *et al.* 2024).

Thermogravimetric analysis (TGA)

The sample preparation involved grinding the materials to pass through a 100 mesh screen followed by one week of air-drying. For TGA testing, approximately 5 mg of each powdered sample was loaded into a platinum crucible. The thermal decomposition profiles were then obtained using a TGA-Q50 analyzer (TA Instruments, USA). The condition of testing: constant nitrogen flow, ramping the temperature from 30 to 850 °C at respective heating rates of 10, 25, and 50 °C/min (Yang *et al.* 2025).

TGA-FTIR analysis

A TGA-FTIR analysis of *Carpinus cordata* wood was performed using a TA Q500 thermogravimetric analyzer (TA Instruments, USA) coupled to a Fourier-transform infrared spectrometer (Nicolet 6700, USA). For each experiment, approximately 5 mg of sample was used. The temperature program involved heating from room temperature to 850 °C at a rate of 10 °C/min under a nitrogen flow of 60 mL/min, followed by a 5-min isothermal hold at the final temperature. Infrared spectra were continuously recorded in the range of 4000 to 400 cm⁻¹ with a resolution of 4 cm⁻¹, generating three-dimensional FTIR spectrograms (Zhang *et al.* 2020).

Cell culture

The human chronic myeloid leukemia cell line K562 was obtained from the Korean Cell Line Bank (Seoul, South Korea). Cells were cultured in RPMI 1640 medium (GenDEPOT Inc., USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Gibco-BRL, USA). Cultures were maintained at 37 °C in a humidified incubator with 5% CO₂.

Cell live/dead staining

Cell viability was assessed using a live/dead cell staining kit (Beyotime, China) to evaluate the cytotoxic effects of the treatments. Cells were seeded and cultivated according to the previous experimental design. After treatment, the culture medium was removed, and the cells were washed once with PBS. An appropriate volume of Calcein AM/PI working solution was then added (100 µL per well for a 96-well plate) and incubated at 37 °C in the dark for 30 min. Following incubation, the stained cells were immediately observed under a fluorescence microscope. Viable cells exhibited green fluorescence (Calcein AM, Ex/Em = 494/517 nm), while dead cells exhibited red fluorescence (PI, Ex/Em = 535/617 nm). The entire procedure was performed under light-protected conditions.

RESULTS AND DISCUSSION

Characterization of Extracts of *Carpinus cordata* Wood

The total ion chromatogram (TIC) of *Carpinus cordata* wood extract is presented in Fig. 1. The GC/MS analysis identified 48 distinct compounds, corresponding to 95 chromatographic peaks. These constituents encompassed a broad spectrum of chemical classes (acids, esters, amides, phenols, saccharides, aromatic compounds, and ethers). The variation in extract composition obtained with different solvents is attributed to the selective solubility of components based on their polarity (Shaheen *et al.* 2022). The main chemical compositions identified in the ethanol extract of *Carpinus cordata* wood were (Table S1): Lupeol (59.05%), β-Amyrin (20.91%), β-Amyrone (11.63%), 24-Noroleana-3,12-diene (3.03%), Cycloecalenyl acetate (1.38%), Cedran-diol, (8S,14)- (0.85%), Dihydro-β-agarofuran (0.41%), Santalcamphor (0.28%), and trans-Sinapyl alcohol (0.22%).

The main bioactive chemical constituents of the ethanol/benzene extracts of *Carpinus cordata* wood were (Table S2): lupeol (42.93%), β-amyrin (26.14%), lupenone (16.29%), γ-sitosterol (5.91%), 1,2-bis(trimethylsilyl)benzene (1.67%), α-amyrin (1.43%), urs-9(11),12-dien-3-ol (1.09%), 26-norcoprostan-16,22-epoxy-3α-ol-25-one (1.22%), cycloecalenyl acetate (0.96%), dibutyl phthalate (0.68%), dihydro-β-agarofuran (0.3%), α-tocopheryl acetate (0.28%), trans-sinapyl alcohol (0.15%), and clopidogrel (0.12%).

The diverse chemical profile of *C. cordata* wood underpins its potential for multiple applications. Specifically, acidic compounds (e.g. 9-hexadecenoic acid) serve as raw materials for soaps, lubricants, and synthetic detergents. Esters function as intermediate in emulsifiers, wetting agents, stabilizers, and plasticizers such as hexadecanoic acid and methyl ester. Phenols can be used as raw material for resins, synthetic fibers, oil refining, plastics, pharmaceuticals, and pesticides. Aromatic compounds can be used as building blocks to synthesize more complex compounds *via* substitution reactions. The olefin compounds are important feedstocks materials in organic synthesis for producing of

polyolefins and synthetic rubbers. Consequently, the extracts of *Carpinus cordata* wood represent valuable feedstocks for bio-oil, pharmaceutical, and biomedical production, with additional prospects as novel materials in various industrial and agricultural applications.

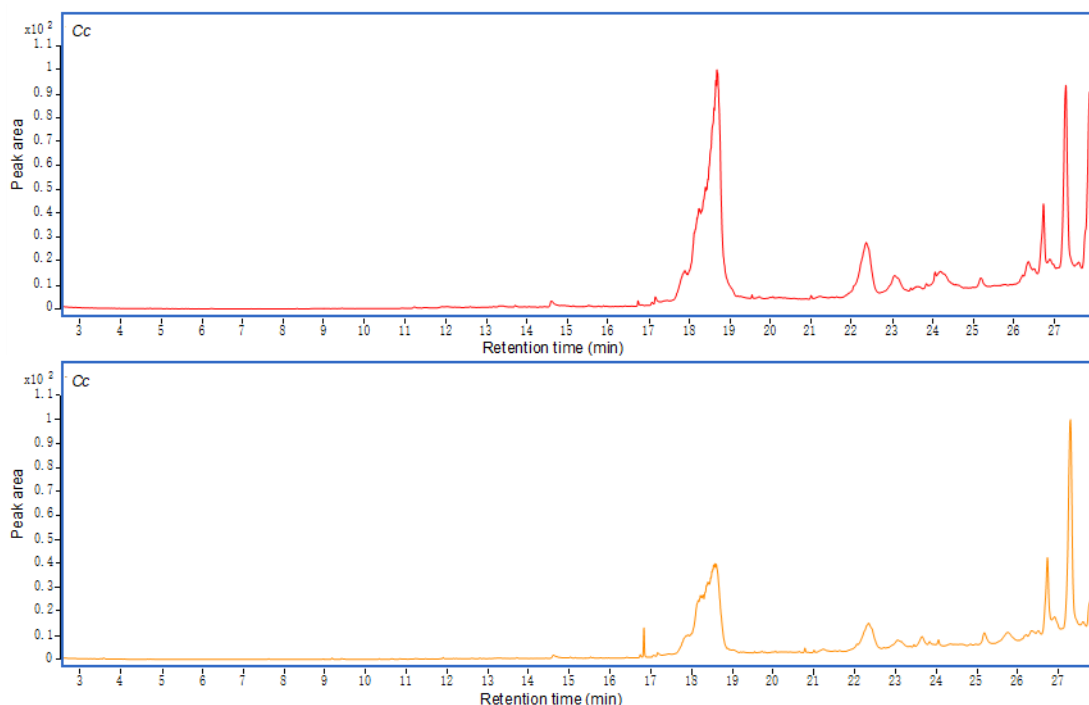


Fig. 1. Typical ion chromatograms of the *Carpinus cordata* wood extractives analysis by GC-MS

In summary, the results demonstrate that *Carpinus cordata* wood encompasses a diverse array of chemical constituents, including aromatics, aliphatics, aldehydes, ketones, carboxylic acids, and esters. These bioactive compounds exhibit promising potential as pharmaceutical and biomedical agents. Furthermore, the extractives of *Carpinus cordata* wood show broad prospects as a renewable raw material for applications across various industrial and agricultural sectors.

TD-GC-MS Analysis

As shown in Fig. 2 and Table S3 (Supplementary information), the TD-GC-MS analysis results identified 111 chemical compounds in 147 peaks of *C. cordata* wood. The high-content pyrolysis products were acetic acid (6.56%), benzyl alcohol (5.48%), undec-10-ynoic acid, decyl ester (4%), phthalic acid, 2-cyclohexylethyl isobutyl ester (3.89%), undec-10-ynoic acid, dodecyl ester (3.64%), trans-isoeugenol (3.58%), glutaric acid, butyl undecyl ester (2.54%), undec-10-ynoic acid, tetradecyl ester (1.81%), DL-arabinose (1.75%), nerolidol acetate (1.72%), l-gala-l-ido-octose (1.66%), oleic acid (1.65%), benzaldehyde (1.59%), dibutylformamide (1.46%), diisobutyl succinate (1.28%), 7-octene-1,2-diol (1.27%), butyl isodecyl phthalate (1.26%), cyclohexene, 1-hexyl- (1.17%), cycloheptasiloxane, tetradecamethyl- (1.09%), tetradecanoic acid (1.05%), E-8-methyl-9-tetradecen-1-ol acetate (0.93%), glycidyl palmitoleate (0.91%), cyclohexanone, 4-hydroxy- (0.9%), triethylene glycol monododecyl ether (0.84%), 2-methyl-Z-4-tetradecene (0.71%), acetylenic glycol (0.7%), 5-hydroxy-7-methoxyflavanone (0.68%), 1-decanamine (0.6%), cis-7-hexadecenoic acid (0.68%), vanillin lactoside (0.65%), 1-

eicosanol, TMS derivative (0.57%), 7-isopropyl-7-methyl-nona-3,5-diene-2,8-dione (0.62%), benzenemethanol, α -(1-ethenylpentyl)- α -methyl- (0.47%), benzene (0.41%), and 2H-pyran, 3,4-dihydro- (0.4%).

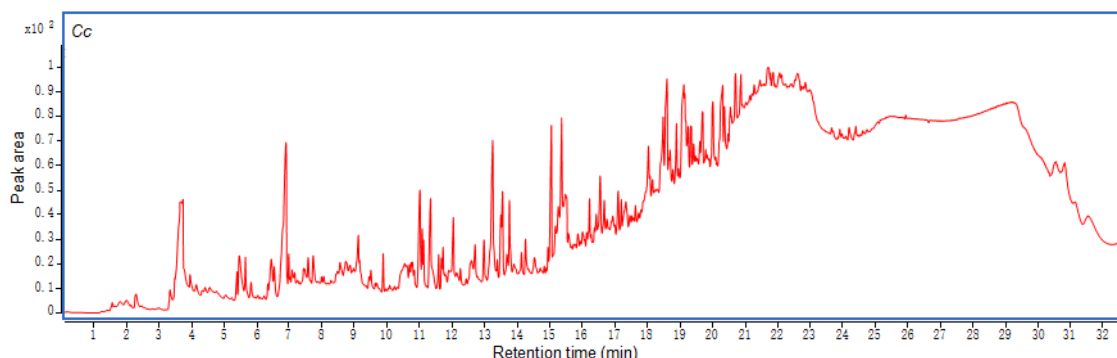


Fig. 2. Typical ion chromatograms of *C. cordata* wood samples analysis by TD-GC-MS

Analysis of the pyrolysis products revealed a diverse profile of constituents with multiple medicinal and bioactive potentials. Key components and their proposed functions include: Benzyl alcohol (5.48%), a widely used antimicrobial preservative; trans-isoeugenol (3.58%), known for its anti-inflammatory and analgesic properties (Zhang *et al.* 2025); oleic acid (1.65%), which exhibits anti-inflammatory activity and enhances transdermal absorption (Hashmat *et al.* 2020); benzaldehyde (1.59%), possessing antimicrobial and antitumor activities (Mota-Gutierrez *et al.* 2019); and DL-arabinose (1.75%), which can inhibit intestinal sucrase activity (Cheng *et al.* 2011), suggesting its potential role in glucose metabolism regulation. A series of Undec-10-ynoic acid esters (collectively > 9%) showed promising antibacterial and wound-healing effects (Al-Warhi *et al.* 2022). Additionally, Glutaric acid esters, phthalate esters, and various higher fatty alcohols may serve as drug carriers or excipients capable of modulating drug release and stability. Collectively, these components underline the potential application of the pyrolysis products in antimicrobial, anti-inflammatory agents, transdermal drug delivery systems, and metabolic regulators.

FTIR and TGA-FTIR Analysis of *Carpinus cordata* Wood

Fourier-transform infrared spectroscopy is a well-established technique for identifying functional groups in chemical constituents. In this study, FTIR was employed to characterize the chemical structures of ethanol (D-1) and ethanol/benzene (D-2) extracts from *C. cordata* wood. As shown in Fig. 3a, the spectra of D-1 and D-2 correspond to the respective extracts. The spectral profiles of D-1 and D-2 were nearly identical, indicating a high similarity in their chemical compositions. The broad absorption band at 3378 cm^{-1} is assigned to the stretching vibration of -OH in aromatics or phenolic (Eze *et al.* 2019; Wang *et al.* 2025; Li *et al.* 2026; Lei *et al.* 2025). The strong peaks in the range of 2921 to 2852 cm^{-1} are attributed to C-H stretching vibrations of -CH_3 , -CH_2 -, and -CH- groups (Pei *et al.* 2023; Li *et al.* 2024). The absorbance peaks at 1734 cm^{-1} and 1710 cm^{-1} correspond to carboxyl-carbonyl, suggesting the existing of esters, ketones or acid compounds in the extracts of *Carpinus cordata* wood (Oyekanmi *et al.* 2021; Wang *et al.* 2025).

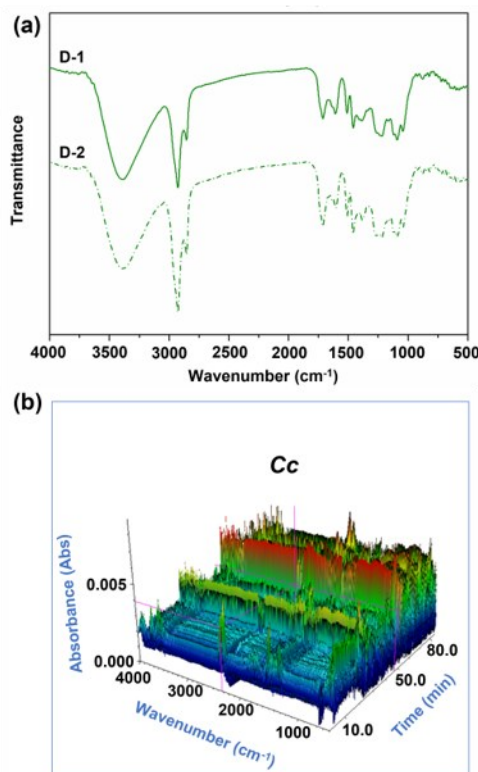


Fig. 3. (a) FTIR spectra of the ethanol and ethanol/benzene extracts of *Carpinus cordata* wood; (b) 3D FTIR spectrograms of pyrolysis volatiles of *C. cordata* wood (Cc)

The peaks at 1610 cm^{-1} , 1509 cm^{-1} , and 1457 cm^{-1} belong to in plane deformation vibrations of the C-H bond in benzene rings (Li *et al.* 2024a), confirming the presence of aromatic compounds. The peak at 1379 cm^{-1} is assigned to phenolic O-H bending or aliphatic C-H deformation in methyl groups, commonly associated with lignin and tannin structures (Ferreira-Santos *et al.* 2020). Bands at 1056 cm^{-1} and 920 cm^{-1} are ascribed to C-O and C-H stretching vibrations, respectively (Li *et al.* 2022). Finally, the band at 821 cm^{-1} is characteristic of C-H out-of-plane deformation in aromatic rings (Sidorowicz *et al.* 2021; Li *et al.* 2024).

A thermogravimetric analyzer coupled with an FTIR spectrometer (TGA-FTIR) was employed for the online monitoring of volatile products during pyrolysis. The three-dimensional FTIR spectrograms of the pyrolysis vapors from *Carpinus cordata* wood are presented in Fig. 3b. The typical functional group vibrations were visible: O-H stretch (peak at 3600 to 3500 cm^{-1}), C-H stretch in aromatic compounds (absorbance at 2950 to 3020 cm^{-1}), C^{2+} aliphatics (peak at 2967 cm^{-1}), CO_2 (peak at 2280 to 2350 cm^{-1}), CO (absorbance at 2090 to 2280 cm^{-1}), C=O (peak at 1750 to 1712 cm^{-1}), aromatic skeleton stretch (1410 to 1680 cm^{-1}), C_2H_4 (peak at 1450 cm^{-1}), and C-O (peak in the range 1000 to 1260 cm^{-1}) (Rodriguez *et al.* 2018; Liu *et al.* 2020; Thepbandit *et al.* 2021; Huwaimel *et al.* 2025; Li *et al.* 2025). Based on Fig. 3b, the composition of gaseous products was identified, including CO_2 , CO, H_2O , carbonyl compounds, and aromatic compounds. Hydrogen (H_2), being a homonuclear diatomic molecule, is infrared-inactive and thus could not be detected by FTIR (Kärkelä *et al.* 2022). The main release of gases occurred between 18 and 60 min, corresponding to a temperature range of approximately 200 to $600\text{ }^\circ\text{C}$, which is consistent with the derivative thermogravimetry (DTG) data.

Py-GC-MS Analysis of *Carpinus cordata* Wood

Pyrolysis of biomass at high temperatures (typically 300 to 800 °C) is a complex process governed by a network of parallel and competing reactions, including decomposition, dehydration, decarboxylation, and decarbonylation, which collectively determine the product distribution (Ren *et al.* 2020). Py-GC-MS analysis of *Cc* sample detected 138 peaks and 97 chemical compounds (Table S4). The high-content pyrolysis products were cyclopropyl carbinol (12.28%), acetic acid (11.32%), 4-pentenol (5.37%), tropilidene (4.49%), butadienylacetylene (4.35%), 1,3-cyclopentadiene (3.35%), pseudoheptyl alcohol (3.31%), 3-vinyl-1-cyclobutene (2.88%), trans,trans-hexa-2,4-dienyl acetate (2.49%), phenol, 3-methyl- (2.46%), 2-penten-1-ol, (E)- (2.21%), 3-hexen-1-ol, acetate, (E)- (2.19%), indane (1.86%), acetaldol (1.76%), acetol (1.75%), benzocyclobutene (1.68%), furfural (1.61%), butanenitrile, 2,3-dioxo-, dioxime, O,O'-diacetyl- (1.34%), dihydrojasnone (1.19%), myrtenol (1.18%), p-xylene (1.15%), phenol (1.14%), tert-hexadecanethiol (0.97%), 1H-indene, 3-methyl- (0.96%), ethylbenzene (0.95%), 2-acetyl-2-methyl-succinonitrile (0.92%), 3,8 dioxatricyclo[5.1.0.0(2,4)]octane, 4-ethenyl- (0.82%), benzene, (1-methylethyl)- (0.77%), 3-cyclohexene-1-methanol (0.75%), 1-decen-4-yne, 2-nitro- (0.69%), 3-carene-10-al (0.69%), 3-methylenecyclohexene (0.67%), 3-ethylphenol (0.66%), methyl isobutylacetylene (0.66%), 2,6-xyleneol (0.65%), 5,7-dodecadiyn-1,12-diol (0.62%), panaxydol (0.62%), tert-hexadecanethiol (0.51%), 1-octanol (0.47%), o-xylene (0.44%), 6-nonynoic acid (0.44%), 2-nitrobutanol (0.44%), butanoic acid, 3-[(1-phenylethyl-2-propynyl)oxy] (0.43%), bicyclo[2.2.0]hex-1-yl-methanol (0.42%), benzene, 1-propenyl- (0.42%), and pyridine borane (0.42%).

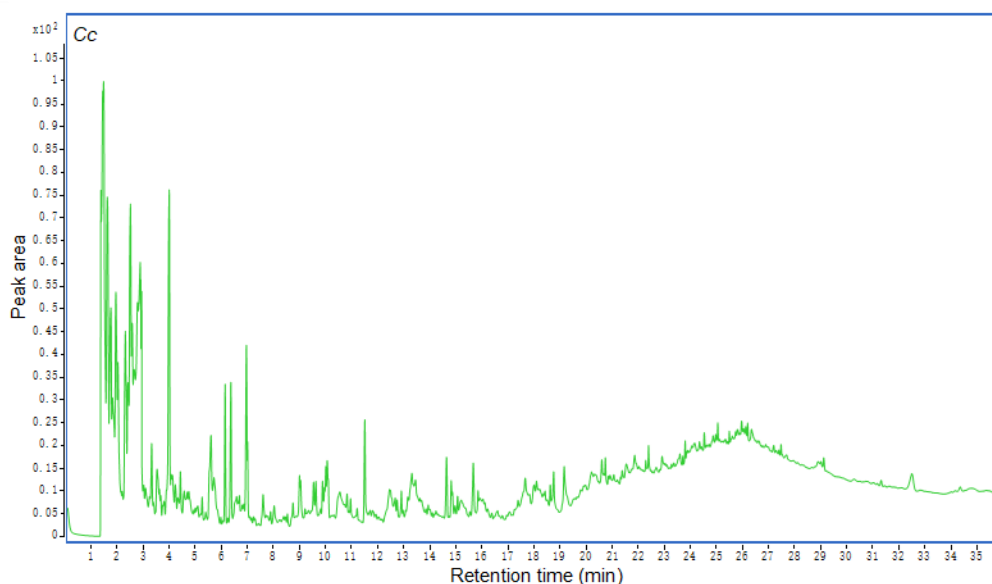


Fig. 4. Ion chromatograms of *C. cordata* wood sample analysis by Py-GC-MS

Notably, over 60% of identified compounds belong to bioactive classes. These included alcohols and carboxylic acids (1-octanol, butanoic acid derivatives) with membrane-disrupting antimicrobial properties; cyclic hydrocarbons (indane, azulene) which are known for cyclooxygenase inhibition (Jan-Roblero *et al.* 2023); and nitrogen-containing compounds (butanenitrile derivatives) which have been implicated in enzyme inhibition (Ibrahim *et al.* 2018). This chemical diversity—particularly the abundance of

antimicrobial alcohols (> 20% total), antioxidant phenolics, and terpenoid intermediates—suggests significant potential for medicinal applications, such as in antibacterial agents, anti-inflammatory formulations, or as precursors for pharmaceutical synthesis.

According to the Py-GC-MS results of *Cc* samples, a total of 97 compounds were identified, respectively. This result indicated that the pyrolysis products of the wood of *Carpinus cordata* have many active compounds.

Thermogravimetric Analysis

The thermogravimetric (TGA) and derivative thermogravimetric (DTG) curves of *Carpinus cordata* wood (*Cc*) at heating rates of 10, 25, and 50 °C/min are presented in Fig. 5. All samples underwent pyrolysis in three distinct stages. The first stage involved the evaporation of moisture and light volatiles. The major mass loss occurred in the second stage, which can be primarily attributed to the decomposition of hemicellulose, cellulose, lignin, and extractives (Burhenne *et al.* 2013; Chen *et al.* 2015). Among these, hemicellulose is the least thermally stable, commencing decomposition around 200 °C due to its lower degree of polymerization compared to cellulose and lignin (Nurazzi *et al.* 2021). In the third stage, weight loss slowed considerably and was relatively minor, resulting from the slow carbonization of residual lignin and solid chars derived from cellulose or hemicellulose (Wang *et al.* 2024b).

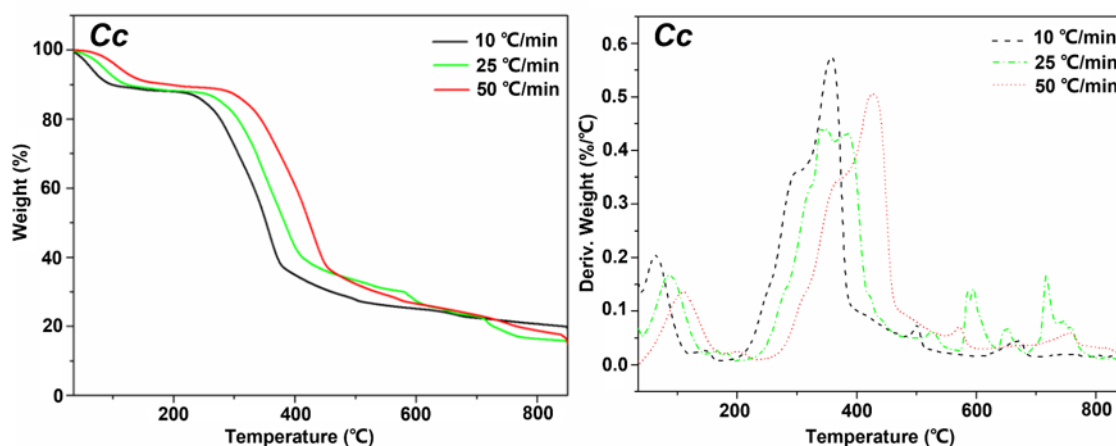


Fig. 5. TGA (a) and DTG (b) curves of *C. cordata* wood (*Cc*) with different heating rates: 10 °C/min; 25 °C/min; 50 °C/min

From Fig. 5b, it can be observed that the residual carbon mass of all samples *Cc* at 850 °C was similar, despite the different heating rates. In addition, as the heating rate increased, the temperatures corresponding to maximum pyrolysis rate of the sample in different stages also increased. For the *Cc* samples, the temperature of the maximum cracking rate increased with increasing heating rate, while the amount of residual carbon showed a decreasing trend. The pyrolysis characteristics differ among samples primarily due to variations in their chemical composition.

Anti-cancer Cell Experiment

The Live/Dead cell staining assay is a fluorescence-based method for assessing viability and cytotoxicity, relying on the parallel detection of intracellular esterase activity and plasma membrane integrity (Vikramdeo *et al.* 2025). In viable cells, the non-

fluorescent calcein AM is hydrolyzed by intracellular esterases to generate green fluorescent calcein (Ex/Em = 494/517 nm). Conversely, propidium iodide (PI), a red fluorescent nucleic acid dye (Ex/Em = 535/617 nm), can only penetrate cells with compromised membranes, thereby selectively staining dead cells (Suo *et al.* 2022). This allows for the direct quantification of viable and dead cell populations. The method has been extensively applied in bioactivity screening of compounds, large-scale anti-tumor drug screening, cytotoxicity assays, and tumor radio sensitivity measurement (De Simone *et al.* 2013; Aveic *et al.* 2018; Li *et al.* 2024b). In this study, the human chronic myeloid leukemia cell line K562 was used to evaluate the anticancer activity of *Carpinus cordata* wood extracts. Preliminary screening indicated that these extracts exhibited activity against K562 cells. It should be noted that other tested extracts did not show inhibitory effects on the growth of these cancer cells.

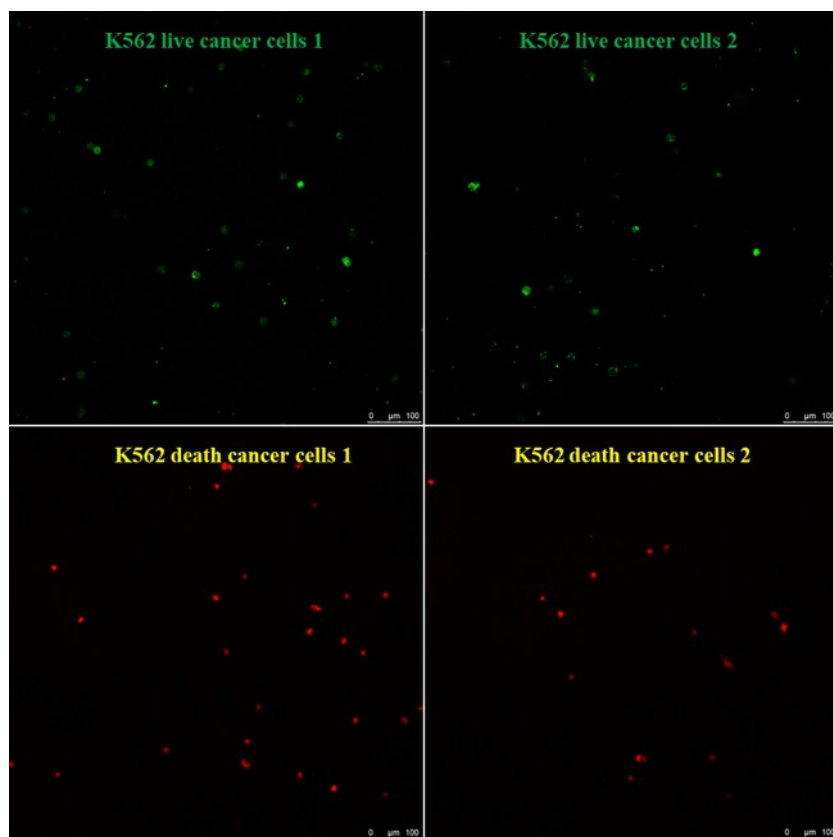


Fig. 6. Fluorescent micrographs of live and dead K562 cells

As shown in Fig. 6, live cells are indicated by green fluorescence (stained with a viable cell dye), while dead cells are indicated by red fluorescence (stained with a dead cell dye). The fluorescent micrographs demonstrate that treatment with *Carpinus cordata* wood extracts resulted in significant cell death in the K562 culture. This suggests that the extracts contain bioactive components capable of inhibiting the growth and viability of K562 cancer cells.

CONCLUSIONS

1. Solvent extraction revealed that *C. cordata* heartwood is rich in a variety of bioactive constituents. Notably, terpenoids and sterols such as lupeol, β -sitosterol, and cyclooleucanyl acetate were identified as major components in both ethanol and benzene/ethanol extracts. These compounds, which are known for their anti-inflammatory and anticancer activities, represent promising targets for subsequent isolation, purification, and pharmacological validation. The significant cytotoxicity exhibited by the ethanol extract against the K-562 human chronic myeloid leukemia cell line further corroborates the presence of potent anticancer constituents. This biological activity highlights high-abundance triterpenoids including lupeol as particularly worthy of in-depth investigation.
2. The significant cytotoxicity exhibited by the ethanol extract against the K-562 human chronic myeloid leukemia cell line further corroborates the presence of potent anticancer constituents. This biological activity highlights high-abundance triterpenoids such as lupeol as particularly worthy of in-depth investigation.
3. Analytical pyrolysis demonstrated that the pyrolysis oil derived from *C. cordata* wood is abundant in phenolic compounds (*e.g.*, phenol, cresols, guaiacol), furfural, and levoglucosan. These compounds, especially phenolics which are characteristic products of lignin decomposition, are not only valuable for chemical fingerprinting but also represent marketable chemicals or precursors for bio-oil refining.
4. From a biorefinery perspective, this work proposes a cascading utilization strategy for *C. cordata*: prioritizing the extraction of high-value bioactive molecules, followed by thermochemical conversion of the residual biomass into chemical feedstocks. Future work should focus on the scaled-up isolation, structural modification, and comprehensive in vitro and in vivo pharmacodynamic evaluation of the aforementioned high-abundance bioactive compounds (*e.g.*, lupeol). Concurrently, process optimization for the integrated valorization pathway—from extractive residues to pyrolysis products—should be pursued.

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REFERENCES CITED

- Al-Warhi, T., Elmaidomy, A.H., Selim, S., Al-Sanea, M.M., Albqmi, M., Mostafa, E.M., Ibrahim, S., Ghoneim, M.M., Sayed, A.M., and Abdelmohsen, U.R. (2022). “Bioactive Phytochemicals of Citrus reticulata Seeds—An Example of Waste Product Rich in Healthy Skin Promoting Agents,” *Antioxidants* 11, 984. <https://doi.org/10.3390/antiox11050984>

- Antoun, K., Tabib, M., Salameh, S. J., Koubaa, M., Ziegler-Devin, I., Brosse, N., and Khelfa, A. (2024). "Isolation and structural characterization of natural deep eutectic solvent lignin from brewer's spent grains," *Polymers* 16(19), article 2791. <https://doi.org/10.3390/polym16192791>
- Aveic, S., Pantile, M., Polo, P., Sidarovich, V., and Tonini, G. P. (2018). "Autophagy inhibition improves the cytotoxic effects of receptor tyrosine kinase inhibitors," *Cancer Cell International* 18(1), article 63. <https://doi.org/10.1186/s12935-018-0557-4>
- Bhadange, Y. A., Carpenter, J., and Saharan, V. K. (2024). "A comprehensive review on advanced extraction techniques for retrieving bioactive components from natural sources," *ACS Omega* 9(29), 31274-31297. <https://doi.org/10.1021/acsomega.4c02718>
- Budzaki, S., Velic, N., Ostojcic, M., Stjepanovic, M., Rajs, B. B., Seres, Z., Maravic, N., Stanojevic, J., Hessel, V., and Strelec, I. (2022). "Waste management in the agri-food industry: The conversion of eggshells, spent coffee grounds, and brown onion skins into carriers for lipase immobilization," *Foods* 11(3), article 409. <https://doi.org/10.3390/foods11030409>
- Burhenne, L., Messmer, J., Aicher, T., and Laborie, M.-P. (2013). "The effect of the biomass components lignin, cellulose and hemicellulose on TGA and fixed bed pyrolysis," *Journal of Analytical and Applied Pyrolysis* 101, 177-184. <https://doi.org/10.1016/j.jaap.2013.01.012>
- Calixto, G. Q., Melo, D. M. A., Melo, M. A. F., and Braga, R. M. (2022). "Analytical pyrolysis (Py-GC/MS) of corn stover, bean pod, sugarcane bagasse, and pineapple crown leaves for biorefining," *Brazilian Journal of Chemical Engineering* 39(1), 137-146. <https://doi.org/10.1007/s43153-021-00099-1>
- Cespi, D., Luque, R., Len, C., and Cucciniello, R. (2023). "Editorial: Biorefinery chemicals: Trend, sources and metrics," *Frontiers in Chemistry* 11, article 1268526. <https://doi.org/10.3389/fchem.2023.1268526>
- Chen, Z., Hu, M., Zhu, X., Guo, D., Liu, S., Hu, Z., Xiao, B., Wang, J., and Laghari, M. (2015). "Characteristics and kinetic study on pyrolysis of five lignocellulosic biomass via thermogravimetric analysis," *Bioresour Technology* 192, 441-150. <https://doi.org/10.1016/j.biortech.2015.05.062>
- Cheng, H., Wang, H., Lv, J., Jiang, M., Lin, S., and Deng, Z. (2011). "A novel method to prepare L-Arabinose from xylose mother liquor by yeast-mediated biopurification," *Microbial Cell Factories* 10(1), 43. <https://doi.org/10.1186/1475-2859-10-43>
- Cherwoo, L., Gupta, I., Flora, G., Verma, R., Kapil, M., Arya, S. K., Ravindran, B., Khoo, K. S., Bhatia, S. K., Chang, S. W., Ngamcharussrivichai, C., and Ashokkumar, V. (2023). "Biofuels an alternative to traditional fossil fuels: A comprehensive review," *Sustainable Energy Technologies and Assessments* 60, article 103503. <https://doi.org/10.1016/j.seta.2023.103503>
- De Simone, U., Manzo, L., Ferrari, C., Bakeine, J., Locatelli, C., and Coccini, T. (2012). "Short and long-term exposure of CNS cell lines to BPA-f a radiosensitizer for boron neutron capture therapy: Safety dose evaluation by a battery of cytotoxicity tests," *NeuroToxicology* 35, 84-90. <https://doi.org/10.1016/j.neuro.2012.12.006>
- Eze, F. N., Tola, A. J., Nwabor, O. F., and Jayeoye, T. J. (2019). "Centella asiatica phenolic extract-mediated bio-fabrication of silver nanoparticles: characterization, reduction of industrially relevant dyes in water and antimicrobial activities against foodborne pathogens," *RSC Advances* 9(65), 37957-37970. <https://doi.org/10.1039/c9ra08618h>

- Ferreira-Santos, P., Genisheva, Z., Botelho, C., Santos, J., Ramos, C., Teixeira, J. A., and Rocha, C. M. R. (2020). "Unravelling the biological potential of *Pinus pinaster* bark extracts," *Antioxidants* 9(4), article 334. <https://doi.org/10.3390/antiox9040334>
- Hashmat, D., Shoaib, M.H., Ali, F.R., and Siddiqui, F. (2020). "Lornoxicam controlled release transdermal gel patch: Design, characterization and optimization using co-solvents as penetration enhancers," *Plos One* 15(2), e0228908. <https://doi.org/10.1371/journal.pone.0228908>
- Hobani, Y. H. (2022). "Cytotoxicity of mahanimbine from curry leaves in human breast cancer cells (MCF-7) via mitochondrial apoptosis and anti-angiogenesis," *Molecules* 27(3), article 971. <https://doi.org/10.3390/molecules27030971>
- Huang, H. Y., Liu, J. Y., Liu, H., Evrendilek, F., and Buyukada, M. (2020). "Pyrolysis of water hyacinth biomass parts: Bioenergy, gas emissions, and by-products using TG-FTIR and Py-GC/MS analyses," *Energy Conversion and Management* 207, article 112552. <https://doi.org/10.1016/j.enconman.2020.112552>
- Huwaimel, B., and Alqarni, S. (2025). "A DFT investigation of the adsorption mechanism of paclitaxel on functionalized graphene oxide for enhanced drug delivery," *Scientific Reports* 15(1), article 13889. <https://doi.org/10.1038/s41598-025-99156-9>
- Ibrahim, B. M. M., Yousuf, A. F., El-Shawwa, M. M., and Mohammed, M. A. (2025). "A pharmaco-metabolomics study of *Glycyrrhiza glabra*, *Boswellia sarca*, and *Acacia nilotica* in acute allergic dermatitis," *Inflammopharmacology* 33(5), 2793-2814. DOI: <https://doi.org/10.1007/s10787-025-01761-7>
- Ibrahim, H.M., Arafa, W.A.A., and Behbehani, H. (2018). "L-Proline catalyzed one-pot synthesis of polysubstituted pyridine system incorporating benzothiazole moiety via sustainable sonochemical approach," *Rsc Advances* 8(66), 37606-37617. <https://doi.org/10.1039/c8ra07013j>
- Jan-Roblero, J., and Cruz-Maya, J.A. (2023). "Ibuprofen: Toxicology and Biodegradation of an Emerging Contaminant," *Molecules* 28, 2097. <https://doi.org/10.3390/molecules28052097>
- Kärkelä, T., Tapper, U., and Kajolinna, T. (2022). "Comparison of 3R4F cigarette smoke and IQOS heated tobacco product aerosol emissions," *Environmental Science and Pollution Research* 29(18), 27051-27069. <https://doi.org/10.1007/s11356-021-18032-x>
- Lei, Z., Shao, J., Li, C., Jiang, S., Yao, M., and Li, J. (2025). "Skin-inspired durable and cost-effective biomass-based supramolecular adhesives," *Advanced Functional Materials* 35(30), article 2501624. <https://doi.org/10.1002/adfm.202501624>
- Li, C., Zhang, X., Zhou, C., Yang, F., Liang, J., Gu, H., Wang, J., Wang, F., Peng, W., Guo, J., and Li, H. (2024). "Performance and mechanism of a novel bamboo-based magnetic biochar composite for efficient removal of norfloxacin," *Advanced Composites and Hybrid Materials* 8(1), article 71. <https://doi.org/10.1007/s42114-024-01142-8>
- Li, C., Tang, Y., Wang, Y., Yuan, X., Zhang, B., Wu, Z., and Tian, H. (2022). "A novel environment-friendly adhesive based on recycling of *Broussonetia papyrifera* leaf forestry waste protein," *Forests* 13, article 291. <https://doi.org/10.3390/f13020291>
- Li, C., Li, M., Li, Z., Guo, P., Zhao, Z., Lu, W., Li, J., Liang, J., Tang, Y., Ge, S., and Wang, F. (2024). "Cleaner production of liquefied biomass-based phenol-formaldehyde resin with improved properties via catalyzed copolymerization," *Advanced Composites and Hybrid Materials* 8(1), article 101. <https://doi.org/10.1007/s42114-024-01195-9>

- Li, C., Li, Z. G., Wang, L. H., Xue, X. B., Xiao, F., Guo, J. H., Li, J. J., Dong, Y. M., Li, J. Z., and Bao, C. Y. (2026). "High-performance phenolic resin reinforced by tannic acid-polyethyleneimine functionalized multi-walled carbon nanotubes for wood-based panels," *Macromolecular Rapid Communications* 47(2), Pmid 9888239. <https://doi.org/10.1002/marc.202500814>
- Li, H. Y., Zhou, C., Wang, L. H., Yang, F., Liang, J. Y., Wang, F., Li, P. Y., Li, C., Wu, Z. G., and Ren, T. B. (2024). "A novel eco-friendly bamboo-based composite biochar for effective removing oxytetracycline hydrochloride," *Advanced Composites and Hybrid Materials* 8(1), article 91. <https://doi.org/10.1007/s42114-024-01186-w>
- Li, J. J., Bian, R. H., Liu, P., Wang, Z. Q., Lyu, Y., Li, X. N., Luo, J., and Li, J. Z. (2024a). "A tough, strong, and fast-curing phenolic resin enabled by quercetin-functionalized hyperbranched polymer," *Industrial Crops and Products* 222, article 119721. <https://doi.org/10.1016/j.indcrop.2024.119721>
- Li, Q.-Z., Chen, Y.-Y., Liu, Q.-P., Feng, Z.-H., Zhang, L., and Zhang, H. (2024). "Cucurbitacin B suppresses hepatocellular carcinoma progression through inducing DNA damage-dependent cell cycle arrest," *Phytomedicine* 126, article 155177. <https://doi.org/10.1016/j.phymed.2023.155177>
- Li, X. D., Li, L., Liu, X. H., Xu, J. Q., Chu, X. Y., Chen, G. B., Li, D. Q., Wang, M. C., Wang, X., Naisa, C., Gao, J., Sun, Y. F., Grätzel, M., and Feng, X. L. (2025). "Designing multi-metal-site nanosheet catalysts for CO₂ photoreduction to ethylene," *Nature Communications* 16(1), article 6500. <https://doi.org/10.1038/s41467-025-61850-7>
- Li, X. J., Guo, M. M., Xue, Y. L., and Duan, Z. Q. (2023). "Effect of extraction methods on the physicochemical properties, chemical composition, and antioxidant activities of samara oil," *Foods* 12(17), article 3163. DOI: <https://doi.org/10.3390/foods12173163>
- Lin, X., Li, H., Wang, T., Xiao, X., and Huang, B. (2025). "The traditional uses, nutrition, phytochemistry, and pharmacology of the medicinal *Abrus* species: A comprehensive review," *Chemistry & Biodiversity* 22(11), article e00182. <https://doi.org/10.1002/cbdv.202500182>
- Liu, S. H., Chen, C. C., Zhang, B., and Wu, J. H. (2020). "Fire and explosion hazards of 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide," *RSC Advances* 10(38), 22468-22479. <https://doi.org/10.1039/d0ra01821j>
- Liu, Y., Zhang, X., Du, Y., Du, X., Zhang, Y., Deng, L., Li, C., and Guo, J. (2025). "Functionalization of wood for the removal of heavy metal ions from waste water: A review," *Forests* 16(11), article 1684. <https://doi.org/10.3390/f16111684>
- Long, B., Zhang, F., Dai, S. Y., Foston, M., Tang, Y. J., and Yuan, J. S. (2025). "Engineering strategies to optimize lignocellulosic biorefineries," *Nature Reviews Bioengineering* 3(3), 230-244. <https://doi.org/10.1038/s44222-024-00247-5>
- Mota-Gutierrez, J., Barbosa-Pereira, L., Ferrocino, I., and Cocolin, L. (2019). "Traceability of Functional Volatile Compounds Generated on Inoculated Cocoa Fermentation and Its Potential Health Benefits," *Nutrients* 11, 884. <https://doi.org/10.3390/nu11040884>
- Nair, R. R., Kissling, P. A., Schaate, A., Marchanka, A., Shamsuyeva, M., Behrens, P., and Weichgrebe, D. (2023). "The influence of sample mass (scaling effect) on the synthesis and structure of non-graphitizing carbon (biochar) during the analytical pyrolysis of biomass," *RSC Advances* 13(20), 13526-13539. <https://doi.org/10.1039/d3ra01911j>

- Neiva, D. M., Araújo, S., Gominho, J., Carneiro, A. D., and Pereira, H. (2018). "An integrated characterization of *Picea abies* industrial bark regarding chemical composition, thermal properties and polar extracts activity," *Plos One* 13(11), article e0208270. <https://doi.org/10.1371/journal.pone.0208270>
- Nurazzi, N. M., Asyraf, M. R. M., Khalina, A., Abdullah, N., Aisyah, H. A., Rafiqah, S. A., Sabaruddin, F. A., Kamarudin, S. H., Norrrahim, M. N. F., Ilyas, R. A., and Sapuan, S. M. (2021). "A review on natural fiber reinforced polymer composite for bullet proof and ballistic applications," *Polymers* 13(4), article 646. <https://doi.org/10.3390/polym13040646>
- Oyekanmi, A. A., Kumar, U. S. U., Khalil, H., Olaiya, N. G., Amirul, A. A., Rahman, A. A., Nuryawan, A., Abdullah, C. K., and Rizal, S. (2021). "Functional properties of antimicrobial neem leaves extract based macroalgae biofilms for potential use as active dry packaging applications," *Polymers* 13(10), article 1664. <https://doi.org/10.3390/polym13101664>
- Park, K. H., Oh, S. Y., Yoo, S., Fong, J. J., Kim, C. S., Jo, J. W., and Lim, Y. W. (2020). "Influence of season and soil properties on fungal communities of neighboring climax forests (*Carpinus cordata* and *Fraxinus rhynchophylla*)," *Frontiers in Microbiology* 11, article 572706. <https://doi.org/10.3389/fmicb.2020.572706>
- Pei, W. J., Wang, J. Q., Zhang, L., Guo, Y. W., Cao, M. J., Liu, R. J., Chang, M., and Wang, X. G. (2023). "Effect of catechin on the formation of polycyclic aromatic hydrocarbons in *Camellia oleifera* oil during thermal processing," *Foods* 12(5), article 980. <https://doi.org/10.3390/foods12050980>
- Ren, X. Y., Guo, J., Li, S. Y., and Chang, J. M. (2020). "Thermogravimetric analysis-fourier transform infrared spectroscopy study on the effect of extraction pretreatment on the pyrolysis properties of eucalyptus wood waste," *ACS Omega* 5(36), 23364-23371. <https://doi.org/10.1021/acsomega.0c03271>
- Rodriguez, P. G., Dral, A. P., van den Nieuwenhuijzen, K. J. H., Lette, W., Schipper, D. J., and ten Elshof, J. E. (2018). "Thermochemical stability and friction properties of soft organosilica networks for solid lubrication," *Materials* 11(2), article 180. <https://doi.org/10.3390/ma11020180>
- Rzepka, Z., Bebenek, E., Chrobak, E., and Wrzesniok, D. (2022). "Synthesis and anticancer activity of indole-functionalized derivatives of betulin," *Pharmaceutics* 14(11), article 2372. <https://doi.org/10.3390/pharmaceutics14112372>
- Shaheen, G., Ashfaq, A., Shamim, T., Asif, H. M., Ali, A., Sajid ur, R., and Sumreen, L. (2022). "Antioxidant, antimicrobial, phytochemical and FTIR analysis of *Peganum harmala* (fruit) ethanolic extract from Cholistan Desert, Pakistan," *Dose-Response* 20(3), article 15593258221126832. <https://doi.org/10.1177/15593258221126832>
- Sidorowicz, A., Szymanski, T., and Rybka, J.D. (2021). "Photodegradation of biohazardous dye Brilliant Blue R using organometallic silver nanoparticles synthesized through a green chemistry method," *Biology-Basel* 10(8), article 784. <https://doi.org/10.3390/biology10080784>
- Suo, L., Liu, C., Zhang, Q.-Y., Yao, M.-D., Ma, Y., Yao, J., Jiang, Q., and Yan, B. (2022). "METTL3-mediated n-6-methyladenosine modification governs pericyte dysfunction during diabetes-induced retinal vascular complication," *Theranostics* 12(1), 277-289. DOI: <https://doi.org/10.7150/thno.63441>
- Thepbandit, W., Papathoti, N. K., Daddam, J. R., Thumanu, K., Siri Wong, S., Thanh, T. L., and Buensanteai, N. (2021). "Identification of salicylic acid mechanism against

- leaf blight disease in *Oryza sativa* by SR-FTIR microspectroscopic and docking studies,” *Pathogens* 10(6), article 652. <https://doi.org/10.3390/pathogens10060652>
- Verma, C., and Devi, B. (2023). “Anti-oxidant, anti-inflammatory and anti-microbial activity of *Pterocarpus santalinus* L. f.” *Pakistan Heart Journal* 56(3), 1020-1027.
- Vikramdeo, K. S., Miree, O., Anand, S., Sharma, A., Srivastava, S. K., Singh, S., Rocconi, R. P., and Singh, A. P. (2025). “MYB/AKT3 axis is a key driver of ovarian cancer growth, aggressiveness, and chemoresistance,” *Journal of Ovarian Research* 18(1), article 179. DOI: <https://doi.org/10.1186/s13048-025-01761-9>
- Wang, L., Li, G., Chen, X., Yang, Y., Liew, R. K., Abo-Dief, H. M., Lam, S. S., Sellami, R., Peng, W., and Li, H. (2024a). “Extraction strategies for lignin, cellulose, and hemicellulose to obtain valuable products from biomass,” *Advanced Composites and Hybrid Materials* 7(6), article 219. <https://doi.org/10.1007/s42114-024-01009-y>
- Wang, L., Gu, W., and Wang, Z. (2025). “Topology-controlled hyperbranched polyesters enable balanced soy adhesive strengthening and toughening,” *Industrial Crops and Products* 237, article 122309. <https://doi.org/10.1016/j.indcrop.2025.122309>
- Wang, Y., Gu, W., and Sui, K. (2025). “Robust Janus hydrogel with wet-tissue adhesive properties for wound dressing and anti-postoperative adhesion,” *ACS Applied Bio Materials* 8(5), 3932-3940. <https://doi.org/10.1021/acsabm.5c00118>
- Wang, S. Q., Hu, X. Y., Lu, R., Yan, S., Li, Y. L., and Zhang, D. D. (2024b). “Research on correlation analysis and multivariate nonlinear regression modeling method for the tensile properties of mugwort petiole,” *Scientific Reports* 14(1), article 31597. <https://doi.org/10.1038/s41598-024-76771-6>
- Xie, Q., Tang, Y. R., Wu, X. Y., Luo, Q. Y., Zhang, W. T., Liu, H. Y., Fang, Y. L., Yue, X. F., and Ju, Y. L. (2023). “Combined ultrasound and low temperature pretreatment improve the content of anthocyanins, phenols and volatile substance of Merlot red wine,” *Ultrasonics Sonochemistry* 100, article 106636. <https://doi.org/10.1016/j.ultsonch.2023.106636>
- Yang, L., Xing, M., Xue, X., Jin, X., Wang, Y., Xiao, F., Li, C., and Wang, F. (2025). “Preparation and characterization of a novel eco-friendly acorn-based wood adhesive with high performance,” *Forests* 16(5), article 853. <https://doi.org/10.3390/f16050853>
- Zhang, S., Chen, H., Zhang, Y., Zhang, Y. M., Kan, W. Y., and Pan, M. Z. (2020). “Flame retardancy of high-density polyethylene composites with P,N-doped cellulose fibrils,” *Polymers* 12(2), article 336. <https://doi.org/10.3390/polym12020336>
- Zhang, S., Yang, M., Xu, T., Yan, Q., Degen, A., and Zhou, X. (2025). “Research advances in *Pimpinella thellungiana*: Nutrients, bioactive compounds, and functional properties benefitting livestock,” *Animal Nutrition* 20, 239-248. <https://doi.org/10.1016/j.aninu.2024.11.005>

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SUPPLEMENTARY INFORMATION

Table S1. Chemical Constituents Identified by GC-MS in the Ethanol Extract of *Carpinus cordata* Wood

No.	Retention Time (min)	Peak area (%)	Components	No.	Retention Time (min)	Peak area (%)	Components
1	14.598	0.33	trans-Coniferyl alcohol	13	26.214	0.28	Santalcamphor
2	17.162	0.22	trans-Sinapyl alcohol	14	26.358	1.38	Cycloeucalenyl acetate
3-6	17.894-18.682	58.41	Lupeol	15	26.489	0.41	Dihydro-β-agarofuran
7	21.228	0.230585	Cedran-diol, (8S,14)-	16	26.727	3.03	24-Noroleana-3,12-diene
8-9	22.361-23.061	10.40	β-Amyrin	17	26.877	0.64	Lupeol
10	24.056	0.62	Cedran-diol, (8S,14)-	18	27.277	10.51	β-Amyrin
11	24.181	1.92	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane	19	27.859	11.09	β-Amyrone

Table S2. Chemical Constituents Identified by GC-MS in the Benzene/ethanol (1:1) Extract of *Carpinus cordata* Wood

No.	Retention Time (min)	Peak area (%)	Components	No.	Retention Time (min)	Peak area (%)	Components
1	14.61	0.35	trans-Coniferyl alcohol	17	24.337	0.12	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane
2	16.831	0.68	Dibutyl phthalate	18	24.638	0.14	β-Amyrin
3	17.169	0.15	trans-Sinapyl alcohol	19	25.188	1.09	Ursa-9(11),12-dien-3-ol
4-9	17.913-18.601	40.71	Lupeol	20	25.757	1.67	1,2-Bis(trimethylsilyl)benzene
10	20.784	0.12	Clopidogrel	21	26.214	0.37	Lupeol
11	21.235	0.28	α-Tocopheryl acetate	22	26.345	0.96	Cycloeucalenyl acetate
12	22.348	6.99	β-Amyrin	23	26.514	0.58	Lupeol
13	23.055	1.43	α-Amyrin	24	26.733	5.91	γ-Sitosterol
14	23.655	1.22	26-Norcoprostan-16,22-epoxy-3α-ol-25-one	25	26.914	1.28	Lupeol
15	23.837	0.37	Santalcamphor	26-27	27.296-27.603	19.01	β-Amyrin
16	24.062	0.30	Dihydro-β-agarofuran	28	27.89	16.29	Lupenone

Table S3. TD-GC-MS Sample of *Carpinus cordata* Wood

No.	Retention Time (min)	Peak area (%)	Components	No.	Retention Time (min)	Peak area (%)	Components
1	1.802	0.20	Ethanol, 2-methoxy-, carbonate (2:1)	41	13.679	0.39	Undec-10-ynoic acid, dodecyl ester
2	1.99	0.29	Hydroperoxide, 1-methylbutyl	42	13.756	1.09	Cycloheptasiloxane, tetradecamethyl-
3	2.287	0.41	Benzene	43	13.899	0.29	trans-Z- α -Bisabolene epoxide
4	3.329	0.33	Hexanal	44	14.132	0.30	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl
5	3.73	6.56	Acetic acid	45	14.254	0.57	Undec-10-ynoic acid, decyl ester
6	3.943	0.20	3-Cyclohexen-1-ol, 1-methyl-	46	14.526	0.36	Ethanone, 1-(1-hydroxy-2,6,6-trimethyl-2,4-cyclohexadien-1-yl)-
7	4.551	0.19	9-Oxabicyclo[6.1.0]nonan-4-ol	47	14.94	0.25	1H-Inden-1-ol, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-
8	5.386	0.40	2H-Pyran, 3,4-dihydro-	48	15.05	2.54	Glutaric acid, butyl undecyl ester
9	5.457	1.59	Benzaldehyde	49-52	15.173-15.477	7.69	Dodecanoic acid
10	5.645	0.60	1-Decanamine	53	15.568	0.23	Undec-10-ynoic acid, octyl ester
11	5.819	0.26	3,10-Dioxatricyclo[4.3.1.0(2,4)]dec-7-ene, (1 α ,2 α ,4 α ,6 α)-	54	16.001	0.26	Dodecanoic acid, 3-hydroxy-
12	6.44	0.90	Cyclohexanone, 4-hydroxy-	55	16.221	0.57	1-Eicosanol, TMS derivative
13	6.531	0.32	7-Octen-2-ol, 2,6-dimethyl-	56-59	16.428-16.751	2.22	Undec-10-ynoic acid, decyl ester
14	6.893	5.48	Benzyl alcohol	60	16.933	0.68	cis-7-Hexadecenoic acid
15	6.977	0.28	Z-3-Methyl-2-hexenoic acid	61	17.101	0.60	Undec-10-ynoic acid, decyl ester
16	7.436	0.53	Dipentene diepoxide	62	17.198	0.20	Naphtho[2,1-b]furan, dodecahydro-3a,6,6,9a-tetramethyl-
17	7.572	0.33	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	63	17.334	0.76	Undec-10-ynoic acid, tetradecyl ester
18	7.728	0.47	Benzenemethanol, α -(1-ethenylpentyl)- α -methyl-	64	18.032	1.05	Tetradecanoic acid
19	8.471	0.37	7-Octene-1,2-diol	65	18.479	1.72	Nerolidol acetate
20	8.556	0.68	DL-Arabinose	66	18.601	2.59	Undec-10-ynoic acid, dodecyl ester
21	8.73	0.89	7-Octene-1,2-diol	67	18.692	0.70	Undec-10-ynoic acid, tetradecyl ester
22-23	8.86-8.97	1.08	DL-Arabinose	68	18.893	0.66	Undec-10-ynoic acid, dodecyl ester
24	9.118	1.66	l-Gala-l-ido-octose	69	19.119	3.89	Phthalic acid, 2-cyclohexylethyl isobutyl ester
25	9.5	0.19	2,6-Dimethyl-1-nonen-3-yn-5-ol	70	19.242	0.79	Undec-10-ynoic acid, decyl ester
26	9.882	0.35	Hexanedioic acid, dimethyl ester	71	19.339	0.71	2-Methyl-Z-4-tetradecene
27	10.541	0.26	L-Lyxose	72	19.417	0.35	Undec-10-ynoic acid, tetradecyl ester
28	11.007	1.46	Dibutylformamide	73	19.481	0.25	E-8-Methyl-9-tetradecen-1-ol acetate
29	11.078	0.38	Cyclohexasiloxane, dodecamethyl-	74	19.611	0.25	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-
30	11.124	0.23	Phenol, p-tert-butyl-	75	19.695	0.94	Oleic Acid
31	11.337	1.17	Cyclohexene,1-hexyl-	76	20.005	0.91	Glycidyl palmitoleate
32	11.583	0.23	Triacetin	77	20.309	1.26	Butyl isodecyl phthalate
33	11.719	0.20	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	78	20.361	0.68	E-8-Methyl-9-tetradecen-1-ol acetate
34	12.036	0.66	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	79	20.549	0.26	Octadecanoic acid
35	12.592	0.65	Vanillin lactoside	80	20.704	0.71	Oleic Acid
36	12.702	0.70	Acetylenic glycol	81-88	20.872-24.404	6.48	n-Hexadecanoic acid
37	12.98	0.62	7-Isopropyl-7-methyl-nona-3,5-diene-2,8-dione	89	29.178	18.01	Octadecanoic acid
38	13.245	3.58	trans-Isoeugenol	90-91	30.556-30.821	0.68	5-hydroxy-7-methoxyflavanone
39	13.491	0.84	Triethylene glycol monododecyl ether	92	31.526	1.30	7-Methyl-Z-tetradecen-1-ol acetate
40	13.549	1.28	Butanedioic acid, bis(2-methylpropyl) ester				

Table S4. Py-GC-MS Sample of *Carpinus cordata* Wood

No.	Retention Time (min)	Peak area (%)	Components	No.	Retention Time (min)	Peak area (%)	Components
1-2	1.438-1.47	12.28	Cyclopropyl carbinol	63	10.81	0.25	Cyclobutane carbonitrile, 3-methyl-3-phenoxy-
3	1.613	5.37	4-Pentenol	64	10.952	0.14	Benzene, 2-propenyl-
4	1.75	3.35	1,3-Cyclopentadiene	65	11.206	0.10	1(4H)-Naphthalenone, 4a,5,8,8a-tetrahydro-4-hydroxy-, (4α,4aβ,8aβ)-
5	1.814	2.21	2-Penten-1-ol, (E)-	66	11.498	1.09	Indene
6	1.944	3.31	Pseudohexyl alcohol	67-68	11.699-11.796	0.32	1-Decen-4-yne, 2-nitro-
7	2.022	2.19	3-Hexen-1-ol, acetate, (E)-	69	12.445	1.18	Myrtenol
8	2.184	0.16	5-Hexen-1-ol	70	12.659	0.31	N-Benzyloxycarbonyl-dl-norleucine
9	2.308	2.88	3-Vinyl-1-cyclobutene	71	12.737	0.19	(-)-Myrtenol
10	2.405	1.76	Acetaldol	72	12.9	0.27	Dipentene diepoxide
11	2.502	4.35	Butadienylacetylene	73	12.964	0.07	7-Methylene-9-oxabicyclo[6.1.0]non-2-ene
12	2.567	2.49	trans,trans-Hexa-2,4-dienyl acetate	74	13.146	0.31	(1R)-(-)-Myrtenal
13-15	2.645-2.879	11.34	Acetic acid	75-76	13.302-13.445	2.46	Phenol, 3-methyl-
16	2.931	1.75	Acetol	77-78	13.613-13.847	0.69	3-Caren-10-al
17	3.009	0.30	Ethanone, 1-(methylenecyclopropyl)-	79	13.931	0.22	5,7-Dodecadiyn-1,12-diol
18	3.087	0.44	Butanenitrile, 2,3-dioxo-, dioxime, O,O'-diacetyl-	80	14.23	0.09	Dipentene diepoxide
19	3.242	0.18	6-Nonynoic acid	81-82	14.632-14.814	0.96	1H-Indene, 3-methyl-
20	3.314	0.74	Butanenitrile, 2,3-dioxo-, dioxime, O,O'-diacetyl-	83	14.86	0.22	Tetracyclo[5.3.0.0<2,6>.0<3,10>]deca-4,8-diene
21	3.385	0.08	Propanoic acid, 2-oxo-	84	14.957	0.11	5,7-Dodecadiyn-1,12-diol
22	3.521	0.75	3-Cyclohexene-1-methanol	85	15.054	0.19	4,7-Methano-1H-inden-1-ol, 3a,4,7,7a-tetrahydro-, acetate
23	3.612	0.21	(1-Allylcyclopropyl)methanol	86	15.203	0.65	2,6-Xylenol
24	3.664	0.12	3-Methylenecyclohexene	87	15.658	0.73	Azulene
25	3.787	0.18	6-Nonynoic acid	88	15.846	0.26	5,8-Decadien-2-one, 5,9-dimethyl-, (E)-
26	3.989	4.49	Tropilidene	89-90	15.93-16.034	0.66	3-Ethylphenol
27	4.067	0.42	Boron, trihydro(pyridine)-, (T-4)-	91	16.099	0.16	5-β,8-β-Epoxy-3,5,8,8a-tetrahydro-1H-2-benzopyran
28	4.118	0.54	3-Methylenecyclohexene	92	16.203	0.08	Bicyclo[3.1.1]hept-3-ene-spiro-2,4'-(1',3'-dioxane), 7,7-dimethyl-
29	4.222	0.42	Bicyclo[2.2.0]hex-1-yl-methanol	93	16.592	0.22	5,7-Dodecadiyn-1,12-diol
30	4.32	0.29	3,4-Hexanediol, 2,5-dimethyl-	94	17.65	0.18	1-Naphthol, 1,2,3,4-tetrahydro-2-methyl-
31	4.417	0.47	1-Octanol	95-96	17.994-18.085	0.70	3,7,7-Trimethyl-8-(2-methyl-propenyl)-bicyclo[4.2.0]oct-2-ene
32	4.566	0.44	2-Nitrobutanol	97-99	18.358-18.611	0.38	Panaxydol
33-34	4.683-4.741	0.92	2-Acetyl-2-methyl-succinonitrile	100	18.741	0.43	Butanoic acid, 3-[(1-phenylethyl-2-propynyl)oxy]
35	4.995	0.09	3-Octyn-1-ol	101	19.149	1.00	9-Methyltricyclo[4.2.1.1(2,5)]deca-3,7-diene-9,10-diol
36	5.085	0.20	6-Methyl-2-heptyne	102	19.967	0.26	Dichloroacetic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester
37	5.254	0.17	Butanenitrile, 2,3-dioxo-, dioxime, O,O'-diacetyl-	103	20.194	0.39	3-Chloro-4-hydroxyiminocarane
38	5.592	1.61	Furfural	104	20.324	0.29	8,11-Octadecadiynoic acid, methyl ester
39	5.709	0.66	Methyl isobutylacetylene	105	20.59	0.24	Benzeneacetic acid, 4-(1H-1,2,3,4-tetrazol-1-yl)-
40	6.137	0.95	Ethylbenzene	106	20.727	0.24	Panaxydol
41	6.358	1.15	p-Xylene	107	20.843	0.07	5,7-Dodecadiyn-1,12-diol
42	6.572	0.27	3,8-Dioxatricyclo[5.1.0.0(2,4)]octane, 4-ethenyl-	108	20.908	0.14	(S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol
43	6.637	0.08	Bicyclo[2.1.1]hexan-2-ol, 2-ethenyl-	109	21.058	0.16	Cyclopropane, 1-(1-hydroxyethyl)-2-methylene-1-phenyl-
44	6.682	0.13	1-Octyne	110-111	21.187-21.376	0.19	Dihydrojasmane
45	6.857	0.08	6-Nonynoic acid	112	21.531	0.24	Spiro[4.4]non-3-en-2-one, 4-methyl-3-(1H-tetrazol-5-yl)-1-oxa-
46	6.955	1.68	Benzocyclobutene	113	21.856	0.26	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-
47	7	0.44	o-Xylene	114	22.388	0.15	Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5R)-
48	7.591	0.18	2-Cyclopenten-1-one, 2-methyl-	115	22.68	0.07	Falcarinol
49	8.019	0.12	3,6-Octadecadiynoic acid, methyl ester	116-117	22.907-23.589	0.50	Spiro[2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane)
50	8.739	0.10	1,3,5-Cycloheptatriene, 7-ethyl-	118-119	23.79-24.134	0.30	Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5R)-
51-52	8.992-9.031	0.77	Benzene, (1-methylethyl)-	120	24.523	0.11	2-Heptanone, 6-(3-acetyl-1-cyclopropen-1-yl)-3-hydroxy-6-methyl-, (R*,R*)-
53	9.2	0.08	cis-p-mentha-1(7),8-dien-2-ol	121-122	24.861-25.042	0.24	Spiro[2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane)
54	9.343	0.18	2-Nonyn-1-ol	123	25.484	0.07	3-buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl)
55	9.531	0.55	3,8-Dioxatricyclo[5.1.0.0(2,4)]octane, 4-ethenyl-	124	25.601	0.18	Spiro[2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane)
56	9.622	0.37	1-Decen-4-yne, 2-nitro-	125-127	25.73-25.958	0.88	(2,3-Diphenyl-aziridin-1-yl)-(1-methyl-7-oxa-bicyclo[4.1.0]hept-2-ylidene)-amine
57	9.797	0.07	Pyrrolidine, 1-nitro-	128	26.035	0.22	Spiro[2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane)
58	9.875	0.36	2-Decenal, (E)-	129-131	26.12-26.34	1.01	Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5R)-
59	9.953	0.25	Benzene, 1,2,3-trimethyl-	132-133	26.607-27.47	0.18	2-(2-Carboxyethyl)-6,6-dimethyl-3-oxocyclohex-1-enecarboxylic acid
60	9.998	0.42	Benzene, 1-propenyl-	134	27.684	0.08	5,8,11,14-Eicosatetraynoic acid, methyl ester
61	10.057	0.78	Indane	135	29.112	0.09	6-Bromohexanoic acid, tridecyl ester
62	10.544	1.14	Phenol	136-137	32.48-34.81	1.48	tert-Hexadecanethiol