Phytochemical Profiling and Apoptosis-Mediated Anticancer and Antimicrobial Activities of Hedychium coronarium Methanolic Extract

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Article

Keywords:

Posted Date: June 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9801208/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on August 12th, 2026. See the published version at https://doi.org/10.1038/s41598-026-66619-6.

Abstract

Hedychium coronarium J. Koenig (*Zingiberaceae*) is a medicinal plant known for its rich phytochemical diversity and pharmacological potential; however, its bioactivities under arid environmental conditions remain insufficiently explored. This study aimed to characterize the phytochemical composition and evaluate the antibacterial, antifungal, and anticancer activities of the methanolic extract of *H. coronarium* (MEHC). Gas chromatography–mass spectrometry (GC–MS) analysis identified 15 bioactive compounds, with (Z)-2-methoxycinnamaldehyde (69.18%) as the predominant constituent, alongside sesquiterpenoids such as δ-cadinene, α-copaene, and α-muurolene. The extract exhibited high total phenolic content (125.17 ± 2.36 mg GAE/g extract) and moderate flavonoid content (78.43 ± 3.25 mg QE/g extract). MEHC demonstrated moderate, dose-dependent cytotoxicity against MCF-7 and HepG2 cancer cell lines, with IC_{50} values of 112.65 ± 1.68 and 98.34 ± 0.58 µg/mL, respectively. Gene expression analysis confirmed apoptosis induction via upregulation of caspase-3, -8, -9, and Bax, and downregulation of Bcl-2 and Bcl-xL. Antibacterial activity was pronounced against Gram-negative bacteria, with minimum inhibitory concentration (MIC) values ranging from 6.25 to 25 µg/mL. Additionally, MEHC exhibited notable antifungal activity against *Candida* species, particularly *C. albicans* (MIC: 12.5 µg/mL). Overall, MEHC displays broad-spectrum biological activities, likely attributed to its cinnamaldehyde derivatives and sesquiterpenoid content. These findings highlight its potential as a natural source of antimicrobial and anticancer agents. However, further studies involving bioactive compound isolation, mechanistic validation, and *in vivo* evaluation are required to confirm its therapeutic applicability.

Introduction

Natural products from medicinal plants have played an important role in drug discovery by providing bioactive molecules with various therapeutic qualities. Plants in the *Zingiberaceae* family, which includes around 50 genera and 1,600 species, are known for their phytochemical diversity and wide range of medicinal effects [1]. Members of this family, including *Curcuma longa* (turmeric), *Zingiber officinale* (ginger), and *Alpinia galanga* (greater galangal), have been extensively studied for their antimicrobial, anti-inflammatory, and anticancer properties, which are attributed to their high phenolic, flavonoid, terpenoid, and essential oils content [2–6].

Hedychium coronarium. Koenig, also known as the white ginger lily, is an important member of the *Zingiberaceae* family prized in traditional medicine for its several health advantages [7]. *H. coronarium* contains a variety of bioactive compounds, including flavonoids (e.g., quercetin, kaempferol), phenolic acids (e.g., gallic acid, caffeic acid), terpenoids (e.g., α-copaene, α-muurolene 1,8-cineole) [8,9]. These chemicals have important biological activity. Research suggests that cinnamaldehydes have antibacterial and antifungal activities [10,11], whereas sesquiterpenoids like α-copaene and α-muurolene have anti-inflammatory and anticancer effects [12–14].

The *Zingiberaceae* family is known for its medicinal potential against several ailments. Essential oils and extracts of *Z. officinale* exhibit antioxidant, anti-inflammatory, antibacterial, antifungal, and anticancer properties [15,16]. *C. longa*, which contains curcuminoids, has been widely explored for its anti-inflammatory, antioxidant, antibacterial, and anticancer properties [17,18]. Similarly, *A. galanga* and *Elettaria cardamomum* have been shown to have antibacterial and anticancer activities due to their high concentrations of 1,8-cineole, galangal acetate, and eugenol [19,20].

Despite this amount of information, the antifungal properties of *H. coronarium* against *Candida* species are mostly unknown. Preliminary results suggest efficacy against fungal strains, although its specific action against pathogenic *Candida* species such as *C. albicans, C. glabrata*, and *C. parapsilosis* has not been thoroughly studied. These fungal pathogens are major causes of systemic and mucosal infections, particularly in immunocompromised people, and their increasing resistance to standard antifungals highlights the need for new treatments [21].

Importantly, research on *H. coronarium* and related species in specific locales, such as Saudi Arabia, is almost nonexistent. This type of research creates a considerable information vacuum, notably about the chemical composition and bioactivities of species that grow in arid and unusual climatic conditions. Given that environmental characteristics such as soil type, climate, and water

availability can have a major impact on the phytochemical profile of medicinal plants, studying *H. coronarium* from Saudi Arabia may yield novel bioactive chemicals or enhanced therapeutic activity.

So, the current study addresses these gaps by conducting a thorough chemical analysis of methanolic extracts of *H. coronarium* (MEHC) using gas chromatography-mass spectrometry (GC–MS) to identify their phytochemical contents. The study investigates its antibacterial, antifungal, and anticancer properties. By linking MEHC's chemical profile to its biological activities, this study sheds new light on *H. coronarium*'s therapeutic applications and its potential as a source of natural bioactive compounds in the *Zingiberaceae* family, particularly in underexplored regions such as Saudi Arabia.

Results

Chemical composition of the MEHC

The MEHC contains 15 distinct compounds by GC-MS analysis. These chemicals were found using the molecular formula (MF), molecular weight (MW), peak retention time (RT), and peak area (%). The Cinnamaldehyde of Z)-2-Methoxycinnamaldehyde (69.18%), and the Sesquiterpenoids of δ-cadinene (4.86%), α-Copaene (4.79%), and α-Muurolene (4.01%), and Benzene, (1-methoxyethyl) (4.42%), and the anisole of Benzene, 1-(1-butenyl)-4-methoxy-, trans (3.39%) were found to be the main constituents (Table 1 and Fig. 1).

Table 1. GC-MS compounds in the MEHC

Peak	Rt	Area	Area%	Name	MF	MW	Classification
1	14.813	35891965	4.42	Benzene, (1-methoxyethyl)	C9H12O	136	Benzylethers
2	15.134	5.62E+08	69.18	(Z)-2-Methoxycinnamaldehyde	C10H10O2	162	Cinnamaldehydes
3	17.837	38890456	4.79	α-Copaene	C15H24	204	Sesquiterpenoids
4	18.391	27522669	3.39	Benzene, 1-(1-butenyl)-4-methoxy-, trans-	C11H14O	162	Anisoles
5	18.782	3817444	0.47	Anisole, p-pentyl-	C12H18O	178	Anisoles
6	20.368	7659974	0.94	γ-Muurolene	C15H24	204	Sesquiterpenoids
7	20.959	32472727	4.01	α-Muurolene	C15H24	204	Sesquiterpenoids
8	21.443	39510993	4.86	δ-cadinene	C15H24	204	Sesquiterpenoids
9	21.516	13210779	1.62	cis-Calamenene	C15H22	202	Sesquiterpenoids
10	21.667	10801082	1.33	(Z)-3-Phenylacrylaldehyde	C9H8O	132	Cinnamaldehydes
11	21.779	16285984	2.06	Cadine-1,4-diene	C15H24	204	Sesquiterpenoids
12	21.982	5027228	0.61	β-Calacorene	C15H20	200	Sesquiterpenoids
13	24.031	5485400	0.67	Di-epi-1,10-cubenol	C15H26O	222	Sesquiterpenoids
14	24.398	9109937	1.12	tau.-Muurolol	C15H26O	222	Sesquiterpenoids
15	24.461	4478779	0.55	α-muurolol (torreyol)	C15H26O	222	Sesquiterpenoids

TPC and TFC of the MEHC

MEHC's TPC (125.17 ± 2.36 mg GAE/g of extract with R^2 = 0.991) was richer than TFC's (78.43 ± 3.25 mg QE/g with R^2 = 0.995).

Cytotoxic Activity and Apoptosis Induction

The cytotoxic effects of the methanolic extract of MEHC were evaluated against human MCF-7 and HepG2 cancer cell lines using the MTT assay (Fig. 2). MEHC exhibited a clear dose-dependent reduction in cell viability across the tested concentration range

(0–800 µg/mL). The calculated IC_{50} values were **112.65 ± 1.68 µg/mL for MCF-7** and **98.34 ± 0.58 µg/mL for HepG2** cells, indicating moderate cytotoxic activity. In comparison, the standard drug Cisplatin (30 µg/mL) showed higher potency. Statistical analysis confirmed that the reduction in cell viability at higher concentrations was significant compared to untreated controls ($p < 0.05$).

To investigate whether the observed cytotoxicity is associated with apoptosis, gene expression analysis was performed using RT-PCR after treating MCF-7 and HepG2 cells with IC_{50} concentrations for 48 h (Fig. 3). The results demonstrated a significant upregulation of pro-apoptotic genes and downregulation of anti-apoptotic markers relative to untreated controls ($p < 0.05$). These findings confirm that MEHC induces apoptosis in both cancer cell lines, suggesting that programmed cell death is a primary mechanism underlying its anticancer activity.

Antibacterial Effects of the MEHC

MEHC showed dose-dependent antibacterial activity, with inhibition zones ranging from 9 to 22 mm at concentrations of 100–800 µg/Ml (Table 3), although these values were lower than those of chloramphenicol (20–23 mm). The MIC values ranged from 6.25 to 25 µg/mL, while MBC values ranged from 12.5 to 50 µg/mL. The highest activity was observed against *E. coli* and *P. aeruginosa* (MIC: 6.25 µg/mL; MBC: 12.5 µg/mL). Moderate activity was recorded against *S. epidermidis* and *K. pneumoniae* (MIC: 12.5 µg/mL; MBC: 25 µg/mL), whereas *S. aureus* and *B. subtilis* were the least sensitive (MIC: 25 µg/mL; MBC: 50 µg/mL). Overall, MEHC demonstrated both bacteriostatic and bactericidal effects, with greater efficacy against Gram-negative bacteria than Gram-positive bacteria.

Table 2. The inhibitory zone (mm), MIC (µg/mL**)**, and MBC (µg/mL) of MEHC

Bacterium/ Dilution	Positive control	800 µg/mL	400 µg/mL	200 µg/mL	100 µg/mL	MIC (µg/mL)	MBC (µg/mL)
S. aureus	21±1.24	19±1.67	17±1.88	14±1.376	10±1.35	25.00±0.37	50.00±0.89
S. epidermidis	22±2.13	18±2.61	17±2.37	13±0.98	9±0.36	12.50±0.76	25.00±0.38
B. subtilis	20±141	19±1.91	17±1.54	14±1.61	9±1.69	25.00±1.72	50.00±1.34
E. coli	23±1.18	22±0.59	19±0.68	15±2.37	13±1.31	6.25.00±0.23	12.50±0.82
K. pneumoniae	23±1.37	21±0.36	17±1.27	14±1.43	12±0.95	12.50±0.39	25.00±0.33
P. aeruginosa	22±2.24	20±0.33	16±0.36	14±1.21	12±0.23	6.25.00±0.69	12.50±0.56

Antifungal activity

MEHC also exhibited antifungal activity against *Candida* species, with inhibition zones ranging from 10 to 22 mm, again lower than those of chloramphenicol (24–26 mm). The MIC values ranged from 12.5 to 25 µg/mL, and MFC values ranged from 25 to 50 µg/mL. *C. albicans* was the most sensitive strain (MIC: 12.5 µg/mL; MFC: 25 µg/mL), while *C. glabrata* and *C. parapsilosis* showed moderate susceptibility (MIC: 25 µg/mL; MFC: 50 µg/mL) (Table 3). Overall, MEHC demonstrated notable antimicrobial activity, particularly against Gram-negative bacteria and *C. albicans*, although its efficacy remained lower than that of the standard antibiotic.

Table 3. The inhibition zone, MIC, and MFC values of MEHC against selected *Candida* strains.

Bacterium/ Dilution	Positive control	800 µg/mL	400 µg/mL	200 µg/mL	100 µg/mL	MIC (µg/mL)	MFC (µg/mL)
C. albicans	24±1.55	20±1.93	16±1.36	12±1.92	10±1.73	12.50±0.38	25±0.82
C. glabrata	26±2.46	19±1.39	17±1.94	14±1.57	11±1.41	25±0.38	50±0.55
C. parapsilosis	25±2.17	22±2.55	20±1.26	18±1.42	14±2.33	25±0.52	50±0.23

Discussion

The current work examines the chemical variety and bioactive potential of the MEHC, showing 15 unique compounds using GC-MS analysis. Among the primary ingredients, (Z)-2-Methoxycinnamaldehyde was discovered as the leading chemical (69.18%), with significant sesquiterpenoids such as δ-cadinene (4.86%), α-Copaene (4.79%), and α-Muurolene. Additionally, phenolic compounds such as benzene, (1-methoxyethyl) (4.42%) and benzene, 1-(1-butenyl)-4-methoxy-, trans (3.39%) were found. These chemicals are widely recognized for their antibacterial, anticancer, and antioxidant capabilities, highlighting MEHC's potential as a source of medicinal agents.

The high concentration of (Z)-2-Methoxycinnamaldehyde in MEHC is notable, as cinnamaldehyde derivatives have been extensively studied for their broad-spectrum antibacterial and anticancer properties. Cinnamaldehyde derivatives, for example, have been shown to suppress viral replication by interfering with viral protein synthesis and host-cell entry mechanisms, most notably against influenza and herpes simplex [22,23]. This chemical most likely contributes to the reported bioactivities of *H. coronarium*, as evidenced by its recognized involvement in breaking microbial cell walls by increasing the reactive oxygen species (ROS) production [24]. A previous study showed that different cinnamaldehyde analogues affected the growth of the fungal *C. albicans* biofilm and nematode *Caenorhabditis elegans* [25]. Sesquiterpenoids including α-Copaene, α-Muurolene, and δ-cadinene have antibacterial properties by disrupting membranes and inhibiting microbial enzymatic pathways, similar to *Zingiberaceae* plants like *Z. officinale* and *C. longa* [26–29]. These findings are consistent with prior research on *H. coronarium*, which has shown comparable antimicrobial capabilities, including efficient activity against harmful bacteria and fungi.

Similar GC-MS investigations of *H. coronarium* extracts in other research have shown comparable patterns, confirming the consistency of its bioactive potential. In the study conducted by Cruz et al. (2023), they found the essential oils extracted from the rhizome and root parts showed higher expression of the monoterpenoid 1,8-cineole. In contrast, the leaf oils had a different composition of the monoterpene β-pinene and (E)-caryophyllene being the most abundant constituents [30]. Sheikh et al. (2023) found that essential oils of *H. coronarium* containing linalool, 1,8-cineole, and β-pinene have strong pesticidal, nematocidal, and antifungal activities against *Spodoptera litura*, *Raphanus raphanistrum* subsp. sativus, *Fusarium oxysporum* and *Curvularia lunata* [31]. Furthermore, a previous study showed that the essential oil analysis of *H. coronarium* rhizome, composed of multiple terpenes and terpenoid compounds including α-Copaene and β-pinene, were responsible for its antioxidant, antibacterial (against *Staphylococcus aureus, B. subtilis, P. aeruginosa*, and *Proteus vulgaris*), and cytotoxic (against K562 cells) activities [32]. Sahu et al. (2024) discovered several terpenoids and phenolics in methanolic extracts, supporting their antifungal and antimicrobial activities. Together with our findings, these investigations highlight the pharmacological flexibility of *H. coronarium* [33].

The phytochemical composition of MEHC, namely its TPC and TFC, sheds light on its bioactive potential. MEHC had a greater TPC (125.17 ± 2.36 mg GAE/g extract, $R^2 = 0.991$) than TFC (78.43 ± 3.25 mg QE/g extract, $R^2 = 0.995$), indicating that the extract contains more phenolic chemicals than flavonoids. Given the well-established bioactivities of phenolics, this phenolic dominance most certainly contributes significantly to MEHC's antioxidant, antibacterial, and anticancer effects. Phenolic chemicals enhance bioactivity by scavenging free radicals, chelating metal ions, and controlling signaling pathways involved in microbial defense and cancer cell death. For example, (Z)-2-Methoxycinnamaldehyde and phenolic derivatives found in MEHC may increase antibacterial and antifungal activity by causing oxidative stress and damaging microbial cell integrity [24]. The greater TPC is consistent with studies showing excellent antioxidant and antibacterial properties in other *H. coronarium* extracts, where phenolics are the key contributors [8,34]. Flavonoids, on the other hand, are necessary for supplementing MEHC's total bioactivity,

despite their modest content. *Zingiberaceae* plants include flavonoids such as quercetin and kaempferol, known for their potential to alter immunological responses, inhibit viral replication, and exert anti-inflammatory effects [35]. While MEHC has a lower TFC than TPC, the synergistic interaction of phenolics and flavonoids may boost the extract's medicinal efficacy. MEHC's greater TPC highlights its relevance as a source of phenolic-rich chemicals for antioxidant and antibacterial compositions. Previous investigations on *H. coronarium* have found similar results, emphasizing the importance of phenolics in conferring bioactive characteristics. For example, Mendez et al. (2023) reported a higher TPC (1.30 ± 0.08 mg GAE/g dried sample) than TFC (0.62 ± 0.02 mg QE/g dried sample) of *H. coronarium* rhizome [34], further supporting our findings. Our findings are further supported. MEHC's substantial phenolic and flavonoid profiles, as seen by high TPC and moderate TFC, support its historical usage to treat infections and other health issues. MEHC's phytochemical richness makes it a promising candidate for pharmaceutical uses, with its phenolic components laying the groundwork for future research in antioxidant and therapeutic investigations.

MEHC exhibited moderate cytotoxicity against MCF-7 and HepG2 cells (IC_{50} = 112.65 ± 1.68 and 98.34 ± 0.58 µg/mL, respectively), indicating limited potency typical of crude extracts. This impact is related to MEHC's bioactive sesquiterpenoids, which are known to induce oxidative stress, mitochondrial malfunction, and death in cancer cells [12]. While the observed cytotoxicity is nonsignificant, it demonstrates MEHC's potential as a source of molecules with specific anticancer characteristics. Comparable research on *H. coronarium* has yielded comparable results. Ray et al. (2019) found that ethanolic extract of *H. coronarium* had significant cytotoxicity against HeLa cancer cells and induced cell cycle arrest at the G1 phase [36]. Another study showed that the coronarin D isolated from *H. coronarium* rhizomes exhibited significant anticancer activities through ERK/JNK phosphorylation in glioblastoma [37]. These results support the cytotoxic activity of *H. coronarium* extracts, albeit with varied IC_{50} values depending on the cell line and extract composition. This suggests that its anticancer efficacy may be cell-type specific. Other members of the *Zingiberaceae* family have cytotoxic properties, which support the plant family's potential as a source of anticancer medicines. For example, *C. longa* (turmeric) includes curcumin, a well-known chemical that causes apoptosis and reduces growth in a variety of cancer cell lines, including breast, colon, and lung malignancies [38,39]. *Z. officinale* (ginger) contains gingerol and shogaol, both of which have shown considerable cytotoxicity against colorectal, ovarian, and pancreatic cancer cells via mechanisms such as ROS production and caspase activation [40,41]. Similarly, *A. galanga* extracts exhibited cytotoxicity against human lung and cervical cancer cell lines, which was attributed to phenolics and terpenoids such as galangin [42,43]. In contrast, *H. coronarium* ethanol extract (HCEE) demonstrated markedly enhanced and selective cytotoxicity, particularly against HeLa cells (IC_{50} = 12.57–17.18 µg/mL), while exhibiting negligible toxicity toward normal HUVEC cells (IC_{50} > 320 µg/mL). This selectivity highlights its potential therapeutic relevance and warrants further mechanistic and *in vivo* investigations [44]. This selectivity highlights its potential therapeutic relevance and warrants further mechanistic and *in vivo* investigations [44].

The antiproliferative effect is likely associated with the phytochemical profile of *H. coronarium*, typically enriched in terpenoids and phenolic compounds known to modulate redox balance, disrupt mitochondrial integrity, and interfere with cell survival signaling [7]. These mechanisms are commonly linked to apoptosis induction in cancer cells. Gene expression analysis confirmed apoptosis as the primary mechanism of action, evidenced by upregulation of caspase-3, -8, -9, and Bax, alongside downregulation of Bcl-2 and Bcl-xL. This profile indicates activation of both intrinsic and extrinsic apoptotic pathways. Increased Bax/Bcl-2 ratio suggests mitochondrial outer membrane permeabilization, promoting cytochrome c release and caspase-9 activation, followed by executioner caspase-3 activation [45]. Concurrent upregulation of caspase-8 further supports involvement of death receptor-mediated signaling.

The ability of MEHC to engage multiple apoptotic pathways is mechanistically relevant, as multi-targeted apoptosis induction can overcome resistance associated with single-pathway chemotherapeutics [46]. This aligns with the recognized advantage of plant-derived mixtures in modulating complex cancer signaling networks [47]. Despite these findings, the relatively high IC_{50} values indicate limited potency, emphasizing the need for bioassay-guided fractionation to isolate active constituents. Moreover, the absence of selectivity evaluation against normal cell lines remains a key limitation, restricting assessment of therapeutic relevance. Future studies should address selectivity indices, validate protein-level expression, and explore *in vivo* efficacy. Overall, MEHC induces apoptosis via coordinated activation of intrinsic and extrinsic pathways, supporting its potential as a source of bioactive compounds for anticancer development, pending further pharmacological optimization.

MEHC's antibacterial activity was tested against Gram-positive and Gram-negative bacteria, and results showed considerable inhibition and bactericidal activities. MEHC effectively inhibited growth and killed *Escherichia coli* and *Pseudomonas aeruginosa*, with MIC values of 6.25 ± 0.00 µg/mL and MBC values of 12.50 ± 0.00 µg/mL. These data imply that MEHC is more sensitive to Gram-negative bacteria than Gram-positive bacteria, possibly because it can permeate the outer membrane of Gram-negative bacteria more effectively [48]. MEHC's antibacterial effects are likely due to bioactive components in its methanolic extract, including (Z)-2-Methoxycinnamaldehyde, α-Copaene, and α-Muurolene. These chemicals have well-documented antibacterial characteristics, such as disrupting bacterial membranes, inhibiting enzyme activity, and inducing oxidative stress. (Z)-2-Methoxycinnamaldehyde, a cinnamaldehyde derivative, is well-known for its capacity to destabilize bacterial cell walls and impede quorum sensing in Gram-negative bacteria such as coliform bacteria and *E. coli* [49]. Another study showed that the Methicillin-resistant *S. epidermidis* was sensitive to (Z)-2-Methoxycinnamaldehyde with MIC and MBC of 220 and 880 µg/mL, respectively [24]. Furthermore, a previous report showed that *Amorpha fruticose* rich in δ-cadinene, γ-Muurolene, and α-Muurolene sesquiterpenoids had a potential antibacterial activity against *S. aureus, B. subtilis, E. coli, K. pneumoniae*, and *E. faecalis* [27].

Other research on *H. coronarium* has shown similar antibacterial properties. Pun et al. (2024) revealed that essential oils extracted from *H. coronarium* (100 mg/ml) had effective antibacterial activity against *S. aureus, B. subtilis, P. aeruginosa*, and *K. pneumoniae*, with a zone of inhibition ranging from 9.3 to 13.73 mm [50]. Mitchaleaw et al. (2024) discovered that ethanolic Rhizome extract of *H. coronarium* has considerable activity against *S. aureus, B. subtilis, M. luteus, P. aeruginosa*, and *E. coli*, demonstrating the extract's broad-spectrum potency[51]. MEHC's antibacterial activity is similarly consistent with data from other *Zingiberaceae* plants. Extracts of *Z. officinale* and *C. longa* have similar inhibitory effects on Gram-negative bacteria, which are attributed to phenolics and terpenoids such gingerol, shogaol, and curcumin [52,53]. Notably, *A. galanga* has shown considerable antibacterial activity against multidrug-resistant pathogens such as *P. aeruginosa* and *E. coli*, owing to the presence of galangin and eucalyptol [20]. MEHC's increased sensitivity to Gram-negative bacteria could be attributed to the bioactive components' unique structural and functional features, which efficiently target the lipid-rich outer membrane of Gram-negative pathogens [48]. This specificity is useful for creating new antibacterial medicines, especially for treating illnesses caused by multidrug-resistant Gram-negative bacteria like *P. aeruginosa*.

MEHC's antifungal activity was tested against *Candida* species, and it showed significant inhibitory and fungicidal effects. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values demonstrate MEHC's ability to inhibit fungal growth and vitality. *C. albicans* was the most sensitive to MEHC, while it showed moderate antifungal activity against *C. glabrata* and *C. parapsilosis*. Similar research on *H. coronarium* supports the antifungal properties of its extracts. Different crude extracts of *H. coronarium* proved significant antifungal activities against *Alternaria* sp., *F. oxysporum, C. lunata*, and *Aspergillus flavus* [7,54]. As previously mentioned, Coronarin D isolated from the essential oils of the Rhizome of *H. coronarium* has exhibited action against *C. albicans* that was even higher than the fungicides clotrimazole and nystatin (Kaomongkolgit et al., 2012). The combination of its phenolic and terpenoid ingredients improves its potency against opportunistic fungal infections. MEHC's potency against *C. albicans* implies that it could be employed as a natural antifungal agent, particularly for infections caused by opportunistic fungal diseases. The slightly lower sensitivity of *C. glabrata* and *C. parapsilosis* to MEHC could be attributed to innate resistance mechanisms, such as altered cell wall composition or efflux pump activity, which are frequent in non-*C. albicans* species.

Despite the above-mentioned novelties, numerous limitations should be considered. The antibacterial, antifungal, and cytotoxic properties were tested *in vitro*, and while promising, the results may not be immediately applicable to in vivo models due to differences in bioavailability, metabolism, and systemic toxicity. Furthermore, the specific molecular processes underlying the reported bioactivities were not identified, limiting our understanding of how MEHC exerts its effects. While GC-MS analysis revealed multiple bioactive chemicals, the study did not isolate or test these compounds individually, limiting the capacity to assign specific activities to individual constituents or investigate potential synergistic interactions. Furthermore, no comparisons with other extracts or chemicals from related plants in the *Zingiberaceae* family were made, which would have offered useful context for these findings. The study also lacked a thorough assessment of MEHC's toxicity to non-target, healthy cells or tissues, making it difficult to determine its safety and therapeutic window. Finally, environmental factors such as soil composition,

climate, and plant development, which can all alter the chemical profile of *H. coronarium*, were not addressed, raising concerns regarding the consistency of the observed bioactivities.

Materials and Methods

Plant material and extraction

The rhizomes of *H. coronarium* were collected from the Al-Kharj region, Saudi Arabia, and identified by Prof. Dr. Mohammed Fasil, King Saud University. A voucher specimen (KSU NO-107424) was deposited in the Botany and Microbiology Herbarium, College of Science, King Saud University. Plant collection and use complied with International Union for Conservation of Nature (IUCN) policies and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) guidelines as well as relevant institutional and national regulations. Rhizome was ground into a powder after being shade-dried for a week in a well-ventilated chamber. The plant powder (20 g) was macerated for three days at room temperature and then extracted using 100 mL of aqueous methanolic extract (20:80). The mixture was centrifuged for 10 minutes at 13,000 rpm after being filtered and passed through a Whatman No. 1 filter paper. Using a freeze-dry drier, the extract was dried and stored at -20°C until needed.

2.1 Identification of bioactive compounds

The Agilent 5977A MSD system from Agilent Technologies (Santa Clara, CA, USA) and a GC-MS system with bioactive components were combined. The system featured a DB5-MS column (30 m length × 0.25 mm internal diameter, phase thickness 0.25 µm). A consistent temperature of 7.5 °C per minute was anticipated, with three-minute intervals of 40 °C, five-minute intervals of 280 °C, and one-minute intervals of 290 °C. With an ionisation voltage of 70 eV and an ionisation mode of EI, the GC-MS was used to gather the data. Injector and detector operating temperatures were set at 200 °C and 300 °C, respectively. The MS specs required a steady flow rate of 1 mL/min for the carrier gas (helium) and a quantity scanning range of 20 to 500 amu. Willey and the Mass Spectral Data Base of the National Institute of Standards and Technology (NIST) served as the search libraries. More than 90% of the components were detected by comparing them with those in the computer NIST libraries linked to the GC-MS apparatus.

2.2 Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

TPC was quantified using the Folin–Ciocalteu method, as was previously noted in the literature [55], with the gallic acid standard. The coefficient of determination (R^2) of the calibration curves was used to evaluate the curve's linearity. Gallic acid equivalent, or mg GAE/g of extract, was used to represent the findings. The TFC was measured using the $AlCl_3$ colorimetric technique, using quercetin as the standard [56], The Results were expressed as mg of quercetin equivalents (mg QE/g of extract).

Cytotoxicity and Apoptosis Assays

Human hepatocellular carcinoma (HepG2, ATCC HB-8065)) and breast adenocarcinoma (MCF-7, ATCC HTB-22) cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM; Qiagen, USA) supplemented with 4.5 g/L glucose, L-glutamine, sodium pyruvate, and 10% fetal bovine serum (FBS), along with penicillin (100 U/mL) and streptomycin. Cells were maintained in a humidified incubator at 37 °C with 5% CO_2 and subcultured in 25 mL culture flasks. The culture medium was replaced every 2–3 days. For cytotoxicity assessment, cells were seeded into 96-well microplates at a density of 1×10^4 cells/well and allowed to reach exponential growth. Cells were then treated with varying concentrations of the MEHC (0–800 µg/mL) and incubated for 24 h under standard conditions. Following treatment, 10 µL of MTT reagent (5 mg/mL) was added to each well and incubated for 30 min. The resulting formazan crystals were dissolved by adding 100 µL of dimethyl sulfoxide (DMSO), and absorbance was measured at 590 nm using a microplate reader. Cell viability (%) was calculated relative to untreated control cells, and experiments were performed in triplicate. The half-maximal inhibitory concentration (IC_{50}) values were determined via GraphPad Prism software (version 5.0; La Jolla, CA, USA).

For apoptosis-related gene expression analysis, MCF-7 and HepG2 cells were treated with selected concentrations of the extract for 24 h. Cells were then harvested using trypsin (0.02%) and centrifuged at 8000 × g for 10 min at 4 °C. Total RNA was extracted using the RNeasy kit (QIAGEN, Germany) according to the manufacturer's instructions. The purified RNA was used as a template

for quantitative real-time PCR (qRT-PCR) using GoTaq qPCR Master Mix and gene-specific primers. Gene expression profiling was conducted using an RT² PCR Array on a real-time PCR system (Applied Biosystems) using the procedures outlined in our recent study [57].

Antibacterial Activity

Disc diffusion assay

The MEHC's antibacterial properties were tested against Gram-negative bacteria (*Escherichia coli*, ATCC-25922; *Pseudomonas aeruginosa*, ATCC-27853; *Klebsiella pneumonia*, ATCC-13883) and Gram-positive bacteria (*Staphylococcus aureus*, ATCC-23235; *S. epidermidis*, ATCC-12228; *Bacillus subtilis*, ATCC-23857) using the disc diffusion technique [58]. The negative control was Muller Hinton Broth (HMB) with 0.01% DMSO, while the positive control was chloramphenicol (25 µg/ml). The antibacterial efficiency was assessed by measuring the zone of inhibition in millimeters (mm) around each plant extract-containing well against the target microorganisms.

Minimum inhibitory/minimum bactericidal concentration (MIC/MBC)

The broth dilution method was used to calculate the MIC and MBC of the aerial portions of the MEHC [59] using the 2,3,5-triphenyl tetrazolium chloride (TTC) method. MEHC was meticulously prepared at concentrations ranging from 1.56 µg/mL to 800 µg/mL using a sterile Mueller-Hinton broth medium. 10 µL of a bacterial culture containing 1×10^6 CFU/mL was then introduced into the medium. The 96-well microtiter plates were then incubated for 24 hours at 37 °C to closely observe the bacterial growth. Following treatment, 20 µL of TTC working solution (2 mg/ml in PBS) was added to each well, and they were incubated at 37°C for 20 minutes. Pink wells were regarded as positive for bacterial growth, whereas colorless wells were seen as negative. To ascertain the inhibitory activity of the MEHC, the concentration at which bacterial growth was first reduced was examined. The lowest concentration of each extract at which bacterial growth may be inhibited was determined. The MBC values were determined using the lowest concentration well of each MEHC, where no bacterial growth was seen [60].

Antifungal activity

The antifungal properties of the MEHC were assessed against *C. glabrata* (ATCC-2001), C. *albicans* (ATCC-10231), and *C. parapsilosis (ATCC-22019)* [61]. The concentration of conidia was measured after the selected fungal strains were cultivated for five days in a Potato Dextrose Agar (PDA) medium. The PDA medium was covered with around 100 µL of conidia at a concentration of 106 conidia/mL. An empty filter paper disc was filled with the methanol extract, which was then allowed to dry for two hours. It was put on PDA plates and incubated for 72 hours at 28 °C after two hours. DMSO (0.01%) served as the negative control, and fluconazole (25 µg/mL) as the positive control. Antifungal activity was assessed by measuring inhibition zone diameters (mm).

Determination of MIC and minimum fungicidal concentration (MFC)

A broth dilution test was used for the determination of the MIC and MFC of the MEHC [62]. using the 2,3,5-triphenyl tetrazolium chloride (TTC) method as mentioned above. The viability (%) was determined. The broth culture was placed on PDA plates, and visible growth was observed.

Statistical analysis

The efficiency of various extract concentrations was analyzed using a one-way ANOVA, and the results were then subjected to a post hoc least significant difference test ($P < 0.05$). The IC_{50} values were obtained using GraphPad Prism 5.

Conclusion

The present study demonstrates that the methanolic extract of MEHC is a rich source of bioactive phytochemicals with broad-spectrum biological activities. GC–MS analysis revealed a predominance of cinnamaldehyde derivatives and sesquiterpenoids, including (Z)-2-methoxycinnamaldehyde, α-copaene, and α-muurolene, which are likely contributors to the observed bioactivities. MEHC exhibited notable antimicrobial activity, with pronounced effects against Gram-negative bacteria and *C. albicans*, alongside

moderate cytotoxic effects against the MCF-7 and HepG2 cells, potentially mediated through apoptosis induction. These findings highlight its potential as a multifunctional natural agent with both antimicrobial and anticancer properties. However, the current study is limited to *in vitro* investigations, and further research is required to validate these findings. Future studies should focus on the isolation and characterization of individual bioactive compounds, elucidation of their molecular mechanisms of action using advanced approaches such as transcriptomics and proteomics, and evaluation of their efficacy, bioavailability, and safety in *in vivo* models. Additionally, comprehensive toxicity assessments and broader-spectrum biological evaluations are essential to determine the therapeutic potential and clinical relevance of MEHC. Overall, MEHC represents a promising natural source for the development of novel therapeutic agents, particularly in addressing antimicrobial resistance and advancing alternative anticancer strategies.

Declarations

Data Availability Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

I.M.A and M.A.F. wrote the main manuscript text. N.A.Y.M. Mainly responsible for providing data. I.M.A. is responsible for charting. N.A.Y.M. and M.A.F. are responsible for writing, reviewing, editing, and the language of manuscript revision. All authors reviewed the manuscript.

Acknowledgments

The authors thank the Ongoing Research Funding Program, (ORF-2026-1743), King Saud University, Riyadh, Saudi Arabia

Funding

This research received no external funding

Competing interest disclosure

The authors declare no conflict of interest.

Ethical conduct of research

The present study did not involve the use of human participants or experimental animals. Collection of the plant material was performed in compliance with the guidelines of the IUCN, regulations concerning research on species at risk of extinction, and the CITES. *H. coronarium* is not classified as an endangered or protected species under national legislation, the IUCN Red List, or CITES trade regulations. All procedures related to plant collection and utilization were carried out in accordance with applicable institutional and national regulations. The cell lines employed in this study were obtained from the Virology Research Group (VRG), College of Science, King Saud University, Saudi Arabia. Cells were maintained at 37 °C under standard culture conditions and routinely verified to be free of mycoplasma contamination using the LookOut® Mycoplasma qPCR Detection Kit (Merck, Darmstadt, Germany).

References

1. Sharifi-Rad, M. et al. Plants of the genus Zingiber as a source of bioactive phytochemicals: From tradition to pharmacy. *Molecules* **22** (12), 2145 (2017).
2. Khattab, A. R. et al. Assessing phytoequivalency of four Zingiberaceae spices (galangals, turmeric and ginger) using a biochemometric approach: A case study. *Ind. Crops Prod.* **188**, 115722 (2022).
3. Razak, A. M., Tan, J. K., Mohd Said, M. & Makpol, S. Modulating effects of zingiberaceae phenolic compounds on neurotrophic factors and their potential as neuroprotectants in brain disorders and age-associated neurodegenerative

disorders: A review. *Nutrients* **15** (11), 2564 (2023).

4. Yuandani, Jantan, I. et al. Immunomodulatory effects and mechanisms of the extracts and secondary compounds of Zingiber and Alpinia species: a review. *Front. Pharmacol.* **14**, 1222195 (2023).
5. Alfuraydi, A. A., Aziz, I. M. & Almajhdi, F. N. Assessment of antioxidant, anticancer, and antibacterial activities of the rhizome of ginger (Zingiber officinale). *J. King Saud University-Science*. **36** (3), 103112 (2024).
6. Aziz, I. M. et al. Phytochemical analysis, antioxidant, anticancer, and antibacterial potential of Alpinia galanga (L.) rhizome. *Heliyon* **10**(17) (2024).
7. Arya, S. et al. Hedychium coronarium J. Koenig: Traditional uses, phytochemistry, biological activities and future aspects. *Curr. Org. Chem.* **26** (18), 1676–1690 (2022).
8. Ray, A. et al. Chemical diversity, antioxidant and antimicrobial activities of the essential oils from Indian populations of Hedychium coronarium Koen. *Ind. Crops Prod.* **112**, 353–362 (2018).
9. Singh, A. P. et al. A comprehensive review on pharmacologically active phyto-constituents from Hedychium species. *Molecules* **28** (7), 3278 (2023).
10. Wang, H., Yuan, H., Li, S., Li, Z. & Jiang, M. Synthesis, antimicrobial activity of Schiff base compounds of cinnamaldehyde and amino acids. *Bioorg. Med. Chem. Lett.* **26** (3), 809–813 (2016).
11. Gan, Z., Huang, J., Chen, J., Nisar, M. F. & Qi, W. Synthesis and antifungal activities of cinnamaldehyde derivatives against Penicillium digitatum causing citrus green mold. *J. Food Qual.* **2020**, 8898692 (2020).
12. Abu-Izneid, T. et al. Sesquiterpenes and their derivatives-natural anticancer compounds: An update. *Pharmacol. Res.* **161**, 105165 (2020).
13. Ogundajo, A. L. et al. Antimicrobial activities of sesquiterpene-rich essential oils of two medicinal plants, Lannea egregia and Emilia sonchifolia, from Nigeria. *Plants* 10(3) 488 (2021).
14. Dhyani, P. et al. Sesquiterpenoid lactones as potential anti-cancer agents: an update on molecular mechanisms and recent studies. *Cancer Cell Int.* **22** (1), 305 (2022).
15. Rinanda, T. & Isnanda, R. P. Chemical analysis of red ginger (Zingiber officinale Roscoe var rubrum) Essential oil and its anti-biofilm activity against candida albicans. *Nat. Prod. Commun.* **13** (12), 1587–1590 (2018).
16. Zammel, N. et al. Antioxidant and Anti-Inflammatory Effects of Zingiber officinale roscoe and Allium subhirsutum: In Silico, Biochemical and Histological Study. *Foods* **10**(6) (2021).
17. Sharifi-Rad, J. et al. Turmeric and its major compound curcumin on health: bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. *Front. Pharmacol.* **11**, 550909 (2020).
18. Fuloria, S. et al. A comprehensive review on the therapeutic potential of Curcuma longa Linn. in relation to its major active constituent curcumin. *Front. Pharmacol.* **13**, 820806 (2022).
19. Almohammed, H. I., Alkhaibari, A. M. & Alanazi, A. D. Antiparasitic effects of Elettaria cardamomum L. essential oil and its main compounds, 1–8 Cineole alone and in combination with albendazole against Echinococcus granulosus protoscoleces. *Saudi J. Biol. Sci.* **29** (4), 2811–2818 (2022).
20. Tian, Y. et al. Antioxidant, antibacterial, enzyme inhibitory, and anticancer activities and chemical composition of Alpinia galanga flower essential oil. *Pharmaceuticals* **15** (9), 1069 (2022).
21. Turner, S. A. & Butler, G. The Candida pathogenic species complex. *Cold Spring Harbor Perspect. Med.* **4** (9), a019778 (2014).
22. Hayashi, K. et al. Inhibitory effect of cinnamaldehyde, derived from Cinnamomi cortex, on the growth of influenza A/PR/8 virus in vitro and in vivo. *Antiviral Res.* **74** (1), 1–8 (2007).
23. Toujani, M. M. et al. Inhibition of HSV-2 infection by pure compounds from Thymus capitatus extract in vitro. *Phytother. Res.* **32** (8), 1555–1563 (2018).
24. Qian, C. et al. Metabolomics-driven exploration of the antibacterial activity and mechanism of 2-methoxycinnamaldehyde. *Front. Microbiol.* **13**, 864246 (2022).
25. Khadke, S. K., Lee, J-H., Kim, Y-G., Raj, V. & Lee, J. Appraisal of cinnamaldehyde analogs as dual-acting antibiofilm and anthelmintic agents. *Front. Microbiol.* **13**, 818165 (2022).

26. Türkez, H., Celik, K. & Toğar, B. Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells in vitro. *Cytotechnology* **66**, 597–603 (2014).
27. Marinas, I. C. et al. Chemical composition, antipathogenic and cytotoxic activity of the essential oil extracted from amorpha fruticosa fruits. *Molecules* **26** (11), 3146 (2021).
28. Li, H-Y. et al. Antibacterial and antifungal sesquiterpenoids: Chemistry, resource, and activity. *Biomolecules* **12** (9), 1271 (2022).
29. Chen, S. et al. Antimicrobial activity and mechanism of α-copaene against foodborne pathogenic bacteria and its application in beef soup. *LWT* **195**, 115848 (2024).
30. Cruz, J. N. et al. Variation in the Chemical Composition of Endemic Specimens of Hedychium coronarium J. Koenig from the Amazon and In Silico Investigation of the ADME/Tox Properties of the Major Compounds. *Plants* 12(14) 2626 (2023).
31. Sheikh, H. I. et al. Promising roles of Zingiber officinale roscoe, Curcuma longa L., and Momordica charantia L. as immunity modulators against COVID-19: A bibliometric analysis. *J. Agric. Food Res.* **14**, 100680 (2023).
32. Tian, M. et al. Phytochemical analysis, antioxidant, antibacterial, cytotoxic, and enzyme inhibitory activities of Hedychium flavum rhizome. *Front. Pharmacol.* **11**, 572659 (2020).
33. Sahu, S. K. et al. Sustainable green synthesis of Hedychium coronarium leaf extract-stabilized silver nanoparticles and their applications: colorimetric sensing of Sn 2 + and Hg 2 + and antifungal and antimicrobial properties. *Nanoscale Adv.* **6** (21), 5361–5374 (2024).
34. Mendez, N. P., Elbit, M. G. D., Villafranca-Tuba, A. R., Lagunday, N. E. & Mendez, R. A. Antioxidant Activity, Phenolic Content, and Flavonoid Content of Genus Hedychium (Hedychieae, Zingiberaceae) in the Philippines. *Philippine J. Science* **152** (2023).
35. Alhazmi, H. A. et al. Medicinal plants and isolated molecules demonstrating immunomodulation activity as potential alternative therapies for viral diseases including COVID-19. *Front. Immunol.* **12**, 637553 (2021).
36. Ray, A. et al. Hedychium coronarium extract arrests cell cycle progression, induces apoptosis, and impairs migration and invasion in HeLa cervical cancer cells. *Cancer Manage. Research* 483–500 (2019).
37. Bailly, C. Anticancer activities and mechanism of action of the labdane diterpene coronarin D. *Pathology-Research Pract.* **216** (6), 152946 (2020).
38. Giordano, A. & Tommonaro, G. Curcumin and cancer. *Nutrients* **11** (10), 2376 (2019).
39. Amaroli, A. et al. The Bright Side of Curcumin: A Narrative Review of Its Therapeutic Potential in Cancer Management. *Cancers* **16** (14), 2580 (2024).
40. Prasad, S. & Tyagi, A. K. Ginger and its constituents: role in prevention and treatment of gastrointestinal cancer. *Gastroenterol. Res. Pract.* **2015**, 142979 (2015).
41. Shanmugam, K. R., Shanmugam, B., Venkatasubbaiah, G., Ravi, S. & Reddy, K. S. Recent Updates on the Bioactive Compounds of Ginger (Zingiber officinale) on Cancer: A Study with Special Emphasis of Gingerol and Its Anticancer Potential: Effect of Ginger and Its Compounds in Cancer Subjects. *Handbook of Oxidative Stress in Cancer: Therapeutic Aspects*: Springer; :1–18. (2022).
42. Basri, A. M., Taha, H. & Ahmad, N. A review on the pharmacological activities and phytochemicals of Alpinia officinarum (Galangal) extracts derived from bioassay-guided fractionation and isolation. *Pharmacogn. Rev.* **11** (21), 43 (2017).
43. Zohmachhuana, A. et al. Alpinia galanga induces caspase-dependent apoptotic cell death in human lung and cervical cancer cells. *J. Herbmed Pharmacol.* **13** (4), 640–650 (2024).
44. Ray, A. et al. Hedychium coronarium extract arrests cell cycle progression, induces apoptosis, and impairs migration and invasion in HeLa cervical cancer cells. *Cancer Management and Research* 11(null) 483–500 (2019).
45. Elmore, S. Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* **35** (4), 495–516 (2007).
46. Fulda, S. Targeting apoptosis for anticancer therapy. *Semin Cancer Biol.* **31**, 84–88 (2015).
47. Atanasov, A. G. et al. Natural products in drug discovery: advances and opportunities. *Nat. Rev. Drug Discovery*. **20** (3), 200–216 (2021).
48. Breijyeh, Z., Jubeh, B. & Karaman, R. Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules* **25** (6), 1340 (2020).

49. Van Liefferinge, E. et al. In vitro and in vivo antimicrobial activity of cinnamaldehyde and derivatives towards the intestinal bacteria of the weaned piglet. *Italian J. Anim. Sci.* **21** (1), 493–506 (2022).
50. Pun, D., Joshi, G. P. & Pant, D. R. Pharmacological activities of six species of Hedychium J. Koenig from Nepal. *J. Nepal. Biotechnol. Association*. **5** (1), 23–30 (2024).
51. Mitchaleaw, M. et al. Antimicrobial Properties Related to Anti-Acne and Deodorant Efficacy of Hedychium coronarium J. Koenig Extracts from Pulsed Electric Field Extraction. *Antibiotics* **13** (1), 108 (2024).
52. Ahmed, N. et al. The antimicrobial efficacy against selective oral microbes, antioxidant activity and preliminary phytochemical screening of Zingiber officinale. *Infection Drug Resistance* 2773–2785 (2022).
53. Yit, K-H. & Zainal-Abidin, Z. Antimicrobial Potential of Natural Compounds of Zingiberaceae Plants and their Synthetic Analogues: A Scoping Review of In vitro and In silico Approaches. *Curr. Top. Med. Chem.* **24** (13), 1158–1184 (2024).
54. Pandya, C. V., Jadeja, A. J. & Golakiya, B. A. Antifungal activity of crude extracts of Hedychium coronarium. *Int. J. Res. Phytochem Pharmacol.* **4**, 4–6 (2014).
55. Wolfe, K. L. & Liu, R. H. Apple peels as a value-added food ingredient. *J. Agric. Food Chem.* **51** (6), 1676–1683 (2003).
56. Ordonez, A., Gomez, J. & Vattuone, M. Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. *Food Chem.* **97** (3), 452–458 (2006).
57. Binobead, M. A., Aziz, I. M., Ibrahim, S. M. & Aljowaie, R. M. Chemical composition and bioactivities of the methanol root extracts of Saussurea costus. *Open. Chem.* **22** (1), 20240002 (2024).
58. Salem, N. et al. Variation in chemical composition of Eucalyptus globulus essential oil under phenological stages and evidence synergism with antimicrobial standards. *Ind. Crops Prod.* **124**, 115–125 (2018).
59. Basri, D. F. & Sandra, V. Synergistic interaction of methanol extract from Canarium odontophyllum Miq. Leaf in combination with oxacillin against methicillin-resistant Staphylococcus aureus (MRSA) ATCC 33591. *International J. Microbiology* **2016** (2016).
60. Aljeldah, M. M., Yassin, M. T., Mostafa, A. A. F. & Aboul-Soud, M. A. Synergistic Antibacterial Potential of Greenly Synthesized Silver Nanoparticles with Fosfomycin Against Some Nosocomial Bacterial Pathogens. *Infection Drug Resistance* 125–142 (2022).
61. Geraldi, A. et al. Tropical Medicinal Plant Extracts from Indonesia as Antifungal Agents against Candida Albicans. *Front. Bioscience-Landmark*. **27** (9), 274 (2022).
62. Rangel, M. L., SGd, A., JMd, L. & Castellano, L. R. Castro RDd. In vitro effect of Cinnamomum zeylanicum Blume essential oil on Candida spp. involved in oral infections. *Evidence-Based Complementary and Alternative Medicine* (2018). (2018).

Figures

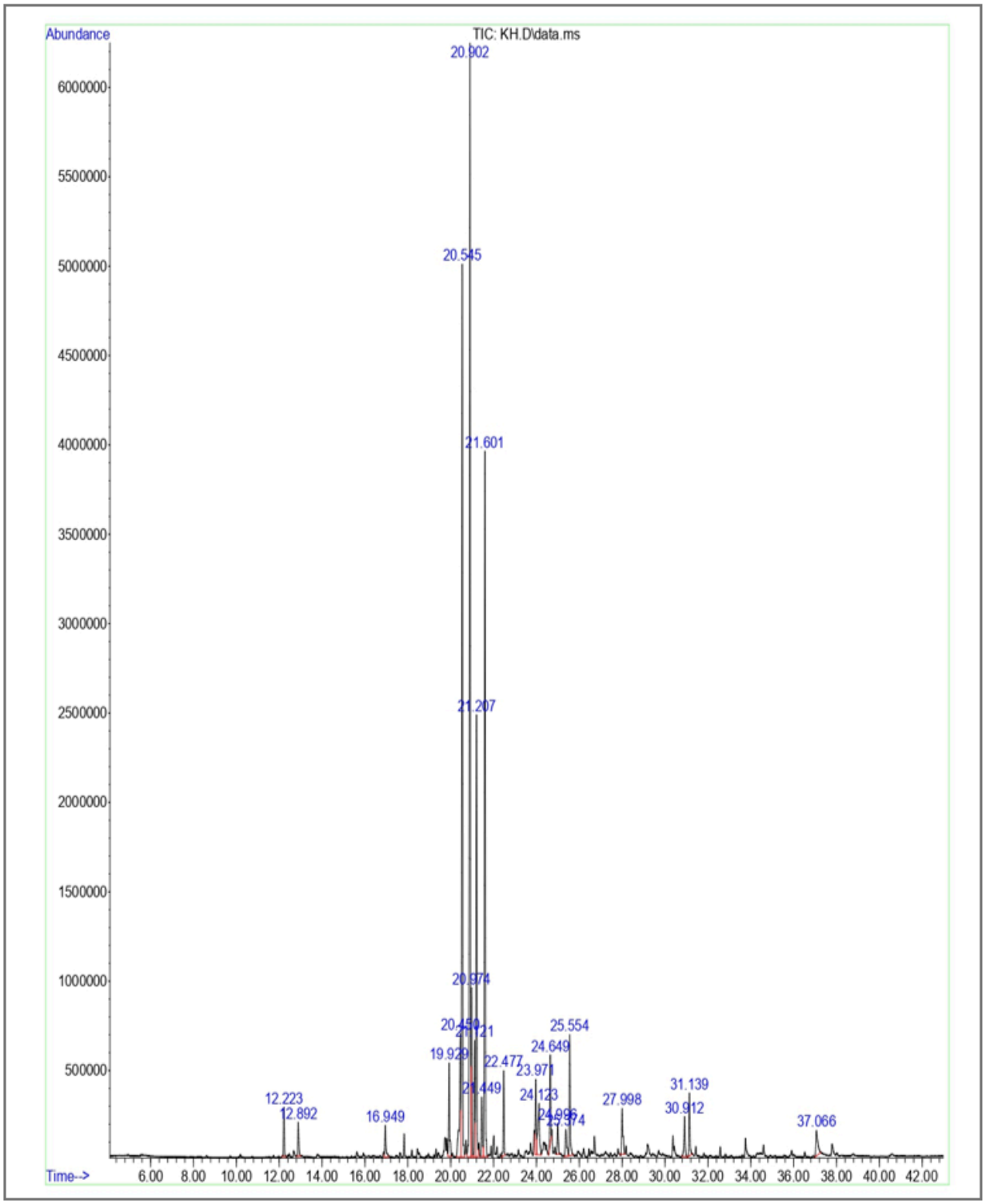

Figure 1

MEHC's GC-MS chromatograms. All spectral peaks correlate with identified chemicals, with a big peak indicating the primary constituent of the extract.

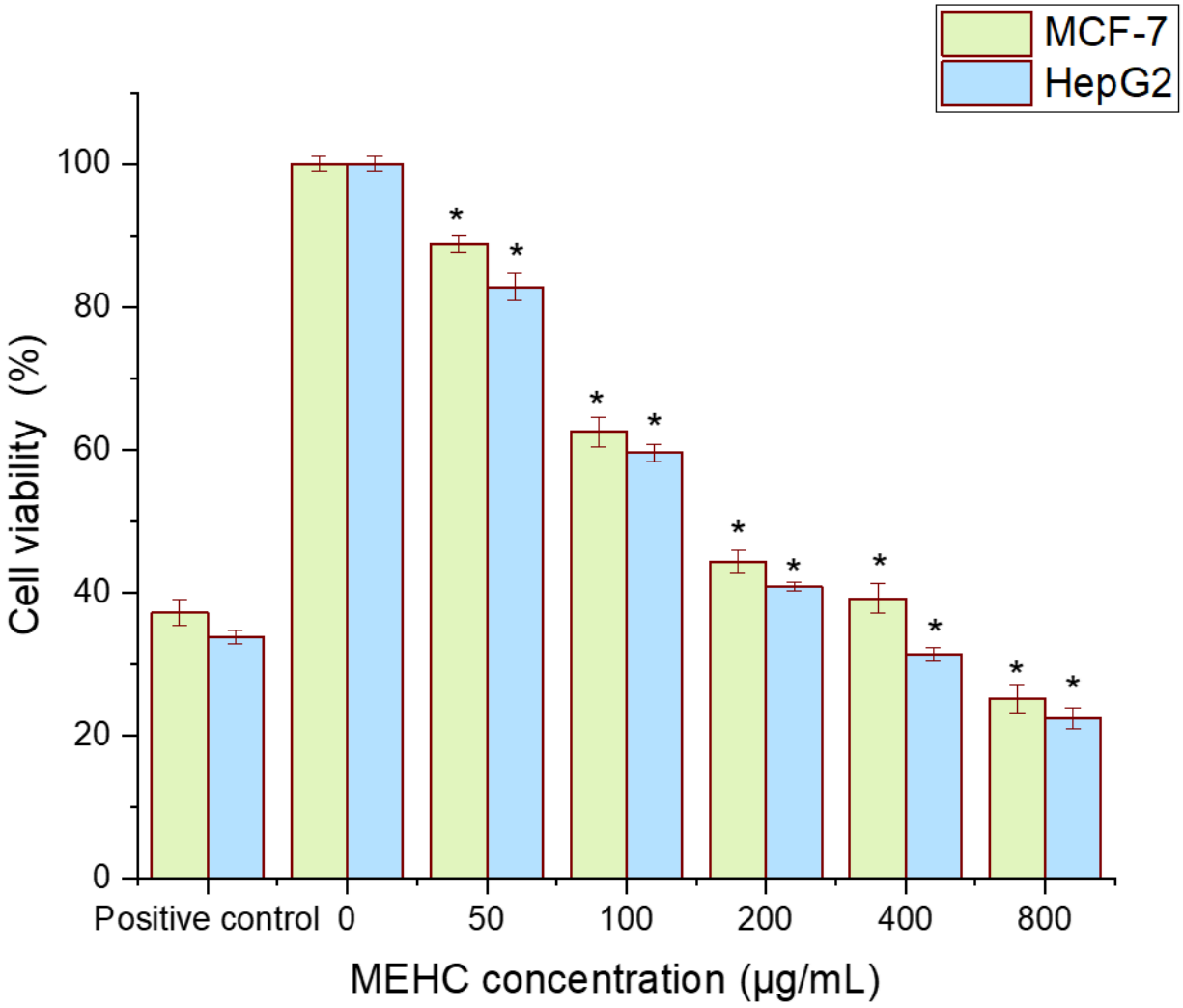

Figure 2

cytotoxic effects of different concentrations (0-800 µg/mL) of MEHC and a positive control (cisplatin 30 µg/mL) on MCF-7 and HepG2 cells viability using the MTT assay. The mean ± SD for the three different investigations is shown (* = $P < 0.05$ relative to the untreated cells (negative control)).

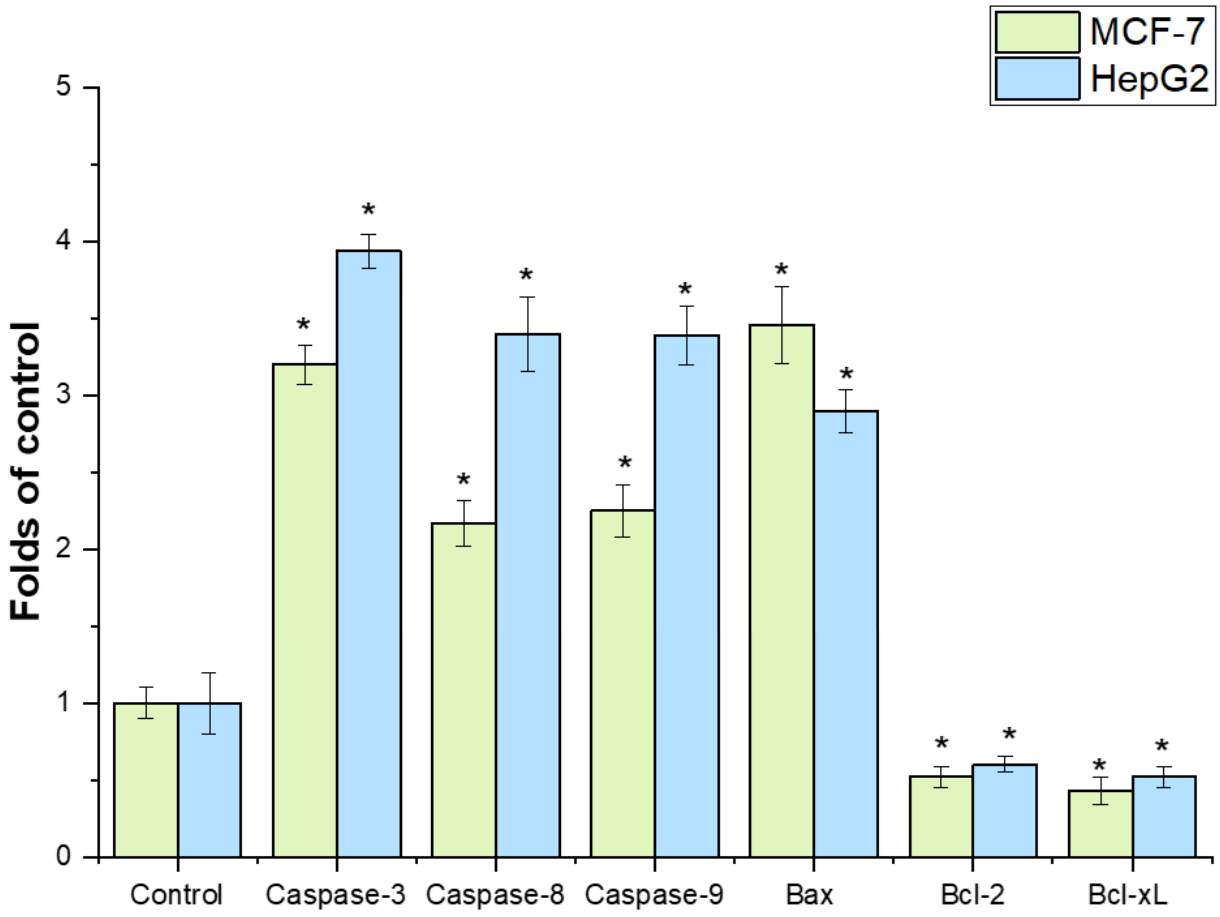

Figure 3

Effect of MEHC on MCF-7 and HepG2 cell lines and analysis of pro- and genes *caspase-3, 8* and 9, and *Bax),* and anti-apoptosis marker *(Bcl-2* and *Bcl-Xl*) genes for 48 h. Gene expression (* = $P < 0.05$ relative to untreated cells, or control) is shown by the mean ± SD of three independent experiments.