Research Article

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In vitro and *in vivo* investigation of polypharmacology of propolis extract as anticancer, antibacterial, anti-inflammatory, and chemical properties

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Abstract: Numerous compounds derived from natural sources such as microbes, plants, and insects have proven to be safe, efficacious, and cost-effective therapeutics for human diseases. This study examined the bioactivities of propolis, a structural sealant and antibacterial/antifungal agent produced by honey bees. Chinese propolis was extracted in methanol or hexane. Propolis significantly reduced the numbers of viable cancer cells when applied as a methanol extract (IC₅₀ values in µg/mL for the indicated cell line: MDA-MB-231, 74.12; LoVo, 74.12; HepG2, 77.74; MCF7, 95.10; A549, 114.84) or a hexane extract (MDA-MB-231, 52.11; LoVo, 45.9; HepG2, 52.11; MCF7, 78.01; A549, 67.90). Hexane extract also induced apoptosis of HepG2 cells according to activated caspase-3/7 expression assays ($17.6 \pm 2.9\%$ at 150 µg/mL and $89.2 \pm 1.9\%$ at 300 µg/mL vs $3.4 \pm 0.4\%$ in vehicle control), suppressed the growth of *Candida albicans* and multiple multidrug-resistant and nonresistant Gram-positive bacteria, and inhibited croton oil-induced skin inflammation when applied as topical treatment. GC-MS identified hexadecanoic acid methyl ester as a major constituent (33.6%). Propolis hexane extract has potential anticancer, antimicrobial, and anti-inflammatory activities.

Keywords: anticancer, antimicrobial, anti-inflammatory, apoptosis, propolis

1 Introduction

Identification of bioactive agents from natural sources, including microbes, insects, and traditional medicinal plants, is a major focus of drug discovery research for infectious diseases and cancer [1,2]. Indeed, there are numerous examples of drugs developed from plants and microbial sources, including the anticancer agents combretastatin, camptothecin, taxanes, geniposide, artesunate, colchicine, salvicine, roscovitine, homoharringtonine, ellipticine, taspargin, maytansin, and bruceantin [3], the antimicrobial polymyxin (colistin) from *Paenibacillus amylolyticus* [4], and the antimicrobial phytochemicals 4-hydroxy-3-methoxybenzoic acid, ellagic acid, gallic acid, caffeic acid, coumaric acid, and 4-methoxycinnamic acid [5]. Several anti-inflammatory compounds were also first isolated from plants, such as resveratrol, curcumin, boswellic acid, baicalein, betulinic acid, oleanolic acid, and ursolic acid [6].

Acquired drug resistance is a major factor limiting the long-term efficacies of both cancer and microbial infection treatments. Further, some of these drug resistance mechanisms are similar [7]. One strategy for overcoming drug resistance is polypharmacy using drugs with distinct mechanisms of action, such as polychemotherapy for cancer. Many raw natural products and extracts contain large numbers of chemical compounds with distinct or shared bioactivities that act synergistically. These compounds may thus help in overcoming resistance, thereby increasing the efficacy of current cancer polychemotherapy treatments and reducing the likelihood of recurrence and secondary malignancies [8].

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Propolis is a resin-like product collected by worker bees (*Apis mellifera* L.) from different plants to protect the hive, seal cracks, maintain internal temperature and humidity, protect against microbial infections, and prevent decomposition of intruder carcasses [9,10]. The major constituents include pollen (5%), oils (10%), wax (30%), and resins and vegetable balsam (50%). In addition, 300 compounds with potential bioactivity have been reported in propolis, including esters, phenolic acids, and flavonoids. While bioactivity varies with geographical origin and time of collection, many reports have documented antibacterial, antifungal, antitumoral, antiprotozoal, anti-inflammatory, hepatoprotective, antioxidant, antiviral, wound healing, and anticancer activities [9,11–14].

The goal of this investigation was to evaluate the chemical composition and anticancer, antimicrobial, and anti-inflammatory properties of propolis hexane and methanol extracts from Chinese propolis, including the first test of anti-inflammatory potential using the croton oil-induced inflammation in mice ear edema model.

2 Experimental

2.1 Solvent extraction

Propolis (30 g) was extracted twice in methanol (300 mL) (Fisher Scientific, UK) in a shaking incubator at 150 rpm for 24 h at 30°C and the two extracts were pooled together. The suspension was filtered using Whatman® Grade 1 filter paper and evaporated using rotary evaporator (Heidolph, Germany). The evaporated suspension was re-extracted twice in 250 mL of hexane (Fisher Scientific, UK) in a shaking incubator similar to methanol. The remaining residue (solid) was re-extracted twice in 250 mL of ethyl acetate (Fisher Scientific, UK) in a shaking incubator and processed similar to methanol extract. The resulting methanol, hexane, and ethyl acetate extracts were evaporated, weighed, and dissolved in methanol at a final concentration of 20 mg/mL and stored at –20°C.

2.2 Cancer cell lines

Breast cancer lines MCF7 and MDA-MB-231, the liver cancer line HepG2, the colon cancer line LoVo, and the lung cancer line A549 were obtained from DSMZ, Germany, and grown in Dulbecco Modified Eagle Medium (DMEM, UFC Biotech, Saudi Arabia, Riyadh). The cell lines were grown at 37°C

using a humidified incubator and 5% CO₂ atmosphere. Cells were incubated under the same conditions during drug treatments unless otherwise specified.

2.3 Viable cell number assay

Number of viable cell during treatment was measured as described previously [15] using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Invitrogen). Increasing concentrations of the extract were prepared from a stock solution (10.00 mg/mL) by diluting in culture medium. The final vehicle (methanol) concentration did not exceed 0.1%. Cells (5×10^5 cells per well) were grown in 24-well plates for 24 h, and then incubated with different doses of propolis (20, 50, 100, 140, and 200 µg/mL). The extract concentration needed to inhibit viable cell number by 50% (IC₅₀) was interpolated from the dose–response curve plotted using Origin 8 software (Northampton, MA). Results are presented as mean ± standard deviation (each with independent triplicate biological repeats).

2.4 Cell morphology and Hoechst 33342 staining

HepG2 cells (5×10^5 cells per well) were grown in 24-well plates, incubated for 24 h, and imaged under phase-contrast microscopy to describe baseline morphology. Cells were then treated with 150 µg/mL extract for 48 h, stained (Hoechst 33342), and then imaged under a fluorescence microscope (EVOS, Carlsbad, USA) according to the method described previously [15].

2.5 Annexin-V, and dead cell assay

HepG2 cells (5×10^5 cells per well) were grown in 6-well plates, incubated until they reach 80% confluence, then treated with 150 µg/mL extract, 300 µg/mL extract, or 0.01% methanol vehicle (control) for 24 h. Apoptosis was assessed by flow cytometry according to the manufacturer's guide. Cells were centrifuged (3,000 rpm, 5 min), washed with PBS, resuspended in DMEM medium 1% bovine serum albumin, and mixed with Annexin V and Dead Cell reagent. The mixture was incubated for 20 min at 25°C in the dark and results were analyzed using the Muse™ Cell Analyzer (Millipore, USA).

2.6 Caspase-3/7 activity

HepG2 cells treated as described for cell apoptosis induction were also examined for caspase activity using the Muse® Caspase3/7 Kit following the user's guide. Briefly, treated HepG2 cells (1×10^5 /well) were trypsinized, washed (phosphate-buffered saline), and collected by centrifugation at 3,000 rpm for 5 min and further processed by flow cytometry as described [15] using the Muse™ Cell Analyzer.

2.7 Induction of dermatitis in mouse ear skin

Adult albino mice (25–30 g) were housed at four animals per group/cage under standard conditions with free access to water and food. Mice were first anesthetized by injection of ketamin HCl (150 mg/kg, i.p.). After 5 min, inflammation was induced by applying croton oil to the inner surface of the ear (0.4 mg/ear). The right ear was left untreated as a negative control. Twenty-four hours later, a 50 μ L solution containing 500 μ g hexane extract in acetone or AVOCOM (0.1% Ointment as a positive control) was applied to the croton oil-treated ear using cotton swaps. Twenty-four hours later, the mice were killed by pentobarbital (200 mg/kg, i.p.) and the ears harvested. Ear samples were cut in 6 mm diameter sections, fixed in 10% formalin, and processed as previously described [16]. The sections were stained with hematoxylin and eosin (HE) and examined under light microscopy.

2.8 Antibacterial activity

A total of eight multidrug-resistant and nonresistant microbial species (Table 1) were examined for propolis sensitivity using agar well diffusion assays as described previously [15]. Muller Hinton agar was poured into Petri plates and allowed to solidify with sterile cork borers to make 6 mm wells. A 100 μ L volume of microbial suspension adjusted to OD = 0.01 in growth medium was swabbed over the growth medium surface and 10 mg/mL of the hexane extract solution in growth medium or methanol (negative control) was carefully pipetted into the wells and left to diffuse for 30 min at 25°C. Plates were then kept for 24 h at 37°C. The zones of growth inhibition were recorded in millimeters. All concentrations and microbial strains were tested in triplicate.

2.9 GC-MS analysis

GC-MS (PerkinElmer Clarus 600, Waltham, MA, USA) fingerprinting was employed to assess the complexity of hexane extract as previously described [15]. The mass spectra were compared to data from the National Institute of Standard and Technology (NIST, Gaithersburg, USA) and WILEY Spectral libraries for species identification.

Ethical approval: All procedures were performed according to the Animal Ethics Committee, Princess 265 Nourah Bint Abdulrahman University, Riyadh, KSA.

Table 1: Suppression of microbial growth by hexane and methanol propolis extracts as measured by zone of inhibition diameter

Microorganism	Origin	R type	Bacterial growth inhibition zones mm $\pm$ std		
			Methanol	Hexane	Ethyl acetate
<i>Candida albicans</i>	ATCC-90028	—	12 $\pm$ 0.7	12 $\pm$ 0.6	0.0 $\pm$ 0.0
MRSA*	ATCC33591	Met	13 $\pm$ 0.6	13 $\pm$ 0.6	0.0 $\pm$ 0.0
<i>S. aureus</i>	Clinical	Ery	12 $\pm$ 0.3	13 $\pm$ 0.3	0.0 $\pm$ 0.0
<i>S. epidermidis</i>	Clinical	Sensitive	13 $\pm$ 0.6	13 $\pm$ 0.8	0.0 $\pm$ 0.0
<i>E. coli</i>	Clinical	Sensitive	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>E. coli</i>	Clinical	fix, amp, amx, cxm,	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>A. baumannii</i>	Clinical	clav, amp, amx, cef, cxm, fix, amox/K	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>A. baumannii</i>	Clinical	Sensitive	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

*MRSA, methicillin-resistant *Staphylococcus aureus*.

Antimicrobial agents: amp, ampicillin; met, amox/K, amoxicillin/clavulanic acid; methicillin; ery, erythromycin; cxm, cefuroxime; amx, amoxicillin; fix, cefixime; clav, clavulanic acid; cef, ceftriaxone.

Figure 1: Suppression of viable cancer cell number by propolis methanol extract (a) and hexane extract (b) against MCF7, MDA-MB-231, HepG2, LoVo, and A549 cancer cell lines.

3 Results

The yield of propolis extract was calculated as weight/weight percent yield of 30 g of propolis (Figure 1). The yields of methanol, hexane, and ethyl acetate extracts were 30, 6.3, and 21.6%, respectively.

3.1 Cell culture

To evaluate the antiproliferative and/or cytotoxic effects of methanol and hexane extracts of propolis on cancer cells, LoVo, MDA-MB-231, HepG2, MCF7, and A549 cell lines were incubated with increasing extract concentrations

Figure 2: Induction of apoptosis in HepG2 cells by treatment with hexane propolis extract for 48 h. (a) Apoptosis as assessed by nuclear staining. Cells treated with 0.1% methanol vehicle displayed even nuclear staining (a). HepG2 cells showing condensation of DNA (a'). The white arrows show apoptotic cells. (b) control cells, (b') treated cells.

and viable cell numbers estimated by MTT assay (Figure 1a and b). These assays yielded IC_{50} values (in $\mu\text{g/mL}$) of 74.12 (MDA-MB-231), 77.74 (LoVo), 74.12 (HepG2), 95.10 (MCF7), and 114.84 (A549) for the methanol extract (Figure 1a) and 53.41 (MDA-MB-231), 52.11 (LoVo), 45.9 (HepG2), 78.01 (MCF7), and 67.90 (A549) for the hexane extract. Due to enhanced antiproliferative or cytotoxic efficacy, subsequent experiments were conducted using hexane extract on HepG2 cells.

3.2 Morphological changes to HepG2 cells

Vehicle-treated HepG2 cells were healthy, spindle-shaped, and attached to the substrate. The cytoplasm was clear

and the cell body smooth with cell filopodia approaching neighboring cells. Following treatment with the propolis hexane fraction, cells detached from the substrate and shrank, suggesting apoptosis. Indeed, Hoechst 33342 staining revealed nuclear condensation and fragmentation consistent with apoptosis (arrows in Figure 2).

3.3 Annexin-V and dead cell assay

To examine the proapoptotic potential of hexane extract, HepG2 cells were incubated with 150 and 300 $\mu\text{g/mL}$ for 48 h, and apoptotic cells were identified by Annexin V staining and a Dead Cell Muse kit. Result revealed substantial dose-dependent apoptosis, with only $60.8 \pm 10.3\%$

Figure 3: Hexane propolis extract induced HepG2 cell apoptosis at 150 and 300 $\mu\text{g/mL}$. (a) In each panel, the lower left quadrant designates live cells (dead cell marker (–) and annexin v (–)), lower-right quadrant designates early apoptosis (dead cell marker (–) and annexin v (+)), upper right quadrant designates late apoptosis (dead cell marker (+) and annexin v (+)), and upper left quadrant designates necrotic cells (dead cell marker (+) and annexin v (–)). (b) Proportions of dead cells (D), live cells (L), early apoptotic cell (EA), and late apoptotic cell (LA). Data presented as the mean $\pm$ SD, obtained from three independent replications. Significance between the treated and control cells is reported at the (**) $p < 0.05$.

of cells remaining after 150 $\mu\text{g/mL}$ incubation and $5.3 \pm 1.64\%$ remaining after 300 $\mu\text{g/mL}$ hexane extract treatment for 48 h, proportions significantly lower than following vehicle treatment ($91.2 \pm 0.8\%$). While there was little difference in the proportion of early apoptotic cells among treatment groups (control: $1.1 \pm 0.17\%$; 150 $\mu\text{g/mL}$: $0.97 \pm 0.5\%$; 300 $\mu\text{g/mL}$: $6.3 \pm 0.65\%$), there were substantial differences in the proportions of late apoptotic cells (control: $3.4 \pm 1.38\%$; 150 $\mu\text{g/mL}$: $29.9 \pm 4.8\%$; 300 $\mu\text{g/mL}$: $87.9 \pm 0.85\%$). These results suggest that the hexane extract of propolis suppressed cancer cell number (Figure 3) by inducing apoptosis.

To further verify the proapoptotic effects of hexane propolis extract on HepG2 cells, caspase-3/7 detection assays were conducted in which apoptotic cells emit a bright fluorescence from the nucleus. Again consistent with proapoptotic efficacy, 150 $\mu\text{g/mL}$ extract induced

markedly brighter fluorescence emission than vehicle. Further, in accord with Annexin V and Muse assays, the increase in caspase-3/7 activity was dose-dependent (control: $3.4 \pm 0.4\%$; 150 $\mu\text{g/mL}$: $17.6 \pm 2.9\%$; 300 $\mu\text{g/mL}$: $89.2 \pm 1.9\%$) (Figure 4).

3.4 Croton-induced mouse ear inflammation

The epidermal layer, dermal layer, and cartilage of the untreated (right) ear exhibited normal histological features with no cell infiltration, while the croton oil-treated (left) ear revealed extensive infiltration of neutrophil in the dermal layer (Figure 5(2)). Treatment with hexane extract (100 mg/kg) after croton oil reduced neutrophil

Figure 4: Hexane extract induced caspase activation in HepG2 cells as revealed by Muse® Caspase 3/7 Kit staining. (a) In each panel, the lower left quadrant designates cells negative for dead cell marker and caspase 3/7, lower-right quadrant cells negative for dead cell marker and positive for caspase 3/7, upper right quadrant cells positive for dead cell marker and caspase 3/7, and upper left quadrant cells positive for dead cell marker and negative for caspase 3/7. (b) Proportions of live, apoptotic, apoptotic/dead, and total apoptotic cells. $**p < 0.05$.

Figure 5: Hexane propolis extract reduced the histopathological alterations associated with ear inflammation/edema induced by croton oil. (1) Normal histological structures of the epidermal layer, dermal layer, and cartilage from an untreated ear. Note the absence of neutrophil infiltration. (2) Tissue treated with croton oil is showing infiltration of neutrophil in the dermis. (3) Tissue of ear treated with hexane propolis extract following croton oil showing virtually undamaged dermis and cartilage with little infiltration of cells. (4) Ear tissue treated with hexane propolis extract showing less of cells in the dermis compared to the croton oil-treated group. H, hair follicle; E, epidermis; D, dermis; C, cartilage; A, adipose tissue.

infiltration (Figure 5(3)) and maintained relatively normal histological features similar to those observed following AVOCAM treatment as a positive control (Figure 5(4)).

3.5 Antimicrobial activity

As shown in Table 1, *C. albicans* was highly susceptible to hexane and methanol extracts as indicated by inhibition zone diameter (12 ± 0.6 mm). However, ethyl acetate extract was inactive at the concentrations tested. Other species demonstrated similar zones of inhibition (12–13 mm) in response to 10 mg/mL of either extract. In contrast, *E. coli* and *A. baumannii* were resistant to both extracts at all concentrations tested.

3.6 GC-MS analysis

GC-MS analysis and comparison with NIST and WILEY Spectral libraries revealed the presence of 18 phytochemical

compounds in hexane extract (Table 2 and Figure 6). The six largest peaks were hexadecanoic acid methylester (33.6%), octadecanoic acid methylester (11.7%), alpha-eudesmo (7.2%), bicyclo[2.2.2]octa-2,5-dien (6.6%), tetradecanoic acid (4.8%), and 5-methyl-2-(1-methylethyl)-phenol (4.6%).

3.7 Statistical analyses

All results represent the means $\pm$ SD from three experiments. Statistical analyses were carried using an unpaired, two-tailed Student's *t*-test. The results were considered statistically different when $**p < 0.05$. All statistical analyses were carried using Microsoft Excel 2016.

4 Discussion

Numerous natural compounds have been demonstrated to inhibit tumor growth, metastasis, and (or) angiogenesis

Table 2: Compounds identified in the hexane extract of propolis by GC-MS

Compound name	Chemical formula	MW (g/mol)	RT	Area %	Biological activity
5-Methyl-2-(1-methylethyl)-phenol	C ₁₀ H ₁₄ O	150.22	13.08	4.630	Anticancer [36]
1-Heptadecanol	C ₁₇ H ₃₆ O	256.5	14.02	1.410	—
Alpha-curcumen	C ₁₅ H ₂₂	202.33	15.29	2.830	Anticancer [37]
7-Methyl-3-methylene -7-octen-1-ol	C ₁₀ H ₁₈ O	154.25	15.69	2.210	—
1-Heptadecene	C ₁₇ H ₃₄	238.5	16.52	1.580	—
Guaïol	C ₁₅ H ₂₆ O	222.37	16.83	1.680	Antibacterial, insecticidal [14,15] and antitumoral [16]
(-)-Alpha-selinene	C ₁₅ H ₂₄	204.35	17.28	2.000	Anticancer [11]
3,9-Epoxy- <i>p</i> -menthane	C ₁₀ H ₁₈ O	154.25	17.35	1.370	—
Alpha.-eudesmol	C ₁₅ H ₂₆ O	222.37	17.59	7.230	Anticancer [38]
Alpha.-bisabolol	C ₁₅ H ₂₆ O	222.37	17.83	3.680	Anticancer [39]
18-Nonadecen-1-ol	C ₁₉ H ₃₈ O	282.5	19.37	2.680	—
Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228.37	19.52	4.880	Anticancer and antioxidant [40]
Bicyclo[2.2.2]octa-2,5-diene	C ₈ H ₁₀	106.16	19.65	6.660	Apoptosis promoters [40]
<i>cis</i> -9,10-Epoxyoctadecan-1-o	C ₁₈ H ₃₆ O ₂	284.5	20.02	0.650	Antioxidant and antibacterial activity [32]
(-)-Alpha-costol	C ₁₅ H ₂₄ O	220.35	20.38	2.120	—
Methyl ester of hexadecanoic acid	C ₁₇ H ₃₄ O ₂	270.5	20.79	33.600	Anticancer and antioxidant [34,35]
Ethyl ester of nonadecanoic acid	C ₂₁ H ₄₂ O ₂	326.6	21.71	3.140	—
Methyl ester of octadecanoic acid	C ₁₉ H ₃₈ O ₂	298.5	23.43	11.790	Antioxidant and antifungal [41,42]

through multi-target actions. This study demonstrates that a hexane (nonpolar) extract of the honey bee product propolis can dose-dependently reduce the number of viable breast, lung, colon, and lung cancer cells *in vitro*. A methanol (polar) extract demonstrated similar anticancer activity, albeit with less potency. Further, these cells demonstrated morphological and biochemical changes indicative of apoptosis. While the precise mechanisms of action remain to be elucidated, according to MTT assay results, the triple negative breast cancer line MDA-MB-231 was more sensitive than the MCF-7 line, suggesting that the antiproliferative and cytotoxic actions of the hexane extract are independent of estrogen receptor signaling.

Targeted apoptosis induction is one of the best promising tactics for cancer therapy [17]. Here, multiple tests demonstrated that hexane extract induced substantial apoptosis, reaching almost 90% of treated cells at 300 µg/mL. Caspases are the main effects of apoptosis, and different caspases are involved in extrinsic and intrinsic pathways [18]. Caspases cleave cellular substrates, such as PARP, during apoptosis [19,20]. We found that hexane extract induced activation of executioner caspases 3 and 7.

Current results also demonstrated the antibacterial potential of propolis extracts against Gram-positive, but not Gram species. This finding is similar to the common reports that Gram-positive bacteria are more sensitive

(compared to Gram-negative) to plant extracts due to the structural differences of their cell walls and the phytochemical composition of plants [13,21–25]. Indeed, propolis is used in different biomedical applications for its antibacterial efficacy [26,27]. However, the mechanisms of bacterial inactivation are still unclear and warrant further study to identify the major active compounds. There are several compounds detected in hexane extract that have been reported to inhibit bacteria (Table 1).

The hexane extract also demonstrated anti-inflammatory potential in the mouse croton oil-induced inflammation, which features both edema and neutrophil infiltration [28]. *n*-Hexadecanoic acid was the most abundant molecule in the exact, accounting for more than 33% of the total phytochemical detected by GC-MS. In a previous enzyme kinetics study, *n*-hexadecanoic acid demonstrated anti-inflammatory activity by inhibiting phospholipase A2 [29]. Further study is required to address if this same mechanism contributes to suppression of ear inflammation induced by croton oil. These findings are in line with Menezes et al. [30], who reported that 14 ethanol extracts of propolis possess anti-inflammatory activity using a mouse ear inflammation model. Moreover, the efficacy of the hexane extract to inhibit neutrophil infiltration was comparable to the clinical topical anti-inflammatory AVOCOM.

Figure 6: Some of the anticancer compounds detected in hexane propolis extract.

GC-MS fingerprinting is employed to characterize the complex composition of plant extract used in traditional medicine [31]. This method was recommended by the World Health Organization (WHO) to control the quality of plant extracts as marker components [32]. GC-MS profiling of the active extracts confirmed the presence of several classes of compounds with documented anticancer or antimicrobial activity [33]. Among these compounds were several saturated fatty acids, which are bioactive compounds found extensively in the plant kingdom. A number of studies have indicated that hexadecanoic acid methyl ester, a major compound present in the hexane extract, is also able to induce apoptosis of gastric cancer cells [34]. Hexadecanoic acid methyl ester also has demonstrated antioxidant, anti-inflammatory, 5- α reductase inhibitory, hypocholesterolemic, pesticidal, and nematocidal activities [35]. In addition, 5-methyl-2-(1-methylethyl)-phenol was found to inhibit the growth of PC3, HCT-116, and AGS cancer cells [36]. Shin and Lee found that alpha-curcumene was highly cytotoxic to human cervical cancer cells (SiHa) [37]. Numerous studies have revealed that the other compounds present in propolis hexane extract have potent cytotoxic

effects on various types of cancer cell lines and microbes (Table 2). These activities may result in part from the synergistic activities of the identified compounds. Studies testing the bioactivities of these individual compounds alone and in specific combinations are warranted to identify the most potent and specific for further clinical development.

5 Conclusion

In this study, the hexane extract of propolis demonstrated potent cytotoxicity against cancer cell lines as well as antimicrobial and anti-inflammatory activities. GC-MS profiling revealed the presence of numerous molecules, such as hexadecanoic acid methyl ester, methyl ester of octadecanoic acid, and Guaiol with documented anticancer and antimicrobial activities. Further research is required to evaluate the subacute toxicity of hexane extract, identify additional bioactive components, and elucidate their mechanisms.

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Conflict of interest: There are no competing interests.

Data availability statement: All data generated are included in this article.

References

- [1] Pejcin B, Iodice C, Tommonaro G, Bogdanovic G, Kojic V, De Rosa S. Further *in vitro* evaluation of cytotoxicity of the marine natural product derivative 4'-leucine-avarone. *Nat Product Res.* 2014;28(5):347–50.

- [2] Katiyar C, Gupta A, Kanjilal S, Katiyar S. Drug discovery from plant sources: an integrated approach. *Ayu*. 2012;33(1):10–9.
- [3] Lichota A, Gwozdinski K. Anticancer activity of natural compounds from plant and marine environment. *Int J Mol sci*. 2018;19(11):3533.
- [4] Ghrairi T, Jaraud S, Alves A, Fleury Y, El Salabi A, Chouchani C. New insights into and updates on antimicrobial agents from natural products. *Hindawi*. 2019;2019:1–3.
- [5] Vandal J, Abou-Zaid MM, Ferroni G, Leduc LG. Antimicrobial activity of natural products from the flora of Northern Ontario, Canada. *Pharm Biol*. 2015;53(6):800–6.
- [6] Gautam R, Jachak SM. Recent developments in anti-inflammatory natural products. *Med Res Rev*. 2009;29(5):767–820.
- [7] Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. *Nature*. 2019;575(7782):299–309.
- [8] Pezzuto JM, Vang O. Natural products for cancer chemoprevention: single compounds and combinations. Switzerland: Springer Nature; 2020.
- [9] Anjum SI, Ullah A, Khan KA, Attaullah M, Khan H, Ali H, et al. Composition and functional properties of propolis (bee glue): a review. *Saudi J Biol Sci*. 2019;26(7):1695–703.
- [10] Pasupuleti VR, Sammugam L, Ramesh N, Gan SH. Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits. *Oxid Med Cell Longev*. 2017;2017:1259510.
- [11] Silva E, Estevam E, Silva T, Nicoletta H, Furtado R, Alves CCF, et al. Antibacterial and antiproliferative activities of the fresh leaf essential oil of *Psidium guajava* L. (Myrtaceae). *Brazilian J Biol*. 2019;79(4):697–702.
- [12] Barraviera B. The journal of venomous animals and toxins including tropical diseases (JVATiD) from 1995 to 2007. *J Venom Anim Toxins Incl Trop Dis*. 2007;13(2):428–9.
- [13] Wagh VD. Propolis: a wonder bees product and its pharmacological potentials. *Adv Pharmacol Sci*. 2013;2013:1–11.
- [14] Lotfy M. Biological activity of bee propolis in health and disease. *Asian Pac J Cancer Prev*. 2006;7(1):22–31.
- [15] Abutaha N, Al-zharani M, Al-Doaiss AA, Baabbad A, Al-Malki AM, Dekhil H. Anticancer, antioxidant, and acute toxicity studies of a Saudi polyherbal formulation, PHF5. *Open Chem*. 2020;18(1):472–81.
- [16] da Silva JM, Conegundes JL, Mendes Rde F, Pinto Nde C, Gualberto AC, Ribeiro A, et al. Topical application of the hexane fraction of *L. acistema pubescens* reduces skin inflammation and cytokine production in animal model. *J Pharm Pharmacol*. 2015;67(11):1613–22.
- [17] Della Corte A, Chitarrini G, Di Gangi IM, Masuero D, Soini E, Mattivi F, et al. A rapid LC-MS/MS method for quantitative profiling of fatty acids, sterols, glycerolipids, glycerophospholipids and sphingolipids in grapes. *Talanta*. 2015;140:52–61.
- [18] Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol*. 2007;35(4):495–516.
- [19] Lazebnik YA, Kaufmann SH, Desnoyers S, Poirier G, Earnshaw W. Cleavage of poly (ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature*. 1994;371(6495):346–7.
- [20] Boulares AH, Yakovlev AG, Ivanova V, Stoica BA, Wang G, Iyer S, et al. Role of poly (ADP-ribose) polymerase (PARP) cleavage in apoptosis Caspase 3-resistant PARP mutant increases rates of apoptosis in transfected cells. *J Biol Chem*. 1999;274(33):22932–40.
- [21] Fokt H, Pereira A, Ferreira A, Cunha A, Aguiar C. How do bees prevent hive infections? The antimicrobial properties of propolis. *Current Res Technol Educ Topics Appl Microbiol Microbial Biotechnol*. 2010;1:481–93.
- [22] Ahuja V, Ahuja A. Apitherapy-a sweet approach to dental diseases. Part II: propolis. *J Adv Oral Res*. 2011;2(2):1–8.
- [23] Harfouch RM, Mohammad R, Suliman H. Antibacterial activity of syrian propolis extract against several strains of bacteria *in vitro*. *World J Pharm Pharma Sci*. 2016;6:42–6.
- [24] Martinotti S, Ranzato E. Propolis: a new frontier for wound healing? *Burn Trauma*. 2015;3(1):9.
- [25] Nedji N, Loucif-Ayad W. Antimicrobial activity of Algerian propolis in foodborne pathogens and its quantitative chemical composition. *Asian Pac J Trop Dis*. 2014;4(6):433–7.
- [26] Tosi B, Donini A, Romagnoli C, Bruni A. Antimicrobial activity of some commercial extracts of propolis prepared with different solvents. *Phytoth Res*. 1996;10(4):335–6.
- [27] Sforzin JM, Bankova V. Propolis: is there a potential for the development of new drugs. *J Ethnopharmacol*. 2011;133(2):253–60.
- [28] Pathak D, Pathak K, Singla A. Flavonoids as medicinal agents-recent advances. *Fitoterapia*. 1991;62(5):371–89.
- [29] Zuzarte M, Gonçalves MJ, Cavaleiro C, Cruz MT, Benzarti A, Marongiu B, et al. Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus herba-barona* essential oils. *Ind Crop Products*. 2013;44:97–103.
- [30] Menezes H, Alvarez JM, Almeida E. Mouse ear edema modulation by different propolis ethanol extracts. *Arzneimittelforschung*. 1999;49(8):705–7.
- [31] Xin Z, Ren D, Zhang X, Yi Z, Yi L. Chromatographic fingerprints combined with chemometric methods reveal the chemical features of authentic radix polygalae. *J AOAC Int*. 2017;100(1):30–7.
- [32] Fan XH, Cheng YY, Ye ZL, Lin RC, Qian ZZ. Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines. *Anal Chim Acta*. 2006;555(2):217–24.
- [33] Bodoprost J, Rosemeyer H. Analysis of phenacyl ester derivatives of fatty acids from human skin surface sebum by reversed-phase HPLC: chromatographic mobility as a function of physico-chemical properties. *Int J Mol Sci*. 2007;8(11):1111–24.
- [34] Yu FR, Lian XZ, Guo HY, McGuire PM, Li RD, Wang R, et al. Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC-7901 cells. *J Pharm Pharm Sci*. 2005;8(3):528–35.
- [35] Kim YH, Chung HJ. The effects of Korean propolis against foodborne pathogens and transmission electron microscopic examination. *N Biotechnol*. 2011;28(6):713–8.
- [36] Islam MT, Khalipha A, Bagchi R, Mondal M, Smrity SZ, Uddin SJ, et al. Anticancer activity of thymol: A literature-based review and docking study with Emphasis on its anticancer mechanisms. *IUBMB life*. 2019;71(1):9–19.
- [37] Shin Y, Lee Y. Cytotoxic activity from *Curcuma zedoaria* through mitochondrial activation on ovarian cancer cells. *Toxicol Res*. 2013;29(4):257–61.

- [38] Wiart C. Lead compounds from medicinal plants for the treatment of cancer. United States: Academic Press; 2012. p. 1
- [39] Seki T, Kokuryo T, Yokoyama Y, Suzuki H, Itatsu K, Nakagawa A, et al. Antitumor effects of α -bisabolol against pancreatic cancer. *Cancer Sci.* 2011;102(12):2199–205.
- [40] Waisundara VY. Traditional herbal remedies of Sri Lanka. Croatia: CRC Press; 2019.
- [41] Sujatha M, Karthika K, Sivakamasungari S, Mariajancyrani J, Chandramohan G. GC-MS analysis of phytocomponents and total antioxidant activity of hexane extract of *Sinapis alba*. *Int J Pharm Chem Biol Sci.* 2014;4(1):112–7.
- [42] Rangel-Sánchez G, Castro-Mercado E, García-Pineda E. Avocado roots treated with salicylic acid produce phenol-2, 4-bis (1, 1-dimethylethyl), a compound with antifungal activity. *J plant Physiol.* 2014;171(3–4):189–98.