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Original Article

COMPARATIVE CYTOTOXICITY OF TURKISH PROPOLIS EXTRACTS: SOLVENT-DEPENDENT EFFECTS ACROSS EIGHT CANCER CELL LINES

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ABSTRACT

Propolis, a natural resinous bee product, has considerable potential as a source of anticancer agents. This study investigated the cytotoxic properties of Turkish propolis extracted with water (PWE) and 70% (v/v) ethanol (PEE) against a panel of eight cancer cell lines representing diverse tissue origins. Cytotoxicity was assessed using the MTT assay after 48 and 72 hours of exposure. Both extracts showed clear time and concentration dependent cytotoxic effects, but the 70% ethanolic extract (PEE) exhibited consistently greater potency across all cell lines. The 72 hour IC_{50} values for PEE ranged from 17.22 ± 0.35 µg/mL to 51.78 ± 1.17 µg/mL, whereas PWE was markedly less active (IC_{50} range from 45.27 ± 0.50 µg/mL to 642.13 ± 3.20 µg/mL). The cell lines most sensitive to PEE were HT-29 (colorectal adenocarcinoma), PC-3 (prostate adenocarcinoma), and MG-63 (osteosarcoma). These findings highlight the critical influence of the extraction solvents on the recovery of bioactive anticancer compounds. The superior efficacy of PEE suggests that the key anticancer components in Turkish propolis are more efficiently extracted with 70% ethanol. Overall, this study underscores the potential of Turkish propolis as a rich source of anticancer agents and contributes to the growing evidence supporting its chemopreventive and therapeutic properties.

KEYWORDS: Anticancer; Cytotoxicity; IC_{50}; MTT assay; Turkish propolis.

1. Introduction

Cancer remains a leading cause of mortality worldwide, characterized by uncontrolled cell proliferation, evasion of apoptosis, and metastatic potential [1–3]. The limitations of conventional chemotherapy, including severe side effects and the development of multidrug resistance, have intensified the search for novel therapeutic agents from natural sources. Propolis, a complex resinous substance collected by honeybees (*Apis mellifera*) from plant exudates, has been used in traditional medicine for centuries and has garnered significant scientific interest due to its broad spectrum of biological activities, including antimicrobial, anti-inflammatory, antioxidant, immunomodulatory, and anticancer properties [4,5].

The chemical composition of propolis is highly complex and varies considerably with geographical origin, botanical sources, and season [6–15]. Indeed, more than 300 distinct compounds have been isolated and identified from propolis, predominantly phenolic acids, flavonoids, and their esters [11]. This variability directly influences its biological efficacy

and presents challenges for standardization [9]. Generally, propolis is rich in polyphenols, flavonoids, phenolic acids, and related derivatives, which are considered the primary bioactive constituents responsible for its therapeutic effects [6]. The anticancer potential of propolis extracts from various regions (e.g., Brazilian, Chinese, Jordanian) has been demonstrated against numerous cancer cell lines through mechanisms such as induction of apoptosis, cell cycle arrest, and inhibition of metastasis [15,16].

Because propolis is a chemically heterogeneous matrix, extraction solvent selection is critical: solvent polarity strongly affects which phenolic acids, flavonoids, and other constituents are recovered, thereby shaping the final extract's bioactivity profile [9,11,15]. While ethanolic propolis extracts (PEE) are most commonly studied due to their high efficiency in extracting lipophilic phenolic compounds such as flavonoids and phenolic acid esters [6,15,17,18], the aqueous extracts (PWE) can also contain bioactive water-soluble components like phenolic acids and glycosides [9,17,18]. Comparative studies of different extracts from the same propolis source are crucial for identifying the most therapeutically valuable fractions.

In this study, we comprehensively evaluate and compare the cytotoxic effects of aqueous (PWE) and 70% ethanolic (PEE) extracts of Turkish propolis on a panel of eight cancer cell lines. These lines were selected to represent prevalent and therapeutically challenging malignancies of diverse tissue origins: prostate (PC-3), cervix (HeLa), lung (CARM-L12 TG3), colon (HT-29, Caco-2, SW48), bone (MG-63), and liver (Huh7). This selection allows for a broad assessment of the extract's potential spectrum of activity and differential cell-type sensitivity. Our findings will contribute to the pharmacological profiling of region-specific propolis and help identify the most effective extraction method for isolating its anticancer components. While the bioactivity of Turkish propolis has been explored in other contexts [18], a direct, systematic comparison of the cytotoxicity of its aqueous and ethanolic extracts across a broad panel of eight cancer cell lines has not been previously reported. This study aims to fill this gap, providing a comprehensive pharmacological profile that highlights the critical role of the extraction solvent.

2. Materials and Methods

2.1. Propolis Collection, Extraction and Composition

Propolis samples were collected from beehives in Kocaeli-Ulaşlı, Türkiye, in August 2023. The raw propolis was stored at -20°C until processing. Prior to extraction, the frozen samples were fragmented using a commercial blender to obtain a homogeneous fine powder (Fig. 1).

The 70% ethanolic extract (PEE) was prepared using an optimized protocol [17,18]. Briefly, 100 g of propolis powder was combined with 300 mL of 70% (v/v) ethanol (a 1:3 w/v solid to solvent ratio). The mixture was shaken at 200 rpm for 30 minutes at 24°C, followed by sonication in an ultrasonic water bath for 30 minutes. It was then incubated at 35°C with continuous shaking (200 rpm) for 1 hour. This ultrasonication and shaking cycle was repeated once to enhance extraction efficiency. The resulting mixture was filtered through a dual layer system (a coarse filter paper over a Whatman No. 1 filter). The filtrate was frozen at –80°C, then lyophilized to dryness and weighed. The amount of PEE obtained per 100 g of raw propolis was approximately 30 g. The dried PEE was stored at –20°C until use and reconstituted in 70% ethanol for experiments [17,18]. The aqueous propolis extract (PWE) was prepared by mixing 100 g of propolis powder with 500 mL of distilled water. The suspension was stirred on a magnetic stirrer at 400 rpm in the dark at 10°C for 4–5 days. The extract was then centrifuged at 4500 rpm for 30 minutes at 4°C. The supernatant was sequentially filtered under vacuum through membranes of 2.5 µm, 0.45 µm, and 0.22 µm pore sizes to remove particulate matter. The filtrate was frozen, lyophilized, and stored under the same conditions as PEE. The extraction yield of PWE was approximately 1.5 g per 100 g of raw propolis. Dried PWE was reconstituted in distilled water for use in cell treatments.

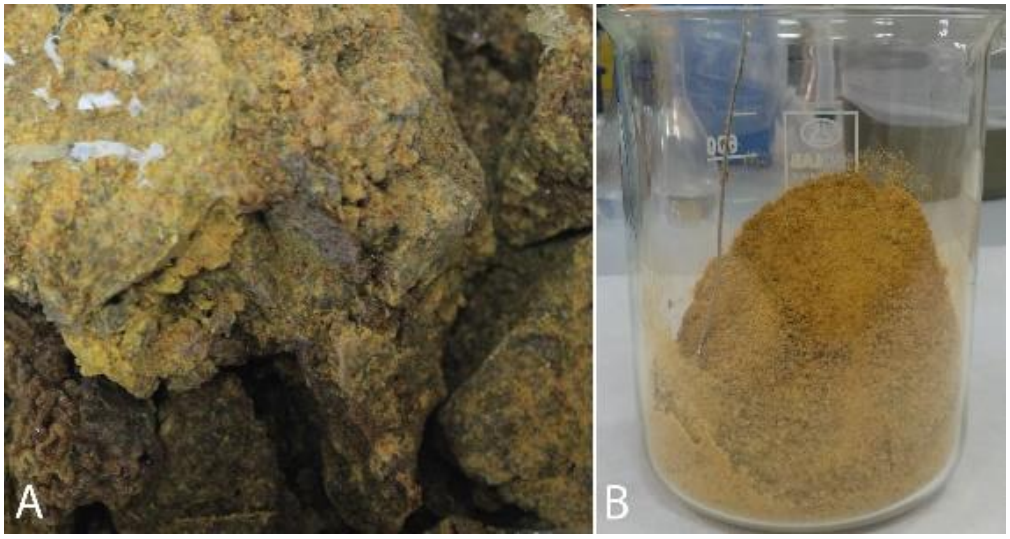

Fig. 1. Propolis samples in raw and powdered form prepared for extraction. (A) Raw propolis as obtained from beehives prior to processing. (B) Propolis after freezing at - 80°C and grinding into a fine powder, which was subsequently used for the preparation of the aqueous and 70% ethanolic extracts.

Additionally, the phenolic composition of both PWE and PEE was analyzed using high performance liquid chromatography (HPLC). This allowed identification and quantification of key phenolic compounds in the extracts, following the methodology as described before [18].

2.2. Cell Culture and Maintenance

Eight cancer cell lines were used in this study: PC-3 (human prostate adenocarcinoma), HeLa (human cervical adenocarcinoma), CARM-L12 TG3 (rat lung mesothelioma), Caco-2 (human colorectal adenocarcinoma), SW48 (human colorectal carcinoma), HT-29 (human colorectal adenocarcinoma), MG-63 (human osteosarcoma), and Huh7 (human hepatocellular carcinoma). All cell lines were cultured in their recommended growth media supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2 as described before [19,20].

2.3. Treatment Condition

Lyophilized PEE and PWE extracts were reconstituted in their respective solvents (70% ethanol for PEE and distilled water for PWE) and then diluted into cell culture medium to achieve the desired final concentrations (31.25, 62.5, 125, 250, 500, 1000, 2000 µg/mL). The final concentration of ethanol in any PEE treated culture did not exceed 0.5% (v/v); this solvent level was confirmed in preliminary experiments to have no effect on cell viability.

2.4. Assessment of Cytotoxicity (MTT Assay)

Cytotoxic effects were evaluated using the MTT colorimetric assay. Cells were seeded in 96-well plates at densities of $5x10^3$ to $1x10^4$ cells/well, depending on the cell line's doubling time, and allowed to adhere for 24 hours. The medium was then replaced with fresh growth medium containing serial dilutions of either PEE or PWE. After treatment for 48 or 72 hours, the media were removed and 100 µL of MTT solution (0.5 mg/mL in PBS) was added to each well. Plates were incubated for 3 hours at 37°C to allow mitochondrial dehydrogenases in viable cells to convert MTT into insoluble formazan crystals. Subsequently, the supernatant was carefully removed, and 100 µl dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan. The absorbance of each well was measured at 570 nm using a microplate reader (BioTek instruments, ELx800, USA). Cell viability was calculated as a percentage of the untreated control wells [19,20]. Each treatment condition was tested in triplicated wells, and each experiment was repeated three times.

2.5. Statistical Analysis

All experiments were performed in triplicate, and data are presented as mean ± standard error of the mean (SEM). Dose response curves were generated and used to calculate the half maximal inhibitory concentration (IC_{50}) values for each extract and time point via nonlinear regression analysis. Statistical comparisons between treatment groups (effects of extract time and exposure time) were carried out using two way analysis of variance (ANOVA). Differences were considered statistically significant at $p < 0.05$.

3. Results

The cytotoxic effects of the aqueous propolis extracts (PWE) and 70% ethanolic extracts (PEE) were evaluated across eight cancer cell lines at 48 hours and 72 hours exposure periods. Both extracts exhibited clear concentration dependent and time dependent cytotoxicity (Fig. 2). However, the 70% ethanolic extract (PEE) was consistently and significantly more potent than the aqueous propolis extract (PWE) in all cell lines, as evidenced by substantially lower IC_{50} values (Table 1).

The sensitivity to propolis extracts varied among the different cancer cell types. At 72 hours, the cell lines most sensitive to PEE were HT-29 (IC_{50} = 17.22 ± 0.35 µg/mL), PC-3 (26.49 ± 0.34 µg/mL), and MG-63 (31.13 ± 0.34 µg/mL). In contrast, for PWE, the cell lines showing the greatest sensitivity at 72 hours were HeLa (45.27 ± 0.50 µg/mL), SW48 (145.08 ± 2.29 µg/mL), and HT-29 (228.52 ± 2.91 µg/mL).

Table 1. IC_{50} values (µg/mL) of aqueous (PWE) and 70% ethanolic (PEE) Turkish propolis extracts against various cancer cell lines[a].

Cell line	PWE (48h) µg/mL	PWE (72h) µg/mL	PEE (48h) µg/mL	PEE (72h) µg/mL
HeLa (cervical)	75.98 ± 1.43	45.27 ± 0.50	48.51 ± 0.76	35.92 ± 0.66
MG-63 (osteosarcoma)	814.68 ± 1.35	437.85 ± 3.46	119.48 ± 2.73	31.13 ± 0.34
HT-29 (colorectal)	908.47 ± 0.94	228.52 ± 2.91	32.18 ± 0.91	17.22 ± 0.35
Caco-2 (colorectal)	539.93 ± 4.81	389.9 ± 4.10	33.43 ± 1.50	39.71 ± 1.30
PC-3 (prostate)	768.32 ± 8.45	562.58 ± 6.60	60.78 ± 0.32	26.49 ± 0.34
Huh7 (hepatic)	679.82 ± 0.90	642.13 ± 3.20	45.10 ± 0.93	29.84 ± 0.39
CARM-L12 TG3 (lung mesothelioma)	355.52 ± 5.14	407.21 ± 5.00	86.43 ± 3.24	46.43 ± 0.83
SW48 (colorectal)	247.09 ± 3.99	145.08 ± 2.29	56.51 ± 1.14	51.78 ± 1.17

[a.] *Values are presented as mean ± SEM (n = 3 independent experiments).*

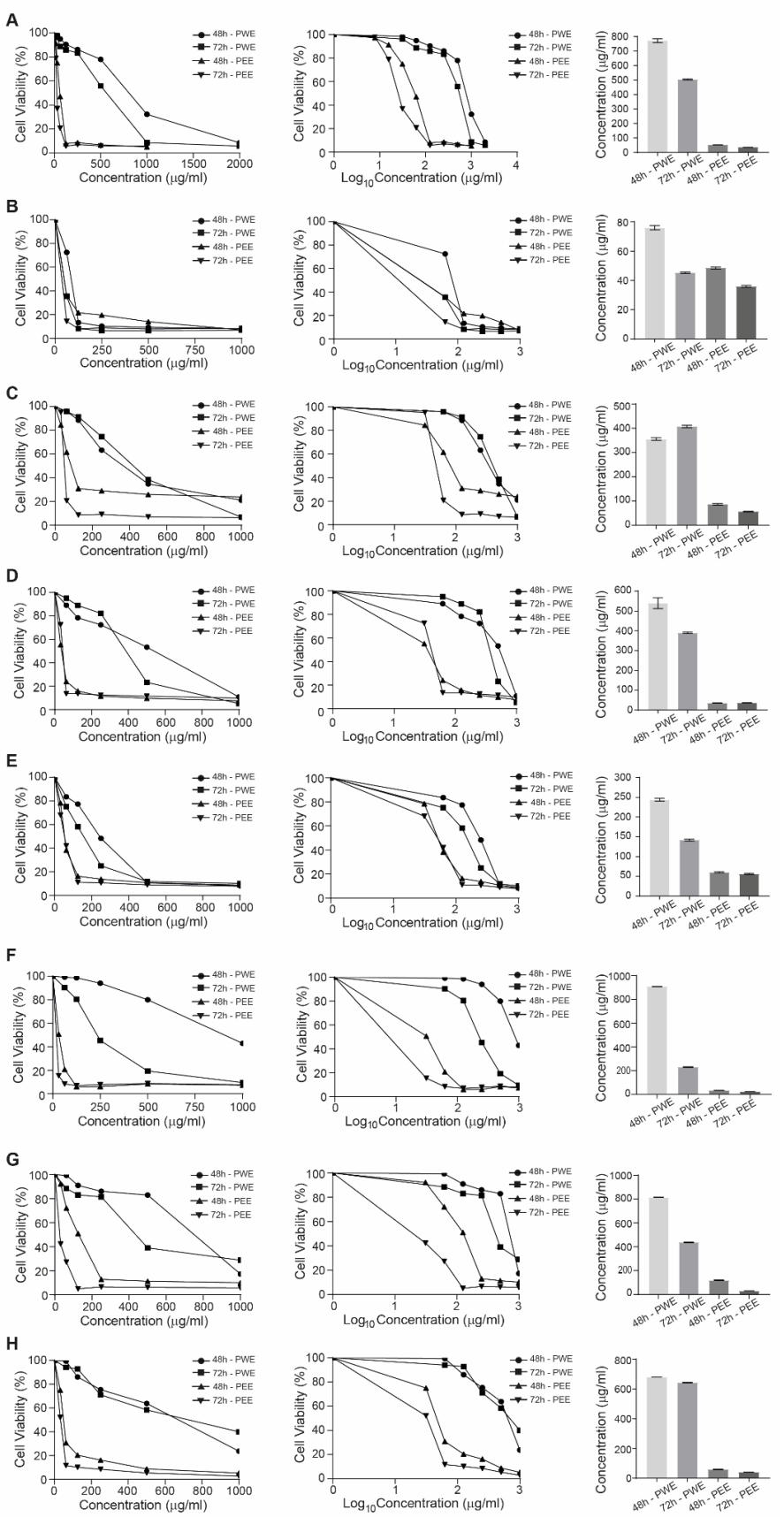

Fig. 2. Dose response curves and IC_{50} values for PWE and PEE in eight cancer cell lines after 48 h and 72 h exposure. (A) PC-3, (B) HeLa, (C) CARM-L12 TG3, (D) Caco-2, (E) SW48, (F) HT-29, (G) MG-63, and (H) Huh7 cells. Data represent mean ± SEM of three experiments. In each set of graphs; the left panel shows the percentage of viable cells (MTT assay) versus extract concentration, the middle panel shows the same viability data on a $\log_{10}$ scale for clarity, and the right panel shows bar graphs of the calculated IC_{50} values. Both PWE and PEE caused a significant reduction in cell viability in a dose and time dependent manner. Notably, PEE yielded much lower IC_{50} values than PWE in all cell lines, indicating greater cytotoxic potency.

A pronounced time dependent enhancement of cytotoxicity was observed for PEE in most cell lines. For instance, in MG-63 osteosarcoma cells, the IC_{50} for PEE decreased from 119.48 ± 2.73 µg/mL at 48 h to 31.13 ± 0.34 µg/mL at 72 h (~74% increase in potency). Similar substantial increases in potency over time were noted in HT-29, PC-3, and Huh7 cells with PEE. In contrast, the aqueous propolis extract (PWE) generally showed either a modest time-dependent effect or, in some cases, a slight reduction in potency over time (as seen with Caco-2 and CARM-L12 TG3 where 72 h IC_{50} values were slightly higher than at 48 h).

As shown above, Table 1 provides a quantitative summary of the IC_{50} data for each extract, cell line, and exposure time. Fig. 3 further illustrates the stark differences in cytotoxic efficacy between PWE and PEE. In both the 48 h and 72 h treatments, PEE achieved a given level of cell killing at substantially lower concentrations than PWE in nearly all cell lines. The potency gap was especially large in certain cell types: for example, at 72 h PEE's IC_{50} was over 20 fold lower than PWE's in PC-3 prostate and Huh7 hepatocellular carcinoma cells (approximately 26–30 µg/mL for PEE vs. 560–640 µg/mL for PWE). Likewise, more than tenfold differences were observed in MG-63 osteosarcoma and HT-29 colorectal cancer cells. Even in the cell line where PWE was most effective (HeLa), PEE still showed greater potency (72 h IC_{50} ~35.9 µg/mL vs. 45.3 µg/mL). These data underscore that PEE is considerably more cytotoxic than PWE across the board (Fig. 3, Table 1).

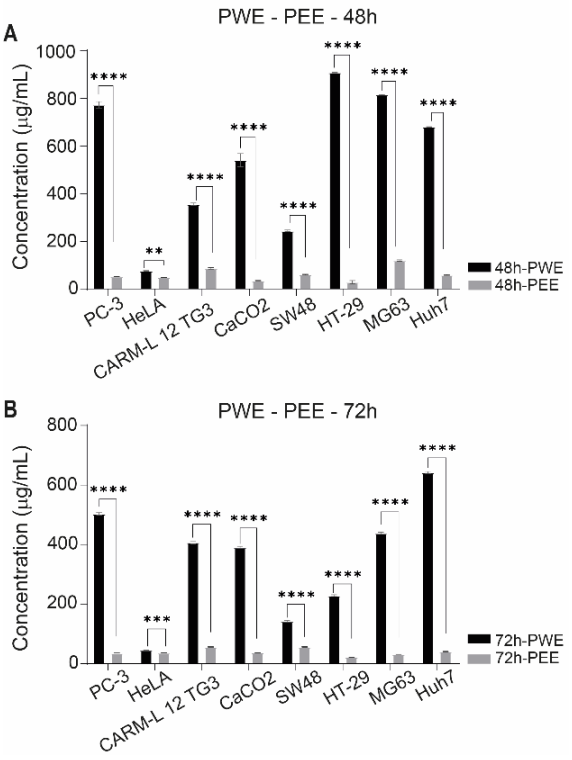

Fig. 3. PEE is more effective than PWE against various cancer cell lines.

In addition, compositional analysis of the extracts identified and quantified several phenolic constituents (Table 2). Although many phenolic compounds were present in both PWE and PEE, their concentrations differed markedly between the two extracts. Notably, PWE contained a far higher level of caffeic acid, whereas PEE was relatively enriched in certain flavonoids such as naringenin and kaempferol. These compositional differences may underlie the observed disparities in bioactivity between propolis aqueous extract (PWE) and the 70% propolis ethanol extract (PEE).

The figure shows the concentrations (µg/mL) of the extracts required to achieve cytotoxic effects on eight different cancer cell lines: PC-3, HeLa, CARM-L12 TG3, Caco-2, SW48, HT-29, MG-63, and Huh7. Panel (A) represents results at 48 hours, while Panel (B) shows results at 72 hours. In both panels, black bars indicate treatment with PWE (propolis water extract), and gray bars represent treatment with PEE (**70%** ethanolic extract). In most cell lines, PEE exhibited significantly lower IC_{50} values compared to PWE, suggesting stronger cytotoxic effects. Statistical analysis revealed significant differences between the two treatments for nearly all cell lines, with significance levels indicated as $p < 0.01$, $p < 0.001$ (*), and $p < 0.0001$ (**). Notably, PEE showed especially enhanced potency in HeLa, CARM-L12 TG3, SW48, HT-29, and MG-63 cells.

Table 2. Selected phenolic compounds identified in the aqueous extract (PWE) and 70% ethanolic extract (PEE) of Turkish propolis[a].

Phenolic Compound	PWE (mg/kg)	PEE (mg/kg)
Quercetin	151.83	102.49
4-Hydroxy benzoic acid	33.81	10.73
3-4-Dihydroxy benzaldehyde	729.46	153.67
trans-Cinnamic acid	17.13	1.43
Vanillin	42.33	38.86
Gentisic acid	380.12	48.19
Protocatechuic acid	283.02	33.96
Caffeic acid	2028.11	274.43
Ferulic acid	2064.09	2985.81
Syringic acid	8.38	9.17
Apigenin	0.05	7.11
Naringenin	20.03	26.03
Kaempferol	8.96	20.44
Hesperetin	0.17	2.90

[a] Concentrations are expressed in mg of compound per kg of dried extract (mg/kg).

4. Discussion

This study provides a comprehensive analysis of the cytotoxic properties of Turkish propolis and reveals that the 70% ethanolic extract (PEE) has significantly greater and broader anticancer activity compared to the aqueous propolis extract (PWE) across a diverse panel of cancer cell lines. These findings are consistent with reports in the literature, where ethanol based propolis extracts generally exhibit higher potency, likely due to their higher content of lipophilic bioactive compounds [21,22]. Propolis extracts in general are known to exert anticancer effects by inducing apoptosis and suppressing the proliferation and metastasis of cancer cells [23].

HPLC analysis revealed distinct compositional profiles: PWE was richer in polar compounds like caffeic acid,

whereas PEE was enriched with less polar flavonoids such as naringenin and kaempferol (Table 2). This likely explains the superior cytotoxicity of PEE, as many documented anticancer constituents of propolis (e.g., chrysin, CAPE) are lipophilic flavonoids and esters more soluble in ethanol [22,24–26]

4.1. PEE vs. PWE Efficacy

In our experiments, PEE consistently achieved much lower IC_{50} values than PWE in all cell lines tested. This superior efficacy of the 70% ethanolic extract can be attributed to more efficient extraction of key anticancer constituents by ethanol. Many flavonoids and phenolic esters in propolis (such as chrysin, galangin, pinocembrin, and CAPE caffeic acid phenethyl ester) are relatively nonpolar and are thus extracted in greater quantities into ethanol than into water. These compounds are well established mediators of propolis's biological effects and are known to induce apoptosis, trigger cell cycle arrest, and inhibit prosurvival signaling pathways like PI3K/Akt and NF-κB [22]. The pronounced time dependent increase in cytotoxicity observed for PEE (e.g. a ~4 fold drop in IC_{50} for MG-63 between 48 h and 72 h) suggests a cumulative or amplifying effect on these critical cellular processes over time, for instance, progressive mitochondrial dysfunction or activation of apoptotic cascades, when the more potent ethanolic extract is used. In contrast, PWE, which lacks high levels of some of these key lipophilic constituents, showed a more modest or negligible time dependent enhancement of cytotoxicity.

4.2. Differential Cell Line Sensitivities

The variation in sensitivity among the cancer cell lines provides interesting insights for potential therapeutic applications. The remarkable sensitivity of HT-29 colorectal cancer cells to PEE aligns with previous studies that demonstrated strong effects of propolis and its components against gastrointestinal cancers [15]. Likewise, the notable responsiveness of PC-3 prostate cancer cells suggests potential utility of propolis extracts in urological oncology. On the other hand, differences observed among the three colorectal cell lines (HT-29, Caco-2, SW48) may reflect their distinct genetic and molecular profiles (e.g. differences in p53 status, oncogenic mutations, or expression of drug efflux transporters). This highlights the importance of tumor heterogeneity in determining treatment response and underscores the potential need for personalized medicine approaches even when considering natural products as therapeutics.

The IC_{50} values observed for PEE in our study (as low as ~17 µg/mL in some cases) are within a pharmacologically relevant range for a complex natural extract. Such potency supports the potential therapeutic relevance of Turkish propolis. However, a recognized limitation of our study is that we tested crude extracts rather than isolated components. Crude propolis contains a complex mixture of active compounds that could have synergistic, additive, or antagonistic interactions. Moreover, the inherent variability in propolis composition and the lack of standardized extracts are known challenges that complicate translational research [23]. To fully exploit propolis as an anticancer agent, future research should pursue bioactivity guided fractionation of the ethanolic extract to identify, isolate, and characterize the specific constituents most responsible for the observed cytotoxic effects. In parallel, more in-depth mechanistic studies are warranted, for example, assays to confirm induction of apoptosis (e.g. Annexin V staining, caspase activation), cell cycle analyses, and investigations into the modulation of key signaling pathways (such as the Hippo/YAP and Wnt/β catenin pathways) by propolis extracts. These efforts will clarify the precise mechanisms of action and could reveal biomarkers for responsiveness. Such investigations have been recommended by other researchers in the field and are supported by our own recent findings on a major propolis component (caffeic acid) [15,27]. Ultimately, in vivo studies (in animal models of cancer) will also be a critical next step to evaluate the therapeutic efficacy and safety of propolis extracts, as well as to verify that the promising in vitro results can translate into antitumor effects in vivo.

5. Conclusions

In summary, this study demonstrates that Turkish propolis is a valuable source of bioactive compounds with significant anticancer potential. The ethanolic extract (PEE) of this propolis exhibits consistently superior and broad spectrum cytotoxicity against cancer cells compared to the aqueous propolis extract (PWE), indicating that the primary anticancer constituents are more effectively extracted by ethanol. PEE showed particular potency against colorectal (HT-29), prostate (PC-3), and osteosarcoma (MG-63) cell lines, with cytotoxic effects intensifying from 48 to 72 hours of exposure.

These findings underscore the critical importance of extraction methodology in propolis research and its eventual therapeutic application. By identifying ethanol as a more efficacious extraction solvent for anticancer activity, our results contribute significantly to the pharmacological profiling of Turkish propolis and provide a strong foundation for future studies. Next steps should include purification and identification of the active anticancer principles within the 70% ethanolic extract, in vivo validation of its anticancer efficacy and safety in appropriate animal models, and detailed mechanistic investigations. Such studies will be essential to fully harness the potential of Turkish propolis as an adjunctive treatment or preventive agent in oncology.

Author Contributions: E.D. performed the experiments and contributed to data analysis and wrote the first and second draft. A.B. and M.S.K. assisted with cell culture and statistical analysis. E.S.E. carried out propolis extraction, A.S. obtained propolis and helped with his knowledge in propolis, D.G. assisted with cell culture and microscopic analysis, İ.K. performed HPLC analysis and compound identification, B.Ç. conceived the study, supervised the project, and wrote the manuscript. All authors reviewed and approved the final version.

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Informed Consent Statement: The study did not involve humans or human-derived materials.

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Conflicts of Interest: The authors declared that there is no conflict of interest.

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