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Polyphenol Contents, Antibacterial and Antioxidant Effects of Four Palestinian Honey Samples, and their Anticancer Effects on Human Breast Cancer Cells

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Abstract

The phenolic compounds of four Palestinian honey samples (PH1-PH4) and their antibacterial effects as well as their cytotoxic, cytostatic, and antimigration effects in human breast cancer cell line (MDA) were evaluated here. HPLC analysis of PH2 (Cornflower), PH3 (Milk thistle), and PH4 (Ziziphus) revealed 15 phenolic compounds, namely, caffeic acid, carvacrol, chrysin, ellagic acid, galangin, gallic acid, kaempferol, p-coumaric acid, pinobanksin, pinocembrin, protocatechuic acid, quercetin, rutin, salicylic acid, and silydamin. The minimum inhibitory concentration (MIC) method applied to *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), and *Escherichia coli* (*E. coli*). A strong positive correlation was detected between antimicrobial activity (*E. coli*) and P coumaric acid, quercetin, and silydamin. IC50 values for DPPH neutralization varied from 0.19 w/w% to 10 w/w%. The potential anticancer properties of the honey samples were evaluated on MDA cells. Samples PH2 and PH3 demonstrated cytostatic activity, reducing cell viability by about 43% at non-toxic concentration of 4 mg/mL. The cytostatic effects were strongly correlated with the presence of caffeic acid, chrysin, protocatechuic acid, rutin, and salicylic acid ($p < 0.01$). Moreover, the cell migration rate was significantly reduced (by up to 85%) with PH2 and PH3 compared to untreated cells ($p < 0.05$). A strong positive correlation was observed between the cytostatic effects of the concentration of carvacrol and Pinocembrin ($p < 0.01$). Our findings validate honey's antibacterial properties and suggest its anticancer benefits may stem from cytostatic and antimigration effects.

Keywords: Antibacterial, Antioxidant, Anticancer, Cytostatic, Cytotoxic, Antimigration

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INTRODUCTION

Honey, crafted by bees from flower nectar, comprises a rich assortment of compounds cherished in traditional medicine across diverse cultures. Ancient civilizations like the Greeks, Romans, and Arab-Islamic societies embraced honey in medicinal contexts, influenced by cultural beliefs, ancient theories, and historical accounts. Within Arab-Islamic medical heritage, honey is revered as a nourishing elixir, commonly applied in wound care, as evidenced in the writings of scholars such as Avicenna and Razes. Numerous varieties of honey, some scientifically substantiated, are regularly employed as natural remedies for sustaining health and addressing a spectrum of ailments.[1-3]

Antibiotic resistance among microbes has surfaced as a major global health crisis in recent times. This concern is especially troubling given that infectious diseases, a primary cause of morbidity and mortality worldwide, are becoming progressively harder to manage. The crux of the matter stems from the excessive use of antibiotics, hastening the emergence of bacteria strains resistant to multiple medications. Adding to the complexity is the marked slowdown in the production of novel antibiotics. Pharmaceutical firms have largely halted the development of new antibiotics, influenced by economic considerations and regulatory challenges.[4,5] Hence, the need to explore new alternatives for treating multi-resistant infections, with minimal side effects, is paramount. Honey, a staple of traditional medicine, is one such alternative.[6] Honey is highly valued for its potent antibacterial properties, which are effective against infections caused by multi-drug resistant bacteria such as *P. aeruginosa, S. aureus,* and *Klebsiella pneumoniae*.[7] This study assessed the antibacterial strength of different kinds of honey through the MIC technique. The efficacy of the MIC of various antimicrobial agents, particularly tetracycline and kanamycin, was tested against bacteria like *P. aeruginosa*, *S. aureus*, and *E. coli*. *S. aureus* as a gram-positive bacteria which is responsible for a wide range of clinical diseases. They are commonly found in both community and hospital settings and can cause serious infections. The treatment of these infections is increasingly difficult due to the emergence of multi-drug resistant strains like MRSA (Methicillin-Resistant *S. aureus*). *P. aeruginosa*, a gram-negative, aerobic, non-spore forming rod-shaped bacterium. It has the ability to infect both individuals with a healthy immune system and those with compromised immunity. Its propensity to infect immunocompromised hosts, its extreme adaptability, resistance to antibiotics, and a broad spectrum of dynamic defenses make it a particularly difficult organism to manage in contemporary medicine. This activity provides an overview of the epidemiology and treatment of *P. aeruginosa* infections. *E. coli,* a gram-negative bacillus, is the primary pathogen causing uncomplicated cystitis. Additionally, it leads to other extraintestinal conditions such as pneumonia, bacteremia, and abdominal infections like spontaneous bacterial peritonitis.

Additionally, honey has been found to possess immuno-modularity properties and can help prevent inflammatory-mediated chronic diseases. This chronic inflammation is associated with a wide range of disorders, including allergies, metabolic syndromes, cardiovascular issues, cancer, and autoimmune diseases. These conditions represent a significant global health challenge.[8] A variety of treatments exist to manage and alleviate inflammation, primarily involving the use of anti-inflammatory medications, both steroidal and non-steroidal, as well as immuno-suppressants. However, these treatments can lead to numerous side effects. These can include gastrointestinal ulcers, cardiovascular toxicity, hormonal imbalances, and other disruptions to the body's normal functions.[9,10] Hence, it's crucial to explore the potential of incorporating natural anti-inflammatory substances into medication therapies. This approach could enhance the pharmacological response while minimizing unwanted side effects. Recent studies have highlighted the effectiveness of various types of honey in treating ulcers, yielding promising results.[11,12]

Honey's strong antioxidant characteristics are a key biological advantage, playing a vital role in lowering the concentration of ROS (reactive oxygen species). High ROS levels are associated with the development of cardiovascular, diabetes, neurodegenerative, and cancerous conditions. Flavonoids, which can neutralize

Reactive Oxygen Species (ROS) and bind with metals such as iron, contribute significantly to the antioxidant effects observed in honey. Moreover, these compounds modulate enzymes like lipoxygenase, cyclooxygenase, phospholipase A2, and NADPH oxidase. This modulation enhances their antioxidant capabilities and impacts other biological functions.[13-15]

Research has been conducted on the potential impact of honey on cancer in the context of prevention, progression, and treatment. These studies have primarily been *in vitro*, utilizing various cell lines and types of honey. Additionally, some *in vivo* studies have been performed on mice/rats, where tumors were either induced or transplanted.[16-18] Honey plays a role in various stages of cancer, including initiation, growth, and advancement. Its anti-cancer properties are typically linked to several mechanisms. These include triggering apoptosis, halting the cell cycle, modulating oxidative stress, improving inflammation, inducing mitochondrial outer membrane permeabilization (MOMP), and inhibiting angiogenesis.[19,20]

For a considerable period in Palestine, honey served as a primary source of sugar. Palestine boasts a rich variety of plants, with descriptions of 2,600 plant species, out of which 393 species hold significant potential as sources of nectar.[21] The cellular and molecular mechanisms behind the alleged anticancer properties of Palestinian honey are still not fully comprehended. This *in vitro* study aims to identify the phenolic compounds in four samples of Palestinian honey and assess their antibacterial, antioxidant, cytotoxic, cytostatic, and antimigration effects on MDA human breast cancer cells.

MATERIALS AND METHODS

The 4 honey samples were purchased from Honey Spring in Tulkarem, located in the Northern West Bank, and were subsequently sealed in cans. These cans were stored at room temperature in a dry location and remained unopened. Table 1 summarizes the samples used, including their botanical origin and collection time.

Free Radical scavenging activity

The scavenging of free radicals was examined using a slightly modified microdilution DPPH assay as described by Masalha et al.[22] The IC50 value for each type of honey was determined by taking the value from the equation that represents the linear part of the graph. Then, 50% was substituted for the y-value to compute the corresponding concentration value on the x-axis.

Antibacterial activities

The Minimum Inhibitory Concentrations (MICs) of different honey samples were established through a micro-dilution test. This process included a broth micro-dilution assay with a two-fold serial dilution in Brain Heart Infusion (BHI) broth for *S. aureus*, and Lysogeny Broth (LB) for *E. coli* and *P. aeruginosa*.[22]

Cell culture

The human breast cancer cell line MDA-MB-231 cell line (ECACC catalogue no. 92020424) was maintained in DMEM, supplemented with 10% vol/vol inactivated fetal calf serum (FCS), 1% nonessential amino acids, 1% glutamine, 100 U/mL penicillin, and 10 g/mL streptomycin.

Cytotoxic and cytostatic effects

For the cytotoxicity tests, 20,000 MDA cells per 100 µL of media from each cell line were placed in 96-microtiter plates for 24 h, the cells were then exposed to different concentrations of honey samples, ranging from 0 to 4000 µg/mL. The MTT assay was then carried out to assess the cytotoxic effects. For the cytostatic tests, 5,000 MDA cells per 100 µL of media were placed in 96-microtiter plates for 24 h, the cells were exposed to different concentrations of honey, ranging from 0 to 4000 µg/mL, for a period of 72 h. The MTT assay was then carried out to assess the cytostatic effects.

MTT assay

The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability test was performed as outlined by Kadan et al.[22] In brief, cells were seeded and after 24 h, they were exposed to escalating doses of honey samples (ranging from 0 to 4000 µg/mL of culture media) for a period of 24 h for the cytotoxic test and 72 h for the cytostatic test at a temperature of 37°C. The absorbance of the MTT formazan was

measured at 570 nm using an ELISA reader. Cell viability was calculated as the percentage ratio of the absorbance of treated cells to that of untreated control cells

Scratch assay

A suspension of MDA cells was made and seeded at a density of 400,000 cells/mL. Each well of a 12-well plate from Corning Costar Corporation (Corning, NY, USA) was filled with 2.5 mL of this cell suspension. The plate was subsequently maintained in a humidified atmosphere with 5% CO2 at 37°C for a duration of 24 hours. Post this interval, the development of a monolayer in each well was verified under a microscope. Utilizing a sterile 200 µL pipette tip, scratches were created in the monolayers along the diameter of the wells. The culture medium from each well was discarded and each well was rinsed four times with DMEM devoid of serum and additives. Each well was then filled with 4 mL of each honey sample (4 mg/mL), as well as just the culture medium (untreated control), in triplicate. Photographs were captured immediately after the scratches were made and then every 24 hours for a total duration of 48 hours. Throughout the experiments, the plates were maintained in a humidified environment with 5% CO2 at 37°C.

Quantitative HPLC determination of phenolic compounds in honey samples

The analysis of polyphenols in honeys samples from Palestine was carried out on a SHIMADZU PROMINANCE UHPLC MS/MS equipped with a SPD A20 type DAD detector, a degasser DGU 20ASR type , LC A20 type quaternary pump as well as a thermostatically controlled automatic injector. The HPLC separation of the polyphenols was carried out on an analytical column, from the Supelco range®C18 Ascentis®4th generation, dimension 4.6 mm x 150 mm, 5um 100A, flow rate 0.5 ml/min of a mobile phase at a ternary gradient of acetonitrile methanol and acidified water (0.2% acetate buffer) temperature of column 45°C, injection volume 10 µl. In the same conditions, solutions of gallic acid s and Tyrosol standard were injected to determine the response factor).

The Honey samples were heated at 37°C bath for 10 min, 250 µl of the mobile phase were added to the samples, centrifuged for 25min 10000 rpm at 10°C, and 10 µl of the filtrate was place in HPLC vials for injection

Statistical analysis

The error limits and error bars depict the simple standard deviations of the mean. Typically, numerical values are reported only to the precision of the least significant digit. The Pearson correlation coefficient was used to establish the correlations between bioactive compounds and cytostatic activity. The significance level was set at 95% ($P<0.01$)

RESULTS AND DISCUSSION

Antimicrobial effects

The antibacterial properties of honey can be attributed to several factors. Its high viscosity, primarily due to a high concentration of sugar and low water content, forms a protective barrier that helps prevent infection. Furthermore, its slight acidity and the presence of hydrogen peroxide enhance its antimicrobial capabilities.[23,24] Numerous scientific studies have examined the impact of honey on a variety of bacterial species. It's clear that honey's antibacterial activity can significantly vary, with different microorganisms

Table 1. Honey samples IDs and their botanic, geographic origins, and harvest year

Sample ID	Traditional name	English name	Scientific name	Location	Year of harvest
PH1	Brasem	Alfalfa	*Medicago sativa*	Jenin	2021
PH2	Morar	Cornflower	*Centaurea dumulosa Boiss*	Jordan Valleys	2021
PH3	Horfesh	Milk thistle	*Silybum*	Tulkarm	2022
PH4	Sader	Ziziphus	*Ziziphusspina-christi*	Jordan Valleys	2021

Table 2. MIC values of the tetracycline and kanamycin antimicrobial agents. Minimum Inhibitory (MIC) values of the tetracycline and kanamycin antimicrobial agents. Data represent the average of three independent experiments conducted in four replications

Pathogenic Bacteria	Antimicrobial Drug	MIC, µg/mL
P. aeruginosa	Tetracycline	0.07±0.01
P. aeruginosa	Kanamycin	0.85±0.1
S. aureus	Tetracycline	0.02±0.008
S. aureus	Kanamycin	0.11±0.08
E. coli	Tetracycline	0.03±0.009
E. coli	Kanamycin	0.47±0.08

exhibiting varying levels of susceptibility to different kinds and concentrations of honey. The antibacterial characteristics of honey have been extensively reviewed, and researchers have observed the growth patterns of various bacteria in the presence of differing honey concentrations.[25-28]

This research evaluated the antibacterial potency of various types of honey using the MIC method. The MIC's effectiveness of different antimicrobial substances, specifically tetracycline and kanamycin, was examined against bacteria such as *P. aeruginosa, S. aureus*, and *E. coli* (Table 2).

All honey samples tested showed a positive effect in combating the bacteria under study. The MICs of each honey sample against the bacterial strain are detailed in Table 3, with values ranging between 0.024 and 1.56 w/w% across the three strains. Among the bacterial strains tested, *E. coli* was found to be the most susceptible, while *P. aeruginosa* proved to be the most resistant (Table 3).

The MICs reported in this study are less than those found by Imtara,[8] Mandal et al.,[29] and Boukraa.[30] However, they align more closely with the findings of Masalha, who reported MICs of honey between 0.52% and 1.0% for *E. coli*.[1] The primary antimicrobial characteristics of honey are largely determined by the levels of hydrogen peroxide, along with non-peroxide elements such as phenolic acids and flavonoids, which contribute to the antibacterial and antioxidant activity of honey.[31,32] Research indicates that the effectiveness of antibacterial properties can differ based on the phytogeographical area, which subsequently influences the production of various compounds[33,34]; newer research has identified the existence of additional antimicrobial elements, specifically, the antimicrobial peptide Bee defensin-1, 5-Hydroxymethylfurfural, and methylglyoxal, along with phenolic compounds like flavonoids.[35]

DPPH radical scavenging assay

Honey is a potent source of antioxidants that help reduce the risk of ailments such as heart disease, cancer, and enhance the immune system.[36,37] The total hydrogen/electron donating activity of dietary antioxidant supplements can be determined with the DPPH radical scavenging method. The differences in antioxidant activity among the tested samples are likely due to the floral source of the honey. It's widely known that the phytochemical makeup, including antioxidant-active molecules, is influenced by the species of the plant and other plant-related factors.[36-40] The analyzed samples' capacity to neutralize DPPH free radicals was assessed and is represented as

Table 3. Antioxidant (IC_{50}) and antimicrobial activity (MIC) of the test honey samples. Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) of the test honey samples against bacterial strains. Data represent the average of three independent experiments conducted in four replications

Kind of Honey	IC_{50} (w/w %)	MIC(w/w %)		
		P. aeruginosa	*S. aureus*	*E. coli*
PH1	0.30	1.56±0.21	0.19±0.11	0.049±0.009
PH2	1.29	0.78±0.11	0.024±0.01	0.049±0.009
PH3	0.35	0.39±0.08	0.39±0.09	0.098±0.009
PH4	0.8	1.56±0.3	0.097±0.01	0.048±0.01

IC50 mg/mL (Table 3). PH3 demonstrated the most effective DPPH free radical neutralization, with IC50 values of 0.7 mg/mL. Honey is typically rich in different types of phytochemicals, particularly phenolics and flavonoids, which contribute to its potent antioxidant properties. Numerous research studies have suggested a strong correlation between the antioxidant capacity of honey and the concentration of its phenolic constituents. The antioxidant activity observed in our honey samples aligns with previous studies that have highlighted the potent antioxidant properties of phenolic compounds.[41-44]

Cytotoxic and cytostatic effects of honey in cell from the human breast cancer cell line, MDA

The pursuit of novel natural anticancer medications is a primary goal in scientific research. Uncontrolled growth is a characteristic feature of cancer cells and is a primary target for both

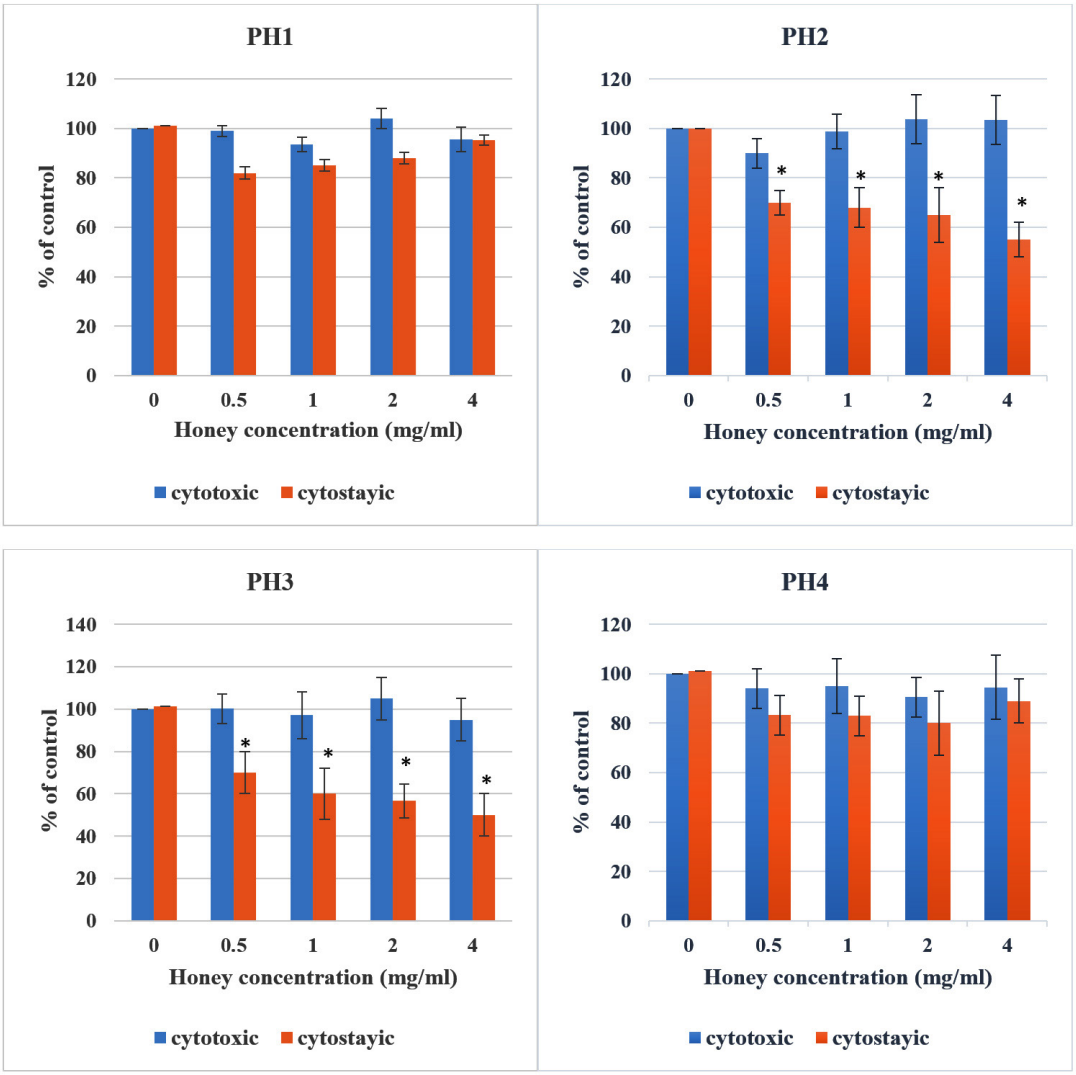

Figure 1. The cytotoxic and cytostatic impacts were evaluated using the MTT Assay on MDA cells after treating them with 0 to 4 mg/mL of honey samples for 24 h (for cytotoxic effects) and 72 h (for cytostatic effects). The cell viability was calculated as the ratio of absorbance of cells treated with honey compared to the untreated cells. Values represent means±SD of three independent experiments carried out in triplicates. *P< 0.05 was considered significant compared to control

traditional chemotherapy and new treatments. The deregulation of the cell cycle leads to this unchecked growth, resulting in the formation of tumors. Changes in DNA can trigger growth arrest at the G0/G1 and G2/M phases or even apoptosis. A lot of chemotherapy drugs aim to inhibit the cell cycle during the S and M phases. Honey has been reported in numerous studies to cause cell arrest in the G0/G1 phase in bladder (T24, 253 J, RT4, and MBT-2), colon (HCT-15 and HT-29), and human melanoma (A375) cell lines.[45-47]

The cytotoxic impacts of various kinds of honey is a phenomenon that has only been acknowledged and detailed in recent scholarly

Table 4. The concentrations (mg/g of DH) of polyphenolic compound determined and total polyphenolic concentration in PH2, PH3, and PH4 were determined by quantitative HPLC

Phenolic compounds	PH2 *Centaura calcirapa honey*	PH3 *Silybum marianum honey*	PH4 *Ziziphus spina-christi honey*
Caffeic acid	6.93±0.61	-	0.25±0.06
Carvacrol	-	-	0.21±0.08
Chrysin	4.021±0.32	-	-
Ellagic acid	15.06±1.1	39.6±5.1	7.15±1.1
Galangin	3.71±0.21	-	11.4±2.3
Gallic acid	28.7±4.3	13.1±2.3	0.16±0.04
Kaempferol	2.1±0.11	8.99±1.1	24.27±2.1
P coumaric acid	4.55±0.21	15.41±3.2	4.1±0.61
Pinobanksin	7.52±0.91	-	4.80±0.51
Pinocembrin	-	-	2.3±0.11
Protocatechuic acid	2.49±0.12	-	-
Quercetin	-	17,073±4.1	-
Rutin	1.09±0.31	-	-
Salicylic acid	2.3±0.51	-	-
Silydamin	-	1.02±0.11	-
Total polyphenol content	78.471±10.6	104.1±11.1	54.64±5.6

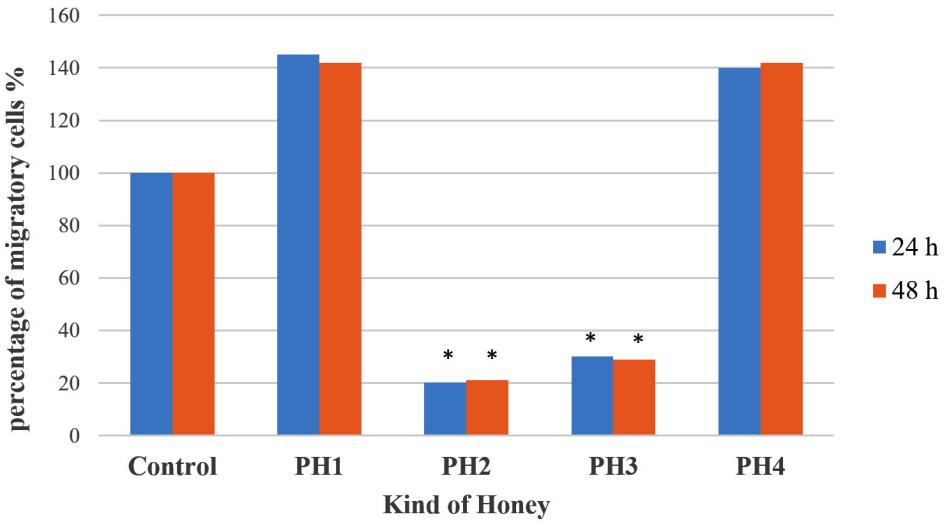

Figure 2. Effect of honey samples on cell migration of MDA cells. The results show the proportion of wound closure after 24 and 48 h of treating with honey samples. Each data point was determined based on the initial wound size at time 0 h and was normalized to the untreated control, which was set as 100%. The asterisk indicates statistical significance compared to the untreated control, with significance recognized when p-value is less than 0.05

Table 5. Correlation between bioactive compounds and biological activities

	Cytostatic	Cytotoxic	Antimigration	Antmigration (24h)	*P. aeruginosa*	*S. aureus* (48h)	*E. coli*
Caffeic acid	0.997*	-0.024	-0.537	-0.523	-0.158	-0.678	-0.512
Carvacrol	-0.537	0.893	0.997*	0.998*	0.945	-0.328	-0.515
Chrysin	0.999*	-0.056	-0.564	-0.550	-0.189	-0.654	-0.485
Ellagic acid	-0.242	-0.942	-0.632	-0.645	-0.888	0.911	0.976
Galangin	-0.239	0.990	0.921	0.927	1.000**	-0.612	-0.762
Gallic acid	0.910	-0.502	-0.877	-0.869	-0.613	-0.241	-0.036
Kaempferol	-0.768	0.714	0.973	0.969	0.801	-0.026	-0.231
P coumaric acid	-0.431	-0.855	-0.465	-0.479	-0.778	0.975	1.000*
Pinobanksin	0.749	0.586	0.083	0.099	0.472	-0.985	-0.928
Pinocembrin	-0.537	0.893	0.997*	0.998*	0.945	-0.328	-0.515
Protocatechuic acid	0.999*	-0.056	-0.564	-0.550	-0.189	-0.654	-0.485
Quercetin	-0.462	-0.837	-0.434	-0.448	-0.756	0.982	1.000*
Rutin	0.999*	-0.056	-0.564	-0.550	-0.189	-0.654	-0.485
Salicylic acid	0.999*	-0.056	-0.564	-0.550	-0.189	-0.654	-0.485
Silydamin	-0.462	-0.837	-0.434	-0.448	-0.756	0.982	1.000*
Total polyphenol content	0.022	-0.997*	-0.814	-0.823	-0.978	0.770	0.885

*. Correlation is significant at the 0.05 level; **. Correlation is significant at the 0.01 level

works.[48,49] This endeavor involved identifying substances in honey that have cytostatic impacts on cancer cells without being cytotoxic. For example, analysis of these active honey samples indicate the presence of rosmarinic acid, tannic acid, caffeic acid, coumaric acid, gallic acid, ferulic acid, syringic acid, catechin, and pyrogallol.[46] Regardless of how honey interacts with cancer cells, our findings demonstrate that the anticancer activities were identified through a cytostatic test on the MDA cell line. The cytostatic and cytotoxic outcomes of the tested samples on MDA cells are illustrated in Figure 1. None of the samples tested exhibited cytotoxic effects at any of the concentrations. The honeys labeled as PH2 and PH3 demonstrated cytostatic activity on MDA cells. The most significant cytostatic effects were observed in the PH2 and PH3 honey samples, which resulted in up to a 43% decrease in cell viability at a concentration of 4 mg/mL when compared to the untreated control cells. The findings of our study align with previously published reports, demonstrating that the impact of honey can differ depending on the variety used.[49-52] In addition, our findings concur with many studies that have documented the cytostatic properties of honey on a variety of cancer cells, suggesting that these effects are influenced by the amount of phenolic content.[49-52] Recently, Imtara and colleagues[46] observed cytotoxic impacts of various honeys sourced from Morocco and Palestine on human colon adenocarcinoma (HCT-116) and breast cancer (MCF-7) cell lines. They discovered a robust association between antioxidant constituents (phenols, flavonoids, and flavonol) and the cytostatic effect in MCF-7 cells, as well as a strong inverse relationship between syringic and tannic acid and the cytostatic activity in HCT-116 cells. Other studies have demonstrated the cytotoxic impacts of STH from Sardinia[51] and a study on Manuka honey from New Zealand[53] against HCT-116 and metastatic colon epithelial adenocarcinoma cells (LoVo), with decreased cytotoxicity observed in non-cancerous cells. It was reported that Chinese jujube honey exhibited a cytotoxic effect on HepG2 cells.[53,54]

Effect of honey samples on cell migration of MDA cells

Cancer is not only defined by the unregulated and escalated growth of cells, but also by the invasive and spreading traits of these multiplying cells. It's well established that the propensity of breast cancer cells to spread to other

tissues elevates the risk of death.[55] We performed a wound healing assay using MDA cells (4 mg/mL) to evaluate the impact of honey samples on cell movement. As depicted in Figure 2, our results indicated that the rate of MDA cell migration is notably diminished after treatment with PH2 and PH3 in comparison to the untreated cells ($p < 0.05$). As illustrated in Figure 2, after a 24-h delay, PH2 hindered MDA cell migration by 90% compared to the control cells. On the other hand, PH1 and PH4 showed a non-significant slight increase in the migration of MDA cells.

The reduction in tumor cell migration due to honey samples could be partially attributed to their cytostatic properties. Honey samples PH2, and PH3 have shown to decrease cell migration and exhibit cytostatic activity on MDA cells. The fact that the suppression of cell migration was more pronounced than the cytostatic effects suggests the involvement of additional cellular and molecular mechanisms. Our findings align with previous studies that reported the inhibitory effects of other honey samples and isolated phytochemicals like resveratrol, kaempferol, and EGCG on the migration of colorectal and OSCC cancer cells. To our knowledge, this is the inaugural study illustrating the anti-metastatic impact of Palestinian honey samples on MDA breast cancer cell lines.[56-58] Metastasis, the most harmful aspect of cancer, involves intricate processes[59] with a multitude of molecules,[60] including matrix metalloproteinases (MMPs), integrins, cadherins, plasminogen activators, PI3Ks, small GTPases similar to Ras (Rho, Rac, Cdc42), phospholipase C (PLCs), and focal adhesion kinases.

The impact of honey on cancer cell metastasis is not well-documented. A study conducted *in vivo* using wildflower honey from Croatia demonstrated a significant reduction in metastasis when administered prior to tumor cell inoculation in CBA mice and Y59 rats.[61] In addition to cytostatic activity of the honey samples, suppression of MMPs might be involved. MMPs are proteases, which assist in breaking down the extracellular matrix, are prominently expressed in cells that are undergoing metastasis.[62] It has been reported that gallic acid can reduce the gelatinolytic activity of MMP-2 and MMP-9, possibly through NF-kB.[63] Additionally, several studies have suggested that honey can decrease both the expression and nuclear translocation of NF-kB *in vivo* and *in vitro.*[64,65] Honey has been shown to reduce the enzymatic activity of MMP-2 and MMP-9.[66] For instance, Fir honey was found to inhibit human keratinocyte migration by decreasing MMP-9 expression.[67] Quercetin has been found to downregulate the expression of both MMP-2 and -9 in PC3 cells.[68,69]

Identification of phenolic compounds of honey samples by HPLC and their correlation with the biological activities

Numerous studies, both *in vitro* and *in vivo*, have highlighted the anti-carcinogenic properties of plant-based polyphenols on tumor cells. These effects include inhibiting angiogenesis and metastasis, acting as anti-proliferative agents, reducing inflammation, and promoting apoptosis. Recently, a variety of new polyphenolic compounds with anticancer potential have been discovered globally. Some of these compounds show promise as anticancer drugs, capable of treating or impeding cancer growth by interfering with the stages of cancer development, including initiation, promotion, and progression.[70]

We utilized liquid-liquid HPLC analysis to isolate phenolic compounds with anticancer properties from the honey samples under study. It's important to note that the polyphenolic profile of honey can vary based on the pollination source as well as geographical and climatic factors.[71,72] Research on Palestinian honey samples, collected from various regions and floral sources in Palestine, revealed that the phenolic content ranged from 26.96 to 70.73 mg equivalence per g of honey.[73] Table 4 presents the concentrations (in mg/g) of various phenolic compounds determined in PH3, PH3, and PH4. The Table includes fifteen identified phenolic compounds: Caffeic acid, carvacrol, chrysin, ellagic acid, galangin, gallic acid, kaempferol, p-coumaric acid, pinobanksin, pinocembrin, protocatechuic acid, quercetin, rutin, salicylic acid, and silydamin. It's noteworthy that ellagic acid, gallic acid, kaempferol, and p-coumaric acid were detected in all the samples, albeit in varying quantities. These compounds are known for their potential health benefits, including anticancer properties. For example,

A significant positive correlation was found between the content of cafeic acid and

protocatechuic and the cytostatic effects ($p < 0.01$) (Table 5). Cafeic acid, a phenolic compound prevalent in many natural sources like honey,[73] exhibits numerous biological properties such as antioxidant, anti-inflammatory, anticancer, and antidiabetic effects.[74,75] The highest concentration of cafeic acid was observed in PH2, while PH3 and PH4 showed negligible to very low concentrations in comparison. The potential therapeutic effects of cafeic acid are mediated via repression and inhibition of transcription and growth factors.[76] Cafeic acid was determined at high levels in PH1 that exhibited significant cytostatic effects. Protocatechuic and its precursor, protocatechuic aldehyde, are components of numerous plants, fruits, and vegetables.[77] Research indicates that protocatechuic acid has the ability to alter the gut bacteria composition, potentially enhancing our health. It also possesses antioxidant properties, exhibits anti-inflammatory effects, and has been shown to inhibit cancer development in lab and animal studies.

p-Coumaric acid, a widely found hydroxycinnamic acid, is present in numerous cereal grains, fruits, and vegetables. Past research has demonstrated that p-Coumaric acid offers a variety of health benefits, including antioxidant, antidiabetic, anti-inflammatory, antiplatelet, antiulcer, and anticancer properties. This hydroxycinnamic acid has also been shown to inhibit growth and induce death in certain colon cancer cells, and prevent cancer in a short-term animal model.[78] Furthermore, this study examined the antibacterial activity of p-coumaric acid, suggesting its potential use in treating microbiome-related inflammation or cancer.[79] p-Coumaric acid was detected in relatively high quantities (Table 4). There was a strong positive relationship between the amount of p-coumaric acid and the antibacterial effects on *E. coli* ($p < 0.01$) (Table 5).

A significant positive relationship was observed between the quantities of carvacrol and pinocembrin and their ability to inhibit cell migration ($p < 0.01$) as shown in Table 5. Carvacrol, which is present in plants such as oregano and thyme, has demonstrated potential medicinal properties, notably against different types of cancer cells. Copper complexes, along with other organometallic compounds, have been recognized as potent anticancer agents effective against a variety of cancer cells, including those of lung and leukemia. This is attributed to the fact that copper, being an endogenous metal, is non-toxic to normal cells.[80] Pinocembrin inhibited the viability, migration, invasiveness, and expressions of matrix metalloproteinase (MMP-2) and N-cadherin in colorectal cancer cells, while promoted E-cadherin and beta-lactamase-like protein (LACTB). The later promotes the viability, migration, and expressions of MMP-2 and N-cadherin in colorectal cancer cells.[81]

Research has shown that salicylic acid, rutin and gallic acid possess properties that can combat cancer. A strong positive relationship was observed between the levels of of these three polyphenols and the cytostatic effects, with a significance level of less than 0.01 (Table 5). Rutin inhibits inflammation and tumor progression.[81] In PH1, where high concentrations of salicylic acid and rutin were detected (Table 4), there was a marked cytostatic impact. Ahmed and his team found that the levels of gallic acid, a compound known for its antioxidant, anti-inflammatory, antimutagenic, anticancer, and cardioprotective properties, varied in two kinds of Malaysian honey.[70,82] The concentration ranged from 0.16 mg/100 g to 28.7 mg/g of honey (Table 4). This acid, which is distinguished by its trihydroxylated phenolic structure, has been identified in several honey samples. There was a significant positive correlation between the amount of gallic acid and the cytostatic effects, with a p-value of less than 0.01 (Table 5).

CONCLUSION

This research showcases four honey samples (PH1-PH4) collected from diverse regions in Palestine, highlighting their potential therapeutic value as natural agents with antibacterial and anticancer properties attributed to their polyphenolic composition. Analysis revealed fifteen phenolic compounds in three honey samples, likely contributing to observed effects. Notably, the samples exhibited significant antibacterial activity against multidrug-resistant strains, particularly *Escherichia coli*. A strong positive correlation was found between antimicrobial activity against *E. coli* and the presence of P coumaric acid, quercetin,

and silydamin. Cytostatic effects were linked to caffeic acid, chrysin, protocatechuic acid, rutin, and salicylic acid ($p < 0.01$), with PH2 and PH3 reducing MDA cell migration rates significantly (up to 80%) compared to controls ($p < 0.05$). Carvacrol and Pinocembrin also showed a strong positive correlation with cytostatic effects ($p < 0.01$). While these findings hold promise, caution is advised in extrapolating them to clinical practice pending further research to address limitations. The study provides valuable insights into the potential health benefits of tested honey samples, particularly their anti-infection, antioxidant, and anticancer properties. Limitations include the focus on *in vitro* assessments, overlooking factors like bioavailability and metabolism of phenolic compounds, and uncertainty regarding generalizability to other cancer cell lines or *in vivo* models. Future research utilizing animal models and clinical trials is crucial for validating these findings and elucidating honey sample mechanisms in humans.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

BL and BS conceptualized and designed the study. BA-F and MM conducted the antibacterial experiments. HH and AK carried out the cytotoxic, cytostatic, and antimigration tests. AA and MEO analysed polyphenol contents. HI calculated the correlation index. BL and BS wrote the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

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

ETHICS STATEMENT

Not applicable.

REFERENCES

1. Nikhat S, Fazil M. History, phytochemistry, experimental pharmacology and clinical uses of honey: A comprehensive review with special reference to Unani medicine. *J Ethnopharmacol.* 2022;282:114614. doi: 10.1016/j.jep.2021.114614
2. Wang H, Li L, Lin X, Bai W, Xiao G, Liu G. Composition, functional properties and safety of honey: a review. *J Sci Food Agric.* 2023;103(14):6767-6779. doi: 10.1002/jsfa.12720
3. Albaridi NA. Antibacterial Potency of Honey. *Int J Microbiol.* 2019;2019:2464507. doi: 10.1155/2019/2464507
4. Wang W, Weng Y, Luo T, Wang Q, Yang G, Jin Y. Antimicrobial and the Resistances in the Environment: Ecological and Health Risks, Influencing Factors, and Mitigation Strategies. *Toxics.* 2023;11(2):185. doi: 10.3390/toxics11020185
5. Bartlett JG, Gilbert DN, Spellberg B. Seven ways to preserve the miracle of antibiotics. *Clin Infect Dis.* 2013;56(10):1445-1450. doi: 10.1093/cid/cit070
6. Kalidasan G, Saranraj P, Ragul V, Sivasakthi S. Antibacterial activity of natural and commercial honey-A comparative study. *Adv Biol Res*. 2017;11:365-372.
7. Nolan VC, Harrison J, Cox JAG. Dissecting the Antimicrobial Composition of Honey. *Antibiotics* (Basel). 2019;8(4):251. doi: 10.3390/antibiotics8040251
8. Imtara H, Elamine Y, Lyoussi B. Honey antibacterial effect boosting using Origanum vulgare L. essential oil. *Evid Based Complement Alternat Med.* 2018: 7842583. doi: 10.1155/2018/7842583
9. Whitehouse MW. Anti-inflammatory glucocorticoid drugs: reflections after 60 years. *Inflammopharmacology.* 2011;19(1):1-19. doi: 10.1007/s10787-010-0056-2
10. Pan MH, Lai CS, Ho CT. Anti-inflammatory activity of natural dietary flavonoids. *Food Funct.* 2010;1(1):15-31. doi: 10.1039/c0fo00103a
11. Oryan A, Alemzadeh E, Moshiri A. Biological properties and therapeutic activities of honey in wound healing: A narrative review and meta-analysis. *J Tissue Viability.* 2016;25(2):98-118. doi: 10.1016/j.jtv.2015.12.002
12. Alfadda AA, Sallam RM. Reactive oxygen species in health and disease. *J Biomed Biotechnol.* 2012;2012:936486. doi: 10.1155/2012/936486
13. Ciappini MC, Stoppani FS, Martinet R, Alvarez MB. Actividad antioxidante y contenido de compuestos fenolicos y flavonoides en mieles de treboles, eucalipto y alfalfa. *Rev Cienc Tecnol.* 2013;19:45-51.
14. Zammit Young GW, Blundell R. A review on the phytochemical composition and health applications of honey. *Heliyon.* 2023;9(2):e12507. doi: 10.1016/j.heliyon.2022.e12507
15. Miguel MG, Antunes MD, Faleiro ML. Honey as a Complementary Medicine. *Integr Med Insights.* 2017;12:1178633717702869. ttps://doi.org/10.1177/1178633717702869
16. Ahmed S, Othman NH. The anti-cancer effects of

Tualang honey in modulating breast carcinogenesis: an experimental animal study. *BMC Complement Altern Med.* 2017;17(1):208. doi: 10.1186/s12906-017-1721-4

17. Afrin S , Giampieri F , Gasparrini M, et al. The inhibitory effect of Manuka honey on human colon cancer HCT-116 and LoVo cell growth. Part 1: the suppression of cell proliferation, promotion of apoptosis and arrest of the cell cycle. *Food Funct.* 2018;9(4):2145-2157. doi: 10.1039/C8FO00164B
18. Qanash H, Bazaid AS, Binsaleh NK, Patel M, Althomali OW, Sheeha BB. In Vitro Antiproliferative Apoptosis Induction and Cell Cycle Arrest Potential of Saudi Sidr Honey against Colorectal Cancer. *Nutrients.* 2023;15(15):3448. doi: 10.3390/nu15153448
19. Escriche I, Kadar M, Juan-Borras M, Domenech E. Suitability of antioxidant capacity, flavonoids and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. *Food Chem.* 2014;142:135-143. doi: 10.1016/j.foodchem.2013.07.033
20. Saad B, Said O. Commonly used herbal medicines in the mediterranean. Greco-Arab and Islamic Herbal Medicine. John Wiley & Sons, Inc. 2011:149-228. doi: 10.1002/9780470944363.ch8
21. Masalha M, Abu-Lafi S, Abu-Farich B, et al. A New Approach for Indexing Honey for Its Heath/Medicinal Benefits: Visualization of the Concept by Indexing Based on Antioxidant and Antibacterial Activities. *Medicines* (Basel). 2018;5(4):135. doi: 10.3390/medicines5040135
22. Kadan S, Saad B, Sasson Y, Zaid H. In vitro evaluation of anti-diabetic activity and cytotoxicity of chemically analysed Ocimum basilicum extracts. *Food Chem.* 2016;196:1066-1074. doi: 10.1016/j.foodchem.2015.10.044
23. Faraz A, Fernando WB, Williams M, Jayasena V. Effects of different processing methods on the antioxidant and antimicrobial properties of honey: a review. *Int J Food Sc Technol.* 2023;58(7):3489-3501. doi: 10.1111/ijfs.16460
24. Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. *Arch Med Res.* 2005;36(5):464-467. doi: 10.1016/j.arcmed.2005.03.038
25. Molan P. Honey: Antimicrobial Actions and Role in Disease Management. In New Strategies Combating Bacterial Infection (eds I. Ahmad and F. Aqil). 2009. doi: 10.1002/9783527622931.ch9
26. Mohammed S. Honey: biological characteristics and potential role in disease management. New strategies combating bacterial infection, 2009;255-274. doi: 10.1002/9783527622931.ch10
27. Pasupuleti VR, Arigela CS, Gan SH, et al. A Review on Oxidative Stress, Diabetic Complications, and the Roles of Honey Polyphenols. *Oxid Med Cell Longev.* 2020;2020:8878172. doi: 10.1155/2020/8878172
28. Mandal S, DebMandal M, Pal NK, Saha K. Antibacterial activity of honey against clinical isolates of *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella enterica* serovar Typhi. *Asian Pac J Trop Med.* 2010;3(12):961-964. doi: 10.1016/S1995-7645(11)60009-6
29. Boukraa L. Additive activity of royal jelly and honey against *Pseudomonas aeruginosa*. *Altern Med Rev.* 2008;13(4):330-333.
30. Montenegro G, Mejias E. Biological applications of honeys produced by *Apis mellifera*. *Biol Res*. 2013;46(4):341-345. doi: 10.4067/S0716-97602013000400005
31. Adeleke OE, Olaitan JO, Okpekpe EL. Comparative antibacterial activity of honey and gentamicin against *Escherichia coli* and *Pseudomonas aeruginosa. Ann Burn Fire Disasters*. 2006;19(4):201-204.
32. Alzahrani HA, Alsabehi R, Boukraa L, Abdellah F, Bellik Y, Bakhotmah BA. Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. *Molecules.* 2012;17(9):10540-10549. doi: 10.3390/molecules170910540
33. Stawiarz E, Wroblewska A. Melissopalynological analysis of multifloral honeys from the sandomierska upland area of Poland. *J Apic Sci J APIC SCI*. 2010;54(1):65-75.
34. Nishio EK, Ribeiro JM, Oliveira AG, et al. Antibacterial synergic effect of honey from two stingless bees: *Scaptotrigona bipunctata* Lepeletier, 1836, and *S. postica* Latreille, 1807. *Sci Rep.* 2016;6(1):21641. doi: 10.1038/srep21641
35. Alshammari GM, Ahmed MA, Alsulami T, Hakeem MJ, Ibraheem MA, Al-Nouri DM. Phenolic compounds, antioxidant activity, ascorbic acid, and sugars in honey from ingenious hail province of Saudi Arabia. *Appl Sci.* 2022;12(16):8334. doi: 10.3390/app12168334
36. Dzugan M, Tomczyk M, Sowa P, Grabek-Lejko D. Antioxidant Activity as Biomarker of Honey Variety. *Molecules*. 2018;23(8):2069. doi: 10.3390/molecules23082069
37. Pleeging CCF, Wagener FADTG, de Rooster H, Cremers NAJ. Revolutionizing non-conventional wound healing using honey by simultaneously targeting multiple molecular mechanisms. *Drug Resist Updat.* 2022;62:100834. doi: 10.1016/j.drup.2022.100834
38. De Sousa JMB, de Souza EL, Marques G, et al. Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region. *LWT-Food Sci Technol*. 2016; 65:645-651. doi: 10.1016/j.lwt.2015.08.058
39. Biluca FC, Braghini F, Gonzaga LV, Costa ACO, Fett R. Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (Meliponinae). *J Food Compos Anal.* 2016;50:61-69. doi: 10.1016/j.jfca.2016.05.007
40. Das A, Datta S, Mukherjee S, Bose S, Ghosh S, Dhar P. Evaluation of antioxidative, antibacterial and probiotic growth stimulatory activities of *Sesamum indicum* honey containing phenolic compounds and lignans. *LWT-Food Sci Technol.* 2015;61(1):244-250. doi: 10.1016/j.lwt.2014.11.044
41. Bueno-Costa FM, Zambiazi RC, Bohmer BW, et al. Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil. *LWT-Food Sci Technol.* 2016;65:333-340. doi: 10.1016/j.lwt.2015.08.018
42. Imtara H, Elamine Y, Lyoussi B. Physicochemical

characterization and antioxidant activity of Palestinian honey samples. *Food Sci Nut*. 2018;6(8):2056-2065. doi: 10.1002/fsn3.754

43. Al-Kafaween MA, Alwahsh M, Mohd Hilmi AB, Abulebdah DH. Physicochemical characteristics and bioactive compounds of different types of honey and their biological and therapeutic properties: a comprehensive review. *Antibiotics.* 2023;12(2):337. doi: 10.3390/antibiotics12020337
44. Swellam T, Miyanaga N, Onozawa M, et al. Antineoplastic activity of honey in an experimental bladder cancer implantation model: *In vivo* and *in vitro* studies. *Int J Urol.* 2003;10(4):213-219. doi: 10.1046/j.0919-8172.2003.00602.x
45. Ichichero E, Cicconi R, Mattei M, Muzi MG, Canini A. Acacia honey and chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. *Int J Oncol.* 2010;37(4):973-981. doi: 10.3892/ijo_00000748
46. Laura MP, Claire S, Mridula C. Honey and Cancer: Current Status and Future Directions. *Diseases*. 2016;4(4):30. doi: 10.3390/diseases4040030
47. Jaganathan KS, Balaji A, Vellayappan M, et al. A review on antiproliferative and apoptotic activities of natural honey. *Anticancer Agents Med Chem.* 2015;15(1):48-56. doi: 10.2174/1871520614666140722084747
48. Mumtaz PT, Bashir SM, Rather MA, et al. Antiproliferative and apoptotic activities of natural honey. In: Rehman MU, Majid S, editors. Therapeutic applications of honey and its phytochemicals, *Springer*. 2020:345-60.27. doi: 10.1007/978-981-15-6799-5_18
49. Imtara H, Kmail A, Touzani S, et al. Chemical analysis and cytotoxic and cytostatic effects of twelve honey samples collected from different regions in Morocco and Palestine. *Evid Based Complement Alternat Med.* 2019;2019:8768210. doi: 10.1155/2019/8768210
50. Afrin S, Forbes-Hernandez TY, Gasparrini M, et al. Strawberry-tree honey induces growth inhibition of human colon cancer cells and increases ROS generation: A comparison with manuka honey. *Int J Mol Sci.* 2017;18(3):613. doi: 10.3390/ijms18030613
51. Afrin S, Giampieri F, Cianciosi D, et al. Strawberry tree honey as a new potential functional food. Part 1: Strawberry tree honey reduces colon cancer cell proliferation and colony formation ability, inhibits cell cycle and promotes apoptosis by regulating EGFR and MAPKs signaling pathways. *J Funct Foods.* 2019;57:439-452. doi: 10.1016/j.jff.2019.04.035
52. Cheng N, Zhao H, Chen S, He Q, Cao W. Jujube honey induces apoptosis in human hepatocellular carcinoma HepG2 cell via DNA damage, p53 expression, and caspase activation. *J Food Biochem.* 2019;43(11):e12998. doi: 10.1111/jfbc.12998
53. Juric A, Karaconji IB, Kopjar N. Homogentisic acid, a main phenolic constituent of strawberry tree honey, protects human peripheral blood lymphocytes against irinotecan-induced cytogenetic damage *in vitro*. *Chem Biol Interact.* 2021;349:109672. doi: 10.1016/j.cbi.2021.109672
54. Thomas HR, Hu B, Boyraz B, et al. Metaplastic breast cancer: A review. *Crit Rev Oncol Hematol.* 2023;182:103924. doi: 10.1016/j.critrevonc.2023.103924
55. Lopez EP-F, Garcia FG, Jornet PL. Combination of 5-Florouracil and polyphenol EGCG exerts suppressive effects on oral cancer cells exposed to radiation. *Arch Oral Biol*. 2019;101:8-12. doi: 10.1016/j.archoralbio.2019.02.018
56. Chung SS, Dutta P, Austin D, Wang P, Awad A, Vadgama JV. Combination of resveratrol and 5-flurouracil enhanced anti-telomerase activity and apoptosis by inhibiting STAT3 and Akt signaling pathways in human colorectal cancer cells. *Oncotarget.* 2018;9(68):32943-32957. doi: 10.18632/oncotarget.25993
57. Riahi-Chebbi I, Souid S, Othman H, et al. The Phenolic compound Kaempferol overcomes 5-fluorouracil resistance in human resistant LS174 colon cancer cells. *Sci Rep.* 2019;9(1):195. doi: 10.1038/s41598-018-36808-z
58. Martin TA, Ye L, Sanders AJ, Lane J, Jiang WG. Cancer invasion and metastasis: molecular and cellular perspective. Madame Curie Bioscience Database [Internet] 2013. Landes Bioscience.
59. Thompson EW, Price JT. Mechanisms of tumour invasion and metastasis: emerging targets for therapy. *Expert Opin Ther Targets*. 2002;6(2):217-233. doi: 10.1517/14728222.6.2.217
60. Orsolic N, Basic I. Antimetastatic effect of honey. Mellifera. 2004;4(7).
61. Reddy RA, Sai Varshini M, Kumar RS. Matrix Metalloproteinase-2 (MMP-2): As an Essential Factor in Cancer Progression. *Recent Pat Anticancer Drug Discov.* 2023. doi: 10.2174/0115748928251754230922095544
62. Ho HH, Chang CS, Ho WC, Liao SY, Wu CH, Wang CJ. Anti-metastasis effects of gallic acid on gastric cancer cells involves inhibition of NF-kappaB activity and downregulation of PI3K/AKT/small GTPase signals. *Food Chem Toxicol.* 2010;48(8-9):2508-2516. doi: 10.1016/j.fct.2010.06.024
63. Batumalaie K, Zaman Safi S, Yusof KM, Ismail IS, Sekaran SS, Qvist R. Effect of gelam honey on the oxidative stress-induced signaling pathways in pancreatic hamster cells. *Int J Endocrinol.* 2013;2013:367312. doi: 10.1155/2013/367312
64. Hussein SZ, Mohd Yusoff K, Makpol S, Mohd Yusof YA. Gelam honey attenuates carrageenan-induced rat paw inflammation via NF-kB pathway. *PLoS One*. 2013;8(8):e72365. doi: 10.1371/journal.pone.0072365
65. Moskwa J, Borawska MH, Markiewicz-Zukowska R, et al. Polish natural bee honeys are anti-proliferative and anti-metastatic agents in human *Glioblastoma multiforme* U87MG cell line. *PLoS One*. 2014;9(3):e90533. doi: 10.1371/journal.pone.0090533
66. Majtan J, Bohova J, Garcia-Villalba R, et al. Fir honeydew honey flavonoids inhibit TNF-a-induced MMP-9 expression in human keratinocytes: a new action of honey in wound healing. *Arch Dermatol Res.* 2013;305(7):619-627. doi: 10.1007/s00403-013-1385-y
67. Vijayababu MR, Arunkumar A, Kanagaraj P, Venkataraman P, Krishnamoorthy G, Arunakaran J. Quercetin downregulates matrix metalloproteinases

2 and 9 proteins expression in prostate cancer cells (PC-3). *Mol Cell Biochem.* 2006;287(1-2):109-116. doi: 10.1007/s11010-005-9085-3

68. Jaganathan SK, Mandal M. Honey constituents and their apoptotic effect in colon cancer cells. *J ApiProd ApiMed Sci.* 2009;1(2):29-36. doi: 10.3896/IBRA.4.01.2.02
69. Rathod NB, Elabed N, Punia S, Ozogul F, Kim SK, Rocha JM. Recent Developments in Polyphenol Applications on Human Health: A Review with Current Knowledge. *Plants* (Basel). 2023;12(6):1217. doi: 10.3390/plants12061217
70. Dong R, Zheng Y, Xu B. Phenolic profiles and antioxidant capacities of Chinese unifloral honeys from different botanical and geographical sources. *Food Bioproc Tech*. 2013;6(3):762-770. doi: 10.1007/s11947-011-0726-0
71. Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta.* 2005;533(2):185-191. doi: 10.1016/j.aca.2004.11.010
72. Bava R, Castagna F, Lupia C, et al. Hive Products: Composition, Pharmacological Properties, and Therapeutic Applications. *Pharmaceuticals*, 2024; 17(5):646.
73. Yim SH, Kim HJ, Park SH, et al. Cytotoxic caffeic acid derivatives from the rhizomes of Cimicifuga heracleifolia. *Arch Pharm Res*. 2012;35(9):1559-1565. doi: 10.1007/s12272-012-0906-0
74. Rajendra Prasad N, Karthikeyan A, Karthikeyan S, Reddy BV. Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. *Mol Cell Biochem.* 2011;349(1-2):11-19. doi: 10.1007/s11010-010-0655-7
75. Alam M, Ahmed S, Elasbali AM, et al. Therapeutic Implications of Caffeic Acid in Cancer and Neurological Diseases. *Front Oncol.* 2022;12:860508. doi: 10.3389/fonc.2022.860508
76. Widy-Tyszkiewicz E. Current Evidence for Disease Prevention and Treatment by Protocatechuic Acid (PCA) and Its Precursor Protocatechuic Aldehyde (PCAL) in Animals and Humans. Plant Antioxidants and Health, Cham,*Springer.* 2022:507-543. doi: 10.1007/978-3-030-78160-6_36
77. Tehami W, Nani A, Khan NA, Hichami A. New Insights Into the Anticancer Effects of p-Coumaric Acid: Focus on Colorectal Cancer. *Dose Response*. 2023;21(1):15593258221150704. doi: 10.1177/15593258221150704
78. Singha K, Banerjee A, Jana A, et al. Molecular exposition of broad-spectrum antibacterial efficacy by p-coumaric acid from an edible mushroom Termitomyces heimii: *in vitro* and *in silico* approach. *Systems Microbiology and Biomanufacturing.* 2023;3(4):750-764. doi: 10.1007/s43393-022-00146-z
79. Singh J, Luqman S, Meena A. Carvacrol as a Prospective Regulator of Cancer Targets/Signalling Pathways. *Curr Mol Pharmacol.* 2023;16(5):542-558. doi: 10.2174/1874467215666220705142954
80. Jiang L, Yang Y, Feng H, Zhou Q, Liu Y. Pinocembrin inhibits the proliferation, migration, invasiveness, and epithelial-mesenchymal transition of colorectal cancer cells by regulating LACTB. *Cancer Biother Radiopharm.* 2022;37(7):527-36. doi: 10.1089/cbr.2020.4052
81. Cruz-Martins N. Advances in Plants-Derived Bioactives for Cancer Treatment. *Cells.* 2023;12(8):1112. doi: 10.3390/cells12081112
82. Perez RA, Sanchez-Brunete C, Calvo RM, Tadeo JL. Analysis of volatiles from Spanish honeys by solid-phase microextraction and gas chromatography-mass spectrometry. *J Agric Food Chem.* 2002;50(9):2633-2637. doi: 10.1021/jf011551r