Arab American University

Faculty of Graduate Studies

In vitro evaluation of cytotoxic, cytostatic, anti-migration and antioxidant effects of extracts of selected honey samples on human breast cancer cell lines MDA, in addition to anti-inflammatory effects on THP-1-derived Macrophages

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This thesis was submitted in partial fulfillment of the requirements for the Master's

degree in

Cellular and Molecular Bio sciences

February/2024

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Thesis Approval

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Declaration

I hereby declare that this thesis represents my work, otherwise referenced, and has not been previously included in a thesis or dissertation submitted elsewhere for a degree, diploma, or other qualifications.

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Dedication

This achievement is presented to My Parents, sister and brothers. To my special kingdom my husband (My King) and daughters (My Queens Mayar and Mayan) ,,,,,,,,

Acknowledgments

Respect and appreciation to my family, friends and everyone who supported me.

Special thanks to my supervisor Prof. Bashar Saad and the Internal Examiner Dr. Siba Shanak and the External Examiner Dr. Walid Basha.

With respect to my supporting college, the College of Science on the campus of the Arab American University-Jenin, and the members of the academic and administrative staff, in addition to my main working college, the College of Medicine.

Abstract

Cancer is a complex disease with high prevalence worldwide and the breast cancer is considered as serious diseases that has become a significant socioeconomic burden cancer type. Many remedies are currently in use in the management of this disease, but they are associated with multiple severe side effects. Hence, the search for alternative herbal-based medicine as a source of high-value bioactive compounds with a minimum side effect is highly appreciated. Based on published literature, the effects of (labelled as PH1 to PH9) floral honey samples were evaluated in this proposed *in vitro* study. Using the MDA human breast cancer cell line, the study examines the potential anticancer effects of the honey samples. MTT assay was used to assess their cytotoxic and cytostatic effects. Furthermore, anti-migration effect was evaluated using scratch-wound assay. No cytotoxic effects were observed in any of the tested samples at all concentrations. The honeys identified as PH2 (Morar), PH3 (Khorfesh), PH5 (Sedr), and PH6 (Kena) showed cytostatic activity on MDA cells, leading to a decrease in cell viability by up to 43% at a concentration of 4mg/mL compared to the untreated control cells. Moreover, the data suggests that the MDA cell migration rate is significantly reduced after treatment with PH2, PH3, PH5, PH6, and PH7 compared to the untreated cells ($p < 0.05$). Specifically, PH2, PH5, and PH6 reduced MDA cell migration by 90%, 80%, and 72% respectively, compared to the control cells. In addition, evaluation of the anti-inflammatory effects of the samples were tested by measuring the nitric oxide (NO) secretion from LPS-activated THP-1-derived macrophages. The results show that Khorfesh, Sedr, Kena Gabali and Aghwar decreased the NO production rate significantly. The evaluation also focuses on measuring their antioxidant (DPPH-based scavenging activity). The IC50 values of the DPPH free radical neutralization ranged from 0.7 μg/mL to 2.87 μg/ml. Results obtained indicate that cytostatic and anti-migration effects may contribute to anticancer benefits. Further future investigations are essential to reveal the molecular mechanisms and the role of isolated phytochemicals in the obtained results. This is an important step in the development of herbal-based anticancer drugs.

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Chapter1: Introduction:

Cancer

Cancer is a complicated disease that affects millions of people around the world. In 2020, there were about 19.3 million new cases and 10.0 million deaths from cancer. The World Health Organization (WHO) estimated in 2019 that cancer is among the top two causes of death before age 70 in most countries (Sung et al., 2021). Cancer alters the structure of DNA in body cells. This can result from various environmental factors such as aging, family history, smoking, sunlight and ionizing radiation, organic and inorganic chemicals, viruses and bacteria, hormone therapy, obesity, air and water pollution (Parsa et al., 2012).

Cancer treatments are being developed based on the findings that changing the signaling pathways that control cell cycle can induce cancer. Normal cells are well regulated; they use external chemical signals to grow and divide only when needed, they die in a programmed way (apoptosis) to keep the tissue balance, and they stick to their proper place. These cells become specialized with different functions even though they have the same DNA. A cancer cell can keep dividing without limit because it loses the balance between cell division and cell differentiation. The cancer cell ignores signals from other tissues, has unstable genes, and avoids cell death (Cisse et al., 2019).

DNA abnormalities in normal cells can result in their transformation into cancerous cells. The selective accumulation of mutated cell through a multistep and long process will induce the development of cancer through an initiation process. The promotion stage is aided by the proliferation of starting cells, which is accompanied by a selection of cells with stimulated growth

characteristics and is followed by a combination of DNA mutations and epigenetic changes that contribute to tumor progression (Cisse et al., 2019).

On the other hand, the cancer cells are classified into either benign or malignant, A malignant tumor can invade any other tissues by metastasis that is the most lethal aspect of carcinogenesis. Metastatic of cancer cells depends on the need of nourishment and an adequate supply of oxygen and nutrients and the removal of waste products. Processes of tumor angiogenesis and lymphangiogenesis are mainly due to angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGF). The tumors activate the angiogenic switch by altering the balance between angiogenesis inducers and inhibitors exerting opposing action (Madu et al., 2020).

Breast cancer:

Breast cancer is characterized by uncontrolled cell growth in tissues of the breast. In addition, it is the most commonly diagnosed cancer among American women. Annually, 40,290 women are estimated to die from breast cancer (Ji et al., 2020).

By using an immunohistological method, invasive BC can be classified into four main molecular subgroups according to the expression of the human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER). About 60% of BC are Luminal A BC (ER+, PR+, and HER2-), which is linked to a favorable prognosis. Thirty percent of BC are Luminal B BC (ER+ and/or PR+ and HER2+), which is linked to a poor prognosis and a high ki67 (>14%) proliferation marker. Ten percent of BC are HER2 BC (ER-, PR-, and HER2+), and these conditions are linked to a dismal prognosis. Last but not least, 15-20% of BC cases are triple-negative BC (TNBC) (ER-, PR-, and HER2-), which is linked to greater aggressiveness and a

poorer prognosis than other BC genetic subtypes and frequently affects younger women. Features of BC according to molecular subtypes (Burguin et al., 2021).

The model for Invasive BC disease is MDA-MBA-231. It is usual practice to model late-stage breast cancer using the MDA-MB-231 cell line, which was obtained at MD Anderson from a pleural effusion of a patient with invasive ductal carcinoma. This cell line expresses mutant p53 and is negative for ER, PR, and E-cadherin. The MDA-MB-231 cell genome clusters with the basal subtype of breast cancer in microarray profiling. The cells are a suitable model of triple-negative breast cancer because they also lack the growth factor receptor HER2 (Welsh et al., 2013).

To provide the most individualized, secure, and effective therapy, treatment options are determined by the grade, stage, and BC molecular subtype. The appearance of tumor cells in comparison to normal cells is described by the grade. It comprises nuclear pleomorphism, tubule differentiation, and the number of mitoses. The TNM system, which includes tumor size, lymph node status, and the existence of metastases, is used to establish the stage, which is used to categorize the degree of cancer in the body. In order to treat non-metastatic breast cancer, a comprehensive or breast-conserving surgical approach is used to remove the tumor. Systemic therapy, which includes chemotherapy and targeted therapy, is also used, along with preoperative (neoadjuvant) or postoperative (adjuvant) radiation therapy. Endocrine therapy for BC that is hormone receptor-positive (HR+) and anti-HER2 therapy for BC that is HER2+ are two types of targeted therapy. Regretfully, the TNBC subtype does not currently have a specific treatment option. Since metastatic BC is still incurable, containing tumor spread is of utmost importance. Metastatic BC is treated with the same systemic treatments (Watkins et al., 2019) (Burguin et al., 2021).

Managing recurrence and treatment resistance are two difficulties in the treatment of BC. In fact, metastases account for 30% of recurrent illness in early-stage BC patients. Therefore, in order to

properly treat each BC subtype, new strategic medicines must be developed and, in this study, we focus in determining treatment by the herbal medicinal product alone or in combined with chemotherapy (Burguin et al., 2021).

Breast cancer prevention involves chemotherapy through hormonal prevention drugs, lifestyle, weight controls, increased physical activity, and reduce alcohol consumption. The most successful preventative approach is reducing the effects of estrogen, progesterone and pSTAT5 (phospho-signal transducer and activator of transcription 5) (Howell et al., 2014) (Clemenceau et al., 2020).

Herein, the research will discuss several other forms of cell death that have been discovered; thus, highlighting that a cell can die via several differing pathways. Most of the anti-cancer therapies can stand out the effects of the honey extract and treat the migration and inflammation destinations that fight and support cancer. Additionally, such therapies also trigger apoptosis induction and related cell death networks to eliminate malignant cells. These are hot topics in current research.

Apoptosis is a natural way of programmed cell death, which plays an essential role in organism development and tissue homeostasis. Apoptosis includes a series of processes that lead to cell death; and a highly regulated and controlled apoptosis is characterized by a number of characteristic morphological changes in the structure of the cell, together with a number of enzyme-dependent biochemical processes including phagocytosis. Cancer cells resist the activation of apoptotic cascade. In this regard, several methods have been developed for the detection of apoptosis. (Fouqué et al., 2016).

Apoptosis can be triggered by intrinsic pathways or extrinsic pathways. Signaling cascades of programmed cell death is caused by both processes by activating caspases, which are cysteine proteases, or enzymes that break down proteins. The activity of caspase-3 gives an index to the

process of apoptosis. The active form of caspase-3 is able to specifically recognize a tetra-peptide sequence (Asp-Glu-Val-Asp, DEVD) on peptide substrates and hydrolyze peptide bonds on the aspartic acid residues at the carboxyl-terminus (C-terminal end). Substrates can be created by appending the appropriate chromophore or fluorophore to the C-terminal residue; and para-nitro-aniline (pNA) tetrapeptide substrates chromophore for caspases are available commercially. Colorimetric assay is simple, and the results can be easily observed, requiring no sophisticated instrument. Many conventional cancer therapies induce apoptosis to remove the cancer cell by engaging the caspases indirectly (Pan et al., 2012). Caspase-3, with its multiple cellular substrates, is one of the most studied and important caspases. Procaspase-3 is activated to caspase-3 through the action of both caspase-8 and caspase-9. Ac-DEVD-pNA and Ac-DEVD-AMC are widely used to monitor caspase-3 activity in cell culture, have been used for the *in vitro* identification of caspase-3 inhibitors, and in the identification and assessment of small molecule activators of procaspase-3. Caspase-3 catalyzes the hydrolysis of Ac-DEVD-pNA, releasing the pNA chromophore which has a λ_{max} of 405 nm (Peterson et al., 2010).

Autophagy: serves to promote cell survival through maintaining intracellular normal homeostasis, so it contributes to the protection of cells against the development of malignant neoplastic lesions. It additionally contributes to lysosomal degradation and the recycling of unnecessary or damaged cellular components. Furthermore, autophagy works closely with the immune system to provide a non-cellular form of defense against cancer. Autophagy can cause cell death (also known as "type II programmed cell death") when triggered by severe cellular stress. Apoptosis and autophagic death may interact in this way (Chmurska et al., 2021).

Necrotic uncontrolled cell death: is a kind of cell death characterized by an increase in cell volume, swelling of organelles, rupture of the plasma membrane, and subsequent loss of intracellular

contents, resulting in inflammation. Necrosis is a consequence of physicochemical stress of harmful substances or physical stressors such as poisons, radiation, heat, trauma, a lack of oxygen owing to an obstruction in blood flow, and other events (Mohammad et al., 2015).

Necroptosis: unlike necrosis, a more controlled form of necrosis that has been demonstrated to protect organisms against internal pathogens and intracellular diseases. Several upstream signaling components of apoptosis and necroptosis are similar (Mohammad et al., 2015).

Phagocytosis: a multi-step cellular process including target cell identification, cellular engulfment, and lysosomal digestion, the capacity of antigen-presenting cells (APCs) to engulf cancer cells is critical to this bridging of innate and adaptive immunity (Sun et al., 2020). As a result, determining cancer phagocytosis checkpoints will open up a new route for creating cancer immunotherapies (Feng et al., 2019).

In summary, targeting the various mechanisms that either promote apoptosis or inhibit survival signals should result in medication resistance and overcoming cancer. This results in limiting the cell migration and arresting cell cycle without interference with the cell viability. The data indicate that honey can inhibit carcinogenesis by modulating the molecular processes of initiation, promotion, and progression stages, and by the modulation of the signaling pathways and inhibition of these vital mechanisms (Erejuwa et al., 2014) (Rahman et al., 2022). Thus, it may serve as a potential and promising anticancer agent which warrants further experimental and clinical studies.

Cancer treatment:

1. Chemotherapy

Nowadays, various methods are used for cancer treatment such as chemotherapy. Because of their lack of selectivity, a high percentage of healthy cells are lost. Furthermore, these treatments are

associated with side effects and in hard cases even cause death. These include headache, weakness, hair loss, diarrhea, memory impairment, bone marrow suppression, neuropathies, and gastrointestinal disorders (Aslam et al., 2014). This motivates the intensive research of new therapies.

2. Herbal medicinal therapy

As aforementioned, due to the side effects of current cancer treatments, there is a need of novel treatment approaches that selectively reduce tumor progression with minimal side effects. Many honey samples contain natural products that exhibit an extensive spectrum of biological activities such as stimulation of the immune system, antibacterial, antiviral, anti-hepatotoxic, anti-ulcer, anti-inflammatory, antioxidant, anti-mutagenic, apoptosis induction, as well as anti-cancer effects. Honey used in human therapy is enhanced via agriculture, nutrition, and scientific research. Honey contains relatively high concentrations of bioactive components such as saponins, flavanoids, tannins and polyphenolic components, that are found in (Zaid et al., 2012) (Varijakzhan et al., 2020) (Barreca et al., 2021).

The main new reveal in this research is to evaluate the anticancer effects of selected ten honey samples on MDA breast cancer cell line by comparing the most effective samples potentials with the least ones in their anti-proliferative, anti-migratory, anti-oxidant and anti-inflammatory effects.

Honey as complementary medicine (Miguel et al., 2017) is a sweet natural material made by honeybees from a variety of plant secretions.

The concentrations and types of several proteins, enzymes, amino acids, phenolic compounds, minerals, vitamins, organic acids, are important contributors to the quality of honey (Lazarević et al., 2017).

Honey's composition is mostly determined by its floral source, although seasonal and environmental factors play a significant role. As a result, honey's chemical composition is exceedingly diverse. Biological activity, chemical composition (volatile compounds, carbohydrates, and phytochemicals), physical features (color, viscosity, hygroscopic properties, and pH), and flavor distinguish different types of honey. As a result, different honey kinds have varied health-promoting effects (Eteraf et al., 2022) (Dżugan et al., 2018).

This origin of the floral source of each honey sample must counted in this field this is due to the importance of the flower and their contribution to the effectiveness of honey samples extraction from and the effect of phytochemicals content and the subsequent environmental effects on the quality of honey and its chemical and physical characteristics that distinguish each type in the search for others. PH1 (Alfalfa (*Medicago sativa* L.)) is the most extensively cultivated forage legume in the world, while its yield and growing range are limited by abiotic stress. Physiology, genetic and molecular level studies have revealed complex regulatory processes that coordinate stress adaptation and tolerance. Saponins, vitamin K, amino acids L-canavanine, and estrogenic isoflavonoids (genistein, daidzein) are all present in alfalfa (Medicago sativa) leaves and sprouts. Alleged to be a galactogogue, alfalfa is a component of certain proprietary blends intended to boost the production of milk (Hadidi etal., 2023). PH2 (*C. calcitrapa)* is a species containing a wide range of phenolic compounds. Previous reports have confirmed the presence of flavonoids such as apigenin, luteolin, kaempferol, kaempferol 3-O-glucoside, eupatorin, jacaeosidin, neptin, protocathechuic acid and others. Traditionally, it was used for the treatment of ophthalmia, common fever, jaundice, digestive and skin disorders. Previous investigations on different extracts from this plant have shown certain bioactivity potential (Dimkić et al. 2020), (Aboul-Soud et al., 2022). PH3 Milk thistle (MT; *Silybum marianum*), a member of the *Asteraceae* family, is a

therapeutic herb. MT fruits contain a mixture of flavonolignans collectively known as silymarin, being silybin (also named silibinin) the main component. The pharmacologically relevant actions for liver diseases (e.g., anti-inflammatory, immunomodulating, antifibrotic, antioxidant, and liver-regenerating properties) as well as the clinical potential in patients with alcoholic liver disease, nonalcoholic fatty liver disease, viral hepatitis, drug-induced liver injury, and mushroom poisoning (Abenavoli et al., 2018). There are 135 perennial herbs in the genus Clinopodium (Lamiaceae family), most of which are abundant in essential oils and can be found throughout the Mediterranean region, Western Asia, and Southern Europe. Numerous preclinical investigations have shown that Clinopodium essential oils suppress a range of bacterial and fungal species. There have also been reports of their insecticidal qualities. Among the many species of Clinopodium, Clinopodium nepeta L. Kuntze (also known as Calamintha nepeta L. Kuntze) is an apolymorphic, fragrant plant that has been used traditionally as a stimulant, antispasmodic, diaphoretic, and tonic medicinal herb; it is also used in many culinary recipes and is thought to resemble mint. A review of the literature on the chemical analysis of the essential oils made from PH4 (C. nepeta) showed that they included a significant amount of oxygenated monoterpenes (Debbabi et al., 2020). In addition to its edible fruits, the PH5 (genus Ziziphus) is grown all over the world for its many phytochemicals, which are mostly used for medical purposes. The genus, which mostly includes Z. jujuba, Z. xylopyrus, Z. spina-christi, Z. lotus, Z. mauritiana, Z. celata, etc., is grown in various parts of the world. The genus Ziziphus has been used traditionally for medical purposes to cure infections, diabetes, hypertension, headaches, obesity, and common colds. Scientific publications focus on the phytochemistry and biological activity of Ziziphus, with a focus on the a range of phytochemicals, such as terpenoids, alkaloids, flavonoids, and saponins. Many of the health advantages of Ziziphus are attributed to its abundant terpenoids and saponins (Sakna et al., 2023).

Experimental findings demonstrate that PH6 (*E. camaldulensis)* essential oil is rich in phytochemicals but has weak antioxidant activity. One of the Eucalyptus species that is most widely distributed is E. camaldulensis (previously Eucalyptus rostrata Schl.). Furthermore, it is also recognized as one of the most widely grown tree species in the world, and it can live for 500–1000 years. Indigenous Australians have been using E. camaldulensis leaves and essential oil as traditional medicines for generations because of their antibacterial, anti-inflammatory, and antipyretic qualities (Jaradat et al., 2023). PH7 (Tebbeyah) is collection of herbal plants with high value of phytochemicals. PH8 (Hairy fleabane [Conyza bonariensis (L.) Cronquist]) is a weed of the Asteraceae family whose main characteristics are high competitivity and prolificacy, producing more than 800 thousand seed per plant. The phytochemical screening of C. bonarensiss exhibited the presence of saponins, tannins, flavonids, steroids, glycosides, diterpenoids and tripernoids that have a positive effect on many biological processes. The crude extract and its fractions were subjected to antioxidant potential by DPPH free radical activity (Shah et al., 2013) (Kaspary et al., 2023). PH9 (Multifloral Vegetation) is an assemblage of mostly citrus trees species and the ground cover they provide. PH10 (Aghwar Vegetation) is an assemblage of Aghwar plant species and the ground cover they provide. PH9 and PH10 details provided by the local market. The botanical source and the geographical origin presented in the Table (1). Based on published literature in this research that proposed to prove the effectiveness of extracted honey samples in fighting cancer.

The immune system's natural, innate response to pathogens is inflammation, where different cellular and humoral immune responses are developed. Oxidative stress, on the other hand, occurs when the excess production of free radicals outweighs the antioxidant components. Many signaling pathways link oxidative stress and inflammation to one another (Battino et al., 2021).

Together with oxidative stress, inflammation is the basis of several common chronic-degenerative diseases, as cancer and metabolic diseases. Inflammatory and oxidative stress pathways are closely interrelated: one of them may appear before or after the other; but when one of them appears, the other one is most likely to appear, taking part together in the pathogenesis of many chronic diseases (Chen et al., 2019).

The product from the bee is collected from different plant sources. Additionally, propolis has several biological properties, including antioxidant and free radical scavenger action. The effects of honey against oxidative stress have been evaluated in several studies. Consuming honey also works well to reduce oxidative stress brought on by various additives or contaminants. Natural honey antioxidants reduce harmful side effects and improve the antitumor activity of anti-cancer medications. The antioxidant properties of honey can be measured in the form of antiradical activity, using oxygen radical absorbance capacity (ORAC) assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, and ferric reducing antioxidant power (FRAP) assay. Antioxidants are substances that prevent damage due to oxidants, which include lipid peroxyl radicals, O2, OH-, superoxide, and/or other oxidants. Oxidative stress is linked to several chronic and degenerative lingering diseases, aging, cancer, mutagen synthesis, and atherosclerosis. Cells have an antioxidant defense mechanism. Free radicals and other oxidative protective agents like peroxidase, catalase, superoxide dismutase, ascorbic acid, tocopherol, and polyphenols make up this defense system. These antioxidants activate biomolecules, including nucleic acids, proteins, lipids, and carbohydrates. This stimulation modifies cells and eventually triggers an antioxidant response. Strong antioxidant activity is shown by honey. Honey's antioxidant properties help to prevent a number of acute and long-term illnesses, including cancer, diabetes, thrombotic, inflammatory, and allergic diseases (Ahmed et al., 2018).

Inflammation is classified into two classes: acute and chronic inflammation. The transformation into chronic inflammation is thought to be a primary contributor to a number of chronic illnesses. Anti-inflammatory action is therefore thought to combat chronic illnesses like cancer, kidney diseases, and liver diseases. A multitude of factors, including cytokines, mitogens, macrophages, cyclooxygenases (COXs), lipoxygenases (LOXs), tumor necrosis factors (TNFs), and numerous other inflammatory pathway components, can contribute to a pro-inflammatory response. There are numerous polyphenols in honey that have been shown to inhibit LOXs. The phenolic compounds and flavonoids found in honey are responsible for its anti-inflammatory properties. There are wide range of causes of inflammation, including endotoxins, viruses, variation in fatty acid levels, growth factors, an imbalance in the oxidative state, oncogenic processes, excess body fat and a poor diet. As a result, eating a diet high in fruits and vegetables and well-balanced is essential to regulate the of inflammatory processes, which are the root cause of many severe illnesses A number of in vitro and in vivo models have been used in recent years to assess the anti-inflammatory properties of honey. For instance, honey was found to reduce the major edema and plasma levels of pro-inflammatory cytokines (inflammatory markers), such as inducible nitric oxide synthase (iNOS), tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6,) and p-38, in macrophages treated with lipopolysaccharide (LPS) by blocking the TLR4/NF-κB pathway while concurrently activating the AMP-activated protein kinase (AMPK) pathway (Ahmed et al., 2018).

Hypothesis:

Extract of selected Palestinian floral honey samples act in a multi-target mechanism and represent a potential anti-breast cancer crude product. These include anti-proliferative, anti-inflammatory, antioxidant as well as anti-metastasis pathways.

Objectives of the study:

The goal of this research is to examine the potential anti-cancer properties of specific floral honey samples: (PH1: Barsem. PH2: Morar, PH3: Khorfesh, PH4: Gabali, Ph5: Sedr, PH6: Kena, PH7: Tebbeyah, PH8: Rabat, PH9: Multifloral, PH10: Aghwar) (Table (1)). This will be done through a series of *in vitro* tests using a cell culture system, conducted in the following five stages:

1. The potential anti-cancer honey samples were chosen based on local traditional knowledge and published research.
2. Assessment of safety (nontoxic) concentrations of the honey extracts.
3. The study examined the anticancer effects of honey extracts on breast cancer cell lines MDA. The extracts' cytotoxic and cytostatic activities were assessed using MTT assay to measure cell proliferation.
4. Evaluation of the anti-migration effect of the honey extracts on breast cancer cell line MDA.
5. Evaluation of the anti-inflammatory effects of the honey extracts in THP-1-derived macrophages (classically activated M1-type macrophages) (Xia et al., 2023).
6. Evaluation of the antioxidant effect of the honey extracts on breast cancer cell line MDA.

Chapter 2: Material and Methods

Plant Material -Natural Products-:

The selected floral honey samples purchased from the beekeepers of local market –Tulkarm, and cultivated in Jenin, Tulkarem, and Jericho in West bank, Palestine, see Table 1.

Table (1): The honey samples were purchased from the beekeepers of local market -Tulkarm in West bank, Palestine

Sample ID	**Honey Samples**	**Geographic Origin**	**Botanical Source**
PH1	Barsem- Alfalfa	Jenin	-Medicago sativa
PH2	Morar- Purple starthisth	Jericho Vally	Centaurea calcitropa
PH3	Khorfesh-Milk thistk	Tulkarm	Silybum marianum
PH4	Gabali-Fothaj Lesser calamint	mountains of Tulkarm	Clinopodium nepeta
PH5	Seder-Christs thornjajuba	Nasaria-Jericho	Ziziphus spina-Chlist
PH6	Kena- Eucalyptus	Jericho	Camaldulensis
PH 7	Tebeyyah- Herdant plant	Jenin	-
PH 8	Rabbat	Kofr Raei	Conyza bonariensis
PH 9	Multiflower	Plains and mountains of Tulkarm	Citrus limon L
PH 10	Aghwar	Jericho Vally	-

.

Preparation of honey samples:

Honey samples were diluted at Dulbecco's Modified Eagle's Medium (DMEM) culture media and were prepared fresh every time when used. And the dilutions were as follows (0.5, 1.0, 2.0, 4.0 mg/ml).

Cell culture:

The human monocytic cell line THP-1 (ATCC 202-TIB) was purchased from American Type Culture Collection (Manassas, VA, USA). Breast cancer MDA-MB-231 cell line (ECACC catalogue no. 92020424) of human cells were cultured in (DMEM) to create human-like conditions, supplemented with 10% fetal calf serum to enhance cell growth, 1% penicillin and streptomycin as antibiotics, 1% amphotricine B as antifungal medication, 1% non-essential amino acids and 1% L-glutamine amino acid used as an energy source. Cell lines were maintained in air, 5% CO2 at 37°C in a humidified atmosphere of 95%. The human monocytic cell line THP-1 cells were cultured in Gibco Roswell Park Memorial Institute (RPMI Medium) with the same supplements.

Counting cell using a hemocytometer protocol:

1. Samples from breast cancer tissue were trypsinized with 5ml of trypsin-EDTA solution for each flask, then incubation period at 37°C for 5%CO2.

2. After 10-minutes, 3 ml media was added, and cell suspension was centrifuged for 5minutes at 3000 rpm for 5 minutes.

3. Later on, the supernatant was removed and 3ml media were added to the pellet and resuspension of the cell with media DMEM.

4. 10 µl of the mixture was loaded in a hemocytometer for cell counting under the microscope.

The MTT assay for cell viability assessment:

The tetrazolium dye, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method is a standard colorimetric assay measuring the activity of enzymes. This assay is based on the conversion of yellow tetrazolium bromide to purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. (Kmail et al., 2015). The absorbance of the MTT formazan was determined at 570 nm in an ELISA reader. Cell viability was defined as the ratio (expressed as a percentage) of absorbance of treated cells to untreated control cells (Control). Viable cells metabolize MTT, producing a purple color. There is a direct correlation between the absorbtion value and cell viability. The test is based on the ability of viable cells to metabolize water-soluble tetrazolium salt (yellow) into a water-insoluble formazan product (purple colour). The purple formazan crystals are produced by dehydrogenases in active mitochondria in viable cells. Dead cells are unable to perform this reaction (Chenoweth et al., 2020).

1.Cytotoxicity assessment, using MTT Assay:

In a flask with 70-80% confluence, cells were detached from the cultured flask by treatment with 2ml/small flask and 5ml/big flask trypsin. Afterwards, a cell suspension of 100 µl (2.0×10^4 cell/well) was added in a 96-well plate and was incubated for 24 hours. Cells were incubated with stock solutions of extracts from honey samples, serially diluted to reach concentrations of (0.5, 1.0, 2.0 and 4.0 mg/mL). After 4 hour of incubation period of 100 µl of MTT (MTT solution was prepared in DMEM without serum (0.5 mg/ml). MTT solution was removed. The formazan product was solubilized with acidified isopropanol solution in isopropanol: formic acid (45ml: 5ml) proportion. The plate was covered with tinfoil and was shaked on orbital shaker for 15 min.

The optical density (OD) was determined at 570 nm by an enzyme-linked immunosorbent assay (ELISA) reader. Conventional microplate-based enzyme-linked immunosorbent assays are widely used in a variety of molecular sensing, disease screening, and nanomedicine applications. When contrasting non-batched or non-standard testing with this multi-well plate batched analysis, it uses a color-changing mechanism based on enzymatic reactions that reduces the volume of biomarkers required for the minimum detectable signal. the ELISA readers rely on large light absorption. A strategy to decrease the ELISA's size while increasing accuracy is to amplify the color change signal using the surface plasmon enhancement effect or use a digital ELISA system with lower device dimensions and extremely accurate femtomolar scale detection (Mirhosseini et al.,2024) (Kmail et al., 2015).

2. Cytostatic effect assessment, using MTT Assay:

The assay is performed to test the cell proliferation activity of the extract. 100 µl (5.0×10^3 cell/well) cells were seeded in each well in a 96-well plate for 24 hours and were incubated with extracts for 72 hours at 37°C. Afterwards, the same procedure of cytotoxicity assessment was followed (Kmail et al., 2015).

The scratch-wound assay:

This *In vitro* transwell-based models' assay is widely used to assess cancer cell migration. Cell migration may be random or directed. Migration, the simplest variation, involves various assays. ***The scratch-wound assay*** is a simple assay that is commonly used. 2.5 mL of cell suspension were grown to confluence. A thin "wound" was introduced by scratching with a 200 µL pipette tip. After removing the culture medium from each well, DMEM devoid of additives and serum

was used to wash each well four times. Next, 4 mL of each honey sample (4 mg/ml) and the culturing medium (untreated control) alone were added to each well in triplicate. Subsequently, an incubation period was performed with honey extract for about 24 hours. Cells at the wound edge were polarized and migrated into the wound space. We tracked the migration of breast cells by visualizing them hourly under inverted microscope during the incubation period at identified magnification over the course of two days (Katt et al., 2016).

Immunoassay for nitric oxide:

The anti-inflammatory activities were assessed by investigating extracts/samples of honey for their ability to alter the production of nitric oxide (NO). THP-1 cell line was co-stimulated. With bacterial lipopolysaccharide (5 μg LPS/mL) was enhanced secretion of nitric oxide in expected high level upon translocation process of NF-kB. The amounts of secreted NO were measured using a commercial ELISA kit. The absorbance at 450 nm was read by a microplate reader, with the wavelength correction at 550 nm. The amounts of NO were calculated with the help of a standard curve, which was constructed using serial dilutions of NO standards was provided with the kit (Touzani et al., 2019).

The DPPH (2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl) Test:

DPPH is a radical that exists in both solid and solution states in its monomer form.

The radical is insoluble in water but is typically soluble in various organic solvents, including methanol, ethanol, or their aqueous mixtures. However, the radical can dissolve more easily if the water content is less than 60%. The characteristic quintet spectrum of the dissolved DPPH• is changed to a singlet spectrum at high water content, which is typical for a solid-state radical. Not all the time is this modification of radical solubility apparent.

The neutralization DPPH test relies on the antioxidants' ability to donate electrons to neutralize the DPPH radical. The DPPH color, which is measured at 517 nm, changes in tandem with the reaction; this discoloration serves as a gauge of antioxidant activity. The effective concentration of the antioxidant required to reduce (neutralize) the initial DPPH concentration by 50% is known as the IC50μg/mL, Furthermore, TIC50—the amount of time required to reach the equilibrium state with IC50—may be applied.

Free radicals are linked to a variety of illnesses, including inflammation, cancer, cardiovascular disease, and neurodegenerative diseases. They induce oxidative stress on living tissues by destroying biomolecules necessary for cell viability. Antioxidants possess the capacity to mitigate these detrimental consequences. It is thought that there is a strong correlation between the antioxidant qualities of natural products that are promoted for their potential benefits to human health and phytochemicals like flavonoids and phenolic acids that are found in honey samples.

A slightly modified micro-dilution DPPH assay was used to investigate the scavenging of free radicals. In 96-well, flat-bottomed micro-titration plates, a two-fold serial dilution in pasteurized water was used for this procedure. The ethanolic DPPH solution (100 ppm) was added in an equivalent volume to each 0.1 milliliter of honey solution. The honey had final concentrations that varied from 0.016% to 16.67% (w/w%). Following mixing, the mixture was allowed to stand at room temperature for 30 minutes in a dark room. Next, the solution's absorbance at 620 nm was measured and transformed into a percentage that showed the solution's ability to scavenge free radicals. The IC50 value for every variety of honey was determined by taking the value that corresponded to the linear part of the graph. Thereafter, in order to determine the matching concentration value on the x-axis, we substituted 50% for the y-value.

Statistical Analysis:

Error limits cited and error bars plotted represent simple standard deviations of the mean. When comparing different samples, results were considered to be statistically significant when $P<0.05$. The values were analyzed by Simple T-test.

Chapter 3: Results and Discussion:

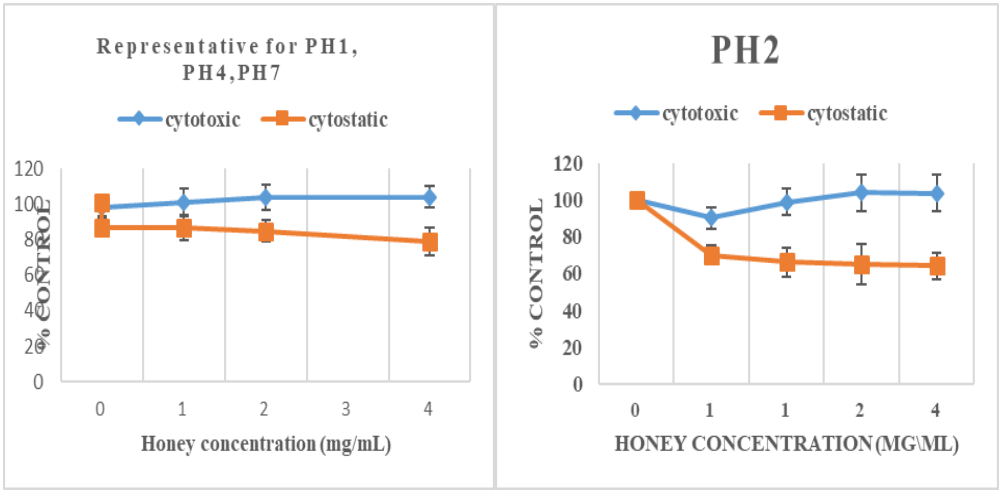
Cytotoxicity and cytostatic effects of the honey samples

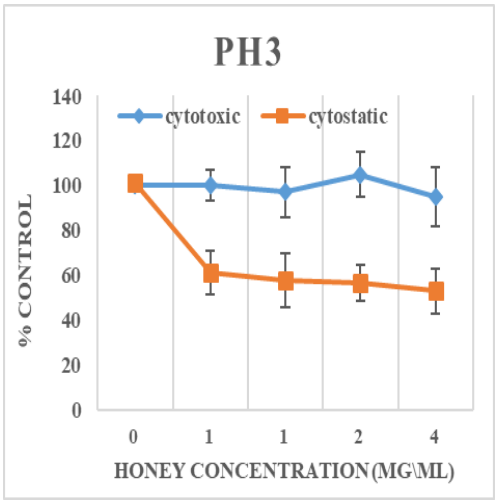
The current investigation aims to screen the anticancer activity of ten honey samples by assessing their cytotoxic and cytostatic activities against the breast cancer cell line (MDA). Honey samples that exert anti-proliferative effects (cytostatic effects) in cancer cells at non-cytotoxic concentrations are illustrated in Fig (1).

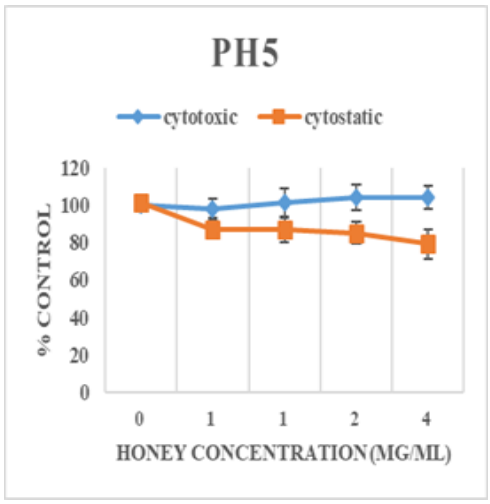
Cell-based *in vitro* culture models are effective research tools for predicting drug metabolism and toxicity. Cells derived from different organs or cell lines are typically used. *In vitro* procedures are often regarded as a very efficient way to conduct toxicity testing due to their advantage of having factors that may be controlled to some extent. The reduction in the number of viable cells may result from two major processes—inhibition of cell metabolism and/or proliferation (cytostatic effect) or actual cell death (cytotoxic effect)—which should be discerned from each other. It is necessary to use a combination of methods to understand exactly what is happening in the cell. MTT and other enzymatic assays evaluate the activity of cell metabolism.

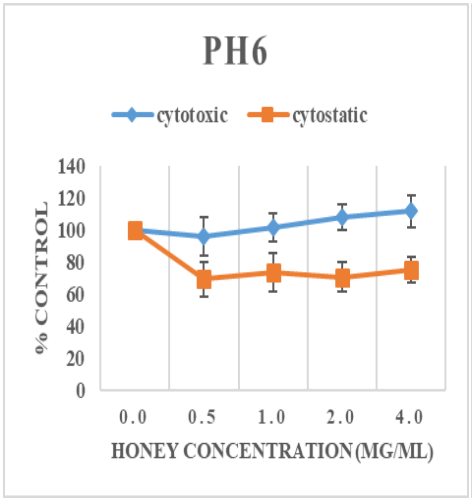
Natural products have a strong track record in the development of anti-cancer agents. Thus, many drug discovery programs and efforts continue to exploit this rich source of natural products**.**
The results obtained here demonstrate a wide range of anticancer activities of the various honey samples on cancer MDA cell lines as measured by MTT assay. The samples that show cytostatic

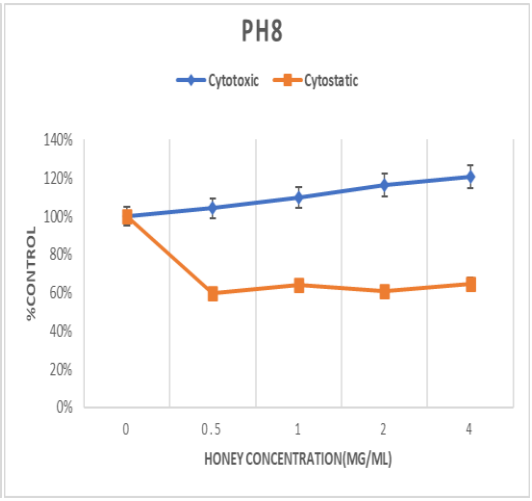
effects at non-cytotoxic concentrations are the most important ones. No cytotoxic effects were found for all the honey samples at all concentrations used (0-4mg/ml). The samples of PH2 (Morar), PH3 (Khorfesh), Sedr (PH5) and alkena (PH6) showed cytostatic activity on MDA cells lines (Figure 1). In contrary, the honey samples of Barsem (PH1) Gabali (PH4) and Tebbeyah (PH7) had a slightly decrease in cell viability values in MDA cells. PH8, PH9 and PH10 show insignificant result in inhibition the growth of MDA. According to the published data, honey can prevent cancer by influencing the molecular processes involved in the initiation, promotion, and progression stages of the disease. Therefore, it could be a viable and promising anticancer agent that needs more research, both in the lab and in the clinic (Samarghandian et al., 2018), (Eteraf et al., 2022).




PH3
cytotoxic
cytostatic
% CONTROL
140
120
100
80
60
40
20
0
0
1
1
2
4
HONEY CONCENTRATION (MG\ML)


PH5
cytotoxic
cytostatic
% CONTROL
120
100
80
60
40
20
0
0
1
1
2
4
HONEY CONCENTRATION (MG/ML)


PH6
cytotoxic
cytostatic
% CONTROL
140
120
100
80
60
40
20
0
0.0
0.5
1.0
2.0
4.0
HONEY CONCENTRATION (MG/ML)


PH8
Cytotoxic
Cytostatic
%CONTROL
140%
120%
100%
80%
60%
40%
20%
0%
0
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1
2
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HONEY CONCENTRATION(MG/ML)

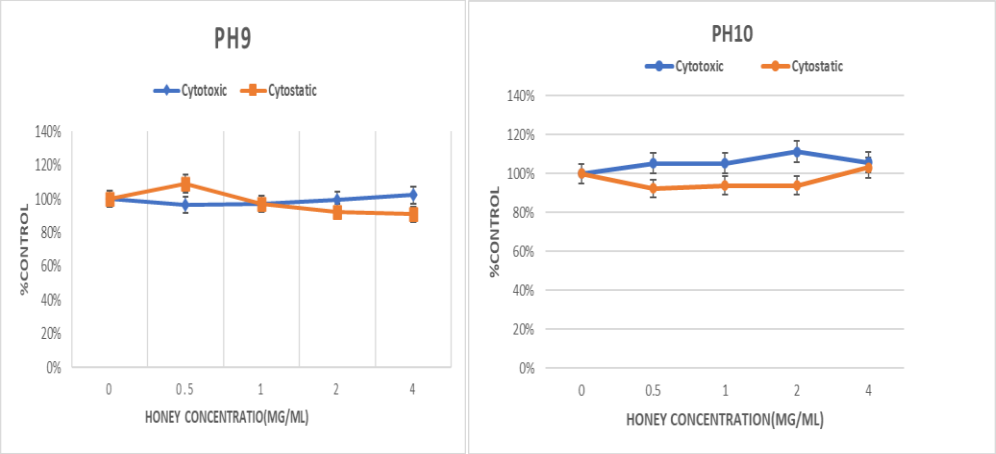


Figure (1): Cytostatic and cytotoxic activity of honey samples. Data represent the MTT values obtained on MDA cells after treating with honey concentrations 0 to 4mg/ml (PH1: Barsem. PH2: Morar, PH3: Khorfesh, PH4: Gabali, Ph5: Sedr, PH6: Kena, Ph7: Tebbeyah, Ph9: Multifloral, PH10: Aghwar) in MDA cell line. The asterisk indicates statistical significance compared to the untreated control, with significance recognized when p-value < 0.05. * p0.05, ** p0.01, ***p0.001 as considered significant compared to the control group one-way ANOVA followed by Dunnett's test.

Several *in vitro* studies showed that the effects of honey on the development and progression of human breast cancer of (MCF-7 & MDA-MB-231) are mediated through many mechanisms. These include the disruption of the signaling pathways of cancer cells by acting on several targets, such as inducing apoptosis, activating the mitochondrial pathway, stopping the cell cycle, modulating insulin signaling and oxidative stress, reducing inflammation, inhibiting angiogenesis and cell proliferation, stimulating immune cells and TNF-α, IL-1β, IFN-γ, and p53, and inhibiting lipoprotein oxidation, IL-1, IL-10, COX-2, lipoxygenases, and prostaglandin E2. Furthermore, honey enhances the quality of life for chemotherapy patients as well as the effectiveness of anti-

neoplastic medications. Before honey is used in clinical interventions for cancer patients, more research needs to be done to confirm its anticancer properties (Masad et al., 2021).

The scratch-wound assay:

Intratumor heterogeneity can complicate cancer diagnosis and treatment and increase the risk of recurrence. While the clinical impacts of intratumor heterogeneity are recognized, less is understood about how intratumor heterogeneity affects phenotypic behaviors such as migration and metastasis. While metastasis is a dynamic, multistep process, numerous studies have focused on the earliest stages of local dissemination, where cancer cells develop a motile phenotype to leave the primary tumor and migrate through the stroma. Collective cell migration is the predominant migration mode observed in clinical samples and is being linked with worsened patient prognosis in numerous cancer types; however, far less is known about this mode of migration compared to single cell migration. In collective migration, the external chemical and physical cues and intracellular signaling and mechano-transduction events that dictate single cell migration are integrated across cohesive sheets, strands, or streams in collective migration. It is still difficult to distinguish the relative contributions of each cell's spatiotemporally distinct interactions with the microenvironment and their intrinsic genetic disposition in order to determine whether this observed spectrum of migration modes reflects cellular plasticity or phenotypic diversity; even though both single and collective cancer cell migration have been simultaneously observed in the same patient's samples (Hapach et al., 2023).

In vitro scratch wound assays are used to examine cellular migration. Here the research discusses scratching of cell monolayers. Besides, we examine the effect of ten honey samples on migration behavior of breast cancer cell line (MDA) and identify possible caveats in experimental design. Results show a significantly reduction of cell migration of MDA when wounds (SCRACHES) are

created using a cell scratching method. The purpose of this study was to determine how honey sample affect whether these weakly and highly migratory cells that interact with each other *in vitro* that are expressed in percentage values.

On MDA cells, honey samples PH2, PH3, PH5, PH6, PH7, PH8, and PH9 have been demonstrated to exhibit reduction in cell migration in addition of their success in cytostatic activity. Whilst PH1, and PH10 show slightly significant reduction the percentage of migratory cells. PH4 present insignificant evidence. Fig. (2) and other figures shows the migration of MDA cell under the effect of each honey sample.

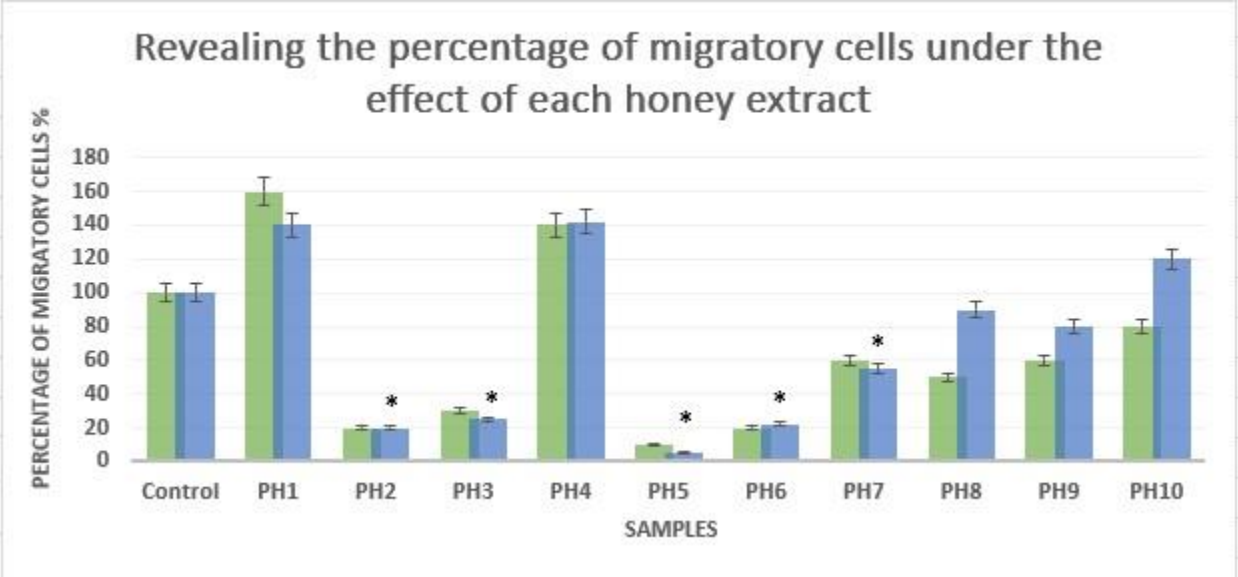


Figure 2. Effect of honey samples on cell migration of MDA cells. The results show the proportion of scratches closure after 24 and 48 h of treating with honey samples. Each data point was determined based on the initial scratch size at time 0 h and was normalized to the untreated control, which was set as 100%. The asterisk indicates statistical significance compared to the untreated control, with significance recognized when p-value < 0.05. * p0.05 as considered significant compared to the control group one-way ANOVA followed by Dunnett's test.

Contrastingly with the control cells, however, there was a non-significant slight increase in MDA cell migration in PH1 and PH4. Other samples did not show any significant effect in the percentage of migration.

The overall net changes brought about by honey and its polyphenolic components modify the tumor microenvironment, inhibit angiogenesis, and reprogram immune cells to become more resistant to the cancer cells' ability to proliferate and migrate.

Numerous studies have also shown the immunomodulatory role of honey polyphenols against cancer. For example, the release of the chemokine CCL2, which is involved in the recruitment of tumor-associated macrophages (TAMs) in the tumor microenvironment, was suppressed by luteolin. This was accompanied by a suppression of Lewis lung cancer cells' migration. The study revealed that apigenin suppresses IFN-γ-induced STAT1 activation, resulting in decreased expression of programmed death-ligand 1 (PD-L1) in A375 melanoma cells. This, in turn, mitigates the inhibition of anti-tumor immune responses mediated by PD-1/PD-L1. Treatment with apigenin decreased PD-L1 expression in DCs, which improved the host's T cell immunity. It has also been demonstrated that quercetin inhibits the PD-1/PD-L1 interaction. By interfering with the IL8/STAT3 signaling axis, genistein has been shown to suppress M2-polarized macrophages and the stemness of ovarian cancer cells SKOV3 and OVCA-3R. Chrysin administered orally suppressed tumor growth in a melanoma model by 60% and 70% after 14 and 21 days of treatment, respectively. Cytotoxic T cells and NK cells showed increased killing activity in tandem with this. Chrysin treatment also enhanced the NK cells' cytotoxicity in separated splenocytes from leukemic BALB/c mice. When considered collectively, these results validate the efficacy of different flavonoid compounds in augmenting anti-tumor immune responses in preclinical cancer models and indicate that multiple mechanisms are probably at play (Erejuwa et al., 2014).

The reduction in tumor cell migration due to honey samples could be partially attributed to their cytostatic properties. Honey samples PH2, PH3, PH5, and PH6 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 suggest the involvement of additional cellular and molecular mechanisms.

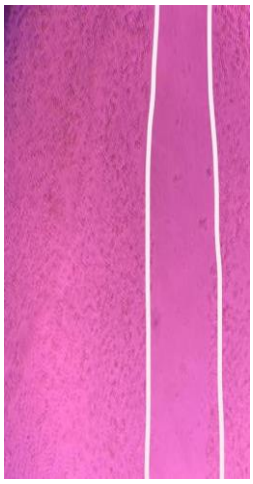
Our findings align with previous studies that reported the inhibitory effects of other honey samples and isolated phytochemicals like resveratrol, kaempferol, and EGCG on the migration of colorectal and OSCC cancer cells. To our knowledge, this is the inaugural study illustrating the anti-metastatic impact of Palestinian honey samples on MDA breast cancer cell lines (Lopez et al., 2019) (Chung et al., 2018) (Riahi et al.,2019). Metastasis, the most harmful aspect of cancer, involves intricate processes (Martin et al., 2013) with a multitude of molecules (Thompson et al., 2002) 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 (Osrolic et al., 2004). 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 (Reddy et al., 2023). It has been reported that gallic acid can reduce the gelatinolytic activity of MMP-2 and MMP-9, possibly through NF-κB (HO et al., 2010). Additionally, several studies have suggested that honey can decrease both the expression and nuclear translocation of NF-κB *in vivo* and *in vitro* (Batumalaie et al., 2013)

(Hussein et al., 2013). Honey has been shown to reduce the enzymatic activity of MMP-2 and MMP-9 (Moskwa et al., 2014). For instance, Fir honey was found to inhibit human keratinocyte migration by decreasing MMP-9 expression (Maitan et al., 2013). Quercetin has been found to downregulate the expression of both MMP-2 and -9 in PC3 cells (Vijayababu et al., 2006) (Jaganathan et al., 2009).

1)AGHWAR **Control**

A)



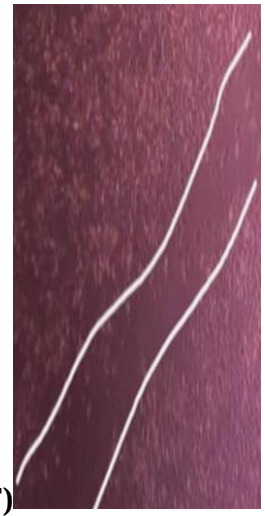
D)

B)

E)

C)

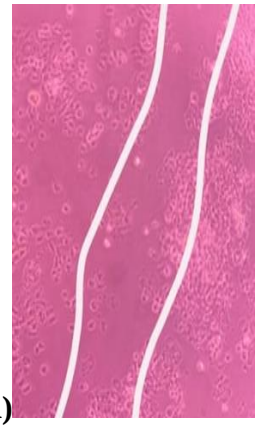
F)



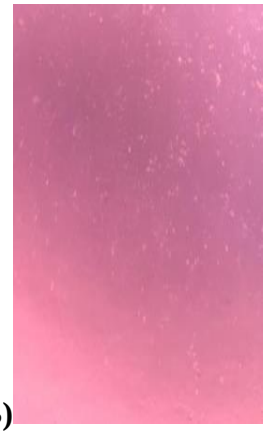
AGHWAR ANTIMIGRATION CAPACITY

Fig. 1. Comparison of wound after gap creation (A) and after 24 h (B), after 48 h(C). Scratched cell monolayer created by means of pipette tip. Gaps created had showed variation in the number of migratory cells. Lines show edges of the cells at the scratched edges.) Reduced migration after 24 h of MDA using scratching monolayer method. Aghwar honey sample delays migration capacity of scratched MDA.

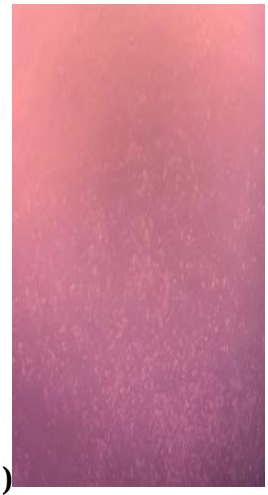
2)BARSEM **Control**

A) 

D) 

B) 

E) 

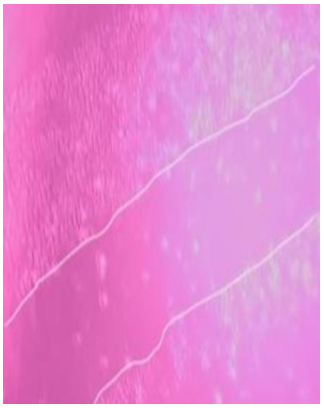
C) 

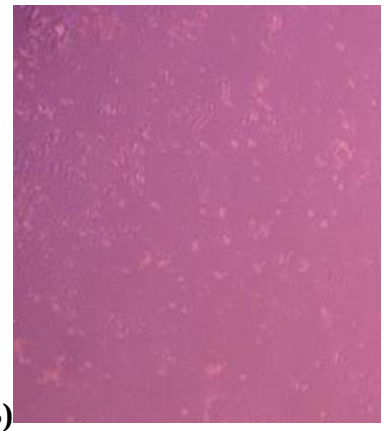
F) 

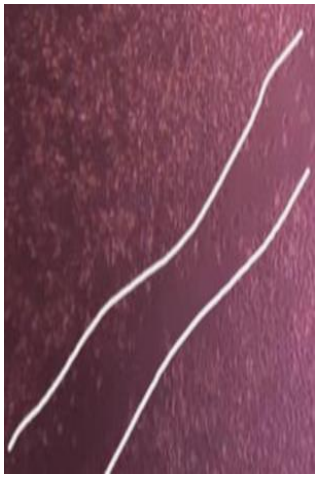
BARSEM CAPACITY

Fig. 2. Comparison of wound after gap creation (A) and after 24 h (B), after 48 h(C). Scratched cell monolayer created by means of pipette tip. Gaps created had showed variation in the number of migratory cells. Lines show edges of the cells at the scratched edges. Reduced migration after 24 h of MDA using scratching monolayer method. Barsem honey sample don't show delays migration capacity of scratched MDA. The cancer cells migrate freely through the scratch.

3)SEDR **Control**

A)  **D)** 

B)  **E)** 

C)  **F)** 

SEDR CAPACITY

Fig. 3. Comparison of wound after gap creation (A) and after 24 h (B), after 48 h(C). Scratched cell monolayer created by means of pipette tip. Gaps created had showed variation in the number of migratory cells. Lines show edges of the cells at the scratched edges. Reduced migration after 24 h of MDA using scratching monolayer method. SEDR honey sample don't show delays migration capacity of scratched MDA. The cancer cells migrate freely through the scratch at three-time levels.

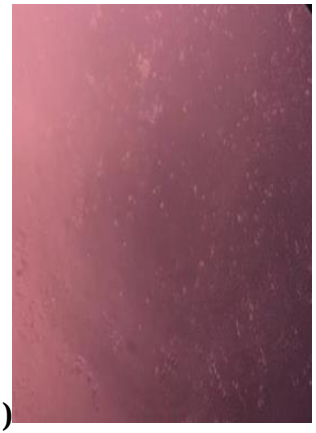
4)GABLI **Control**

A) 

D) 

B) 

F) 

C) 

D) 

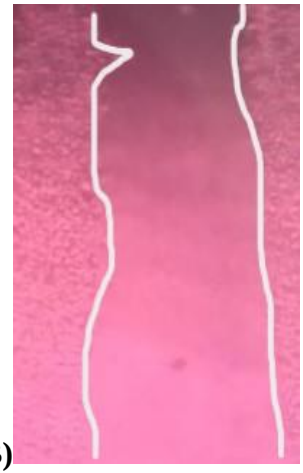
SEDR CAPACITY

Fig. 4. Comparison of wound after gap creation (A) and after 24 h (B), after 48 h(C). Scratched cell monolayer created by means of pipette tip. Gaps created had showed variation in the number of migratory cells. Lines show edges of the cells at the scratched edges. Reduced migration after 24 h of MDA using scratching monolayer method. SEDR honey sample don't show delays migration capacity of scratched MDA. The cancer cells migrate freely through the scratch at three-time levels.

5)KHORFESH **Control**

A) 

D) 

B) 

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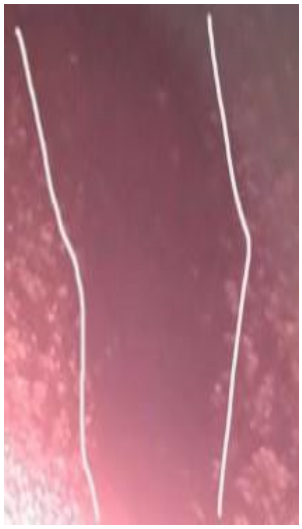
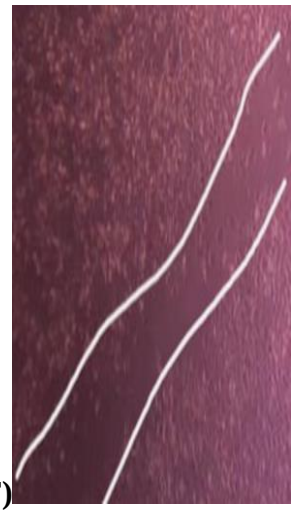
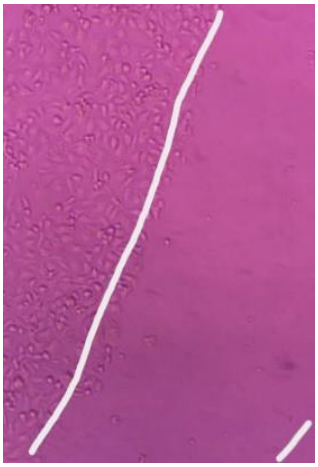
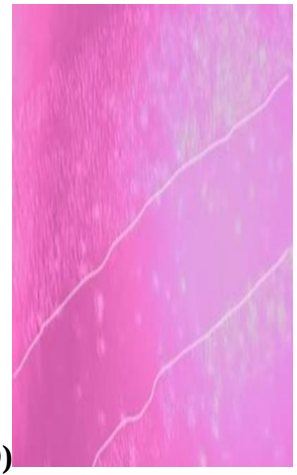


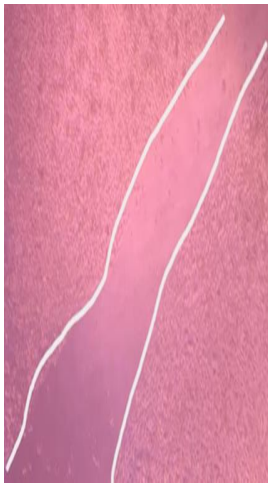
F) 

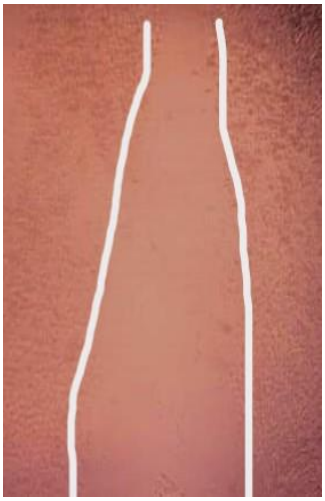
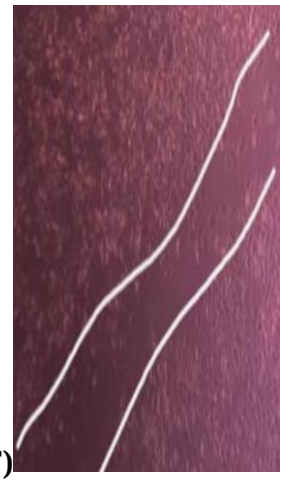
KHORFESH ANTIMIGRATION CAPACITY

Fig. 5. Comparison of wound after gap creation (A) and after 24 h (B), after 48 h(C). Scratched cell monolayer created by means of pipette tip. Gaps created had showed variation in the number of migratory cells. Lines show edges of the cells at the scratched edges. Reduced migration after 24 h of MDA using scratching monolayer method. KHORFESH honey sample don't show delays migration capacity of scratched MDA. The cancer cells migrate freely through the scratch at three-time levels.

6)KENA **Control**

A)  **D)** 

B)  **E)** 

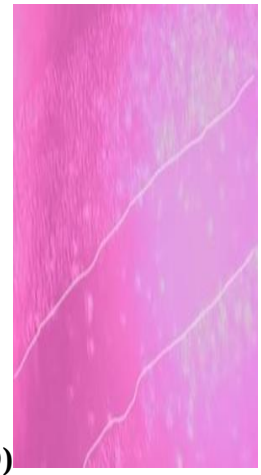
 **F)** 

KENA ANTIMIGRATION CAPACITY

Fig. 6. Comparison of wound after gap creation (A) and after 24 h (B), after 48 h(C). Scratched cell monolayer created by means of pipette tip. Gaps created had showed variation in the number of migratory cells. Lines show edges of the cells at the scratched edges. Reduced migration after 24 h of MDA using scratching monolayer method. KENA honey sample don't show delays migration capacity of scratched MDA. The cancer cells migrate freely through the scratch at three-time levels.

7)Morar **Control**

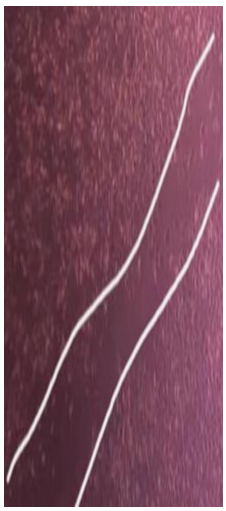
A) 

D) 

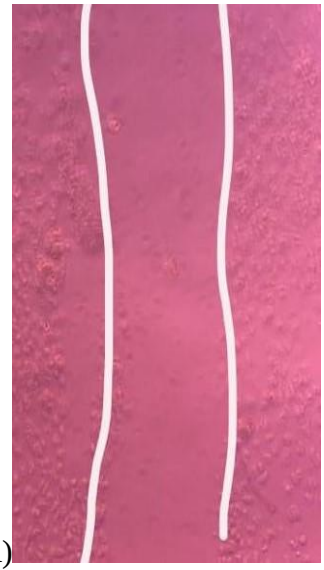
B) 

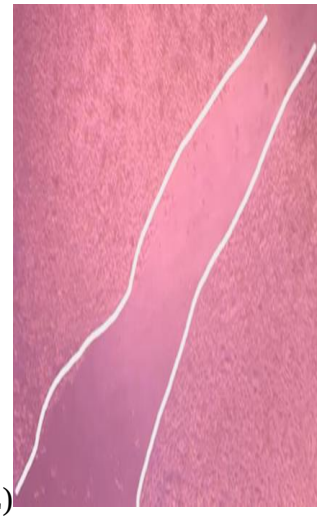
E) 

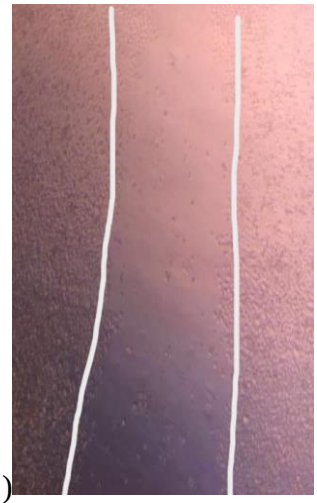
C) 

F) 

8)Multifloral **Control**

A)  D) 

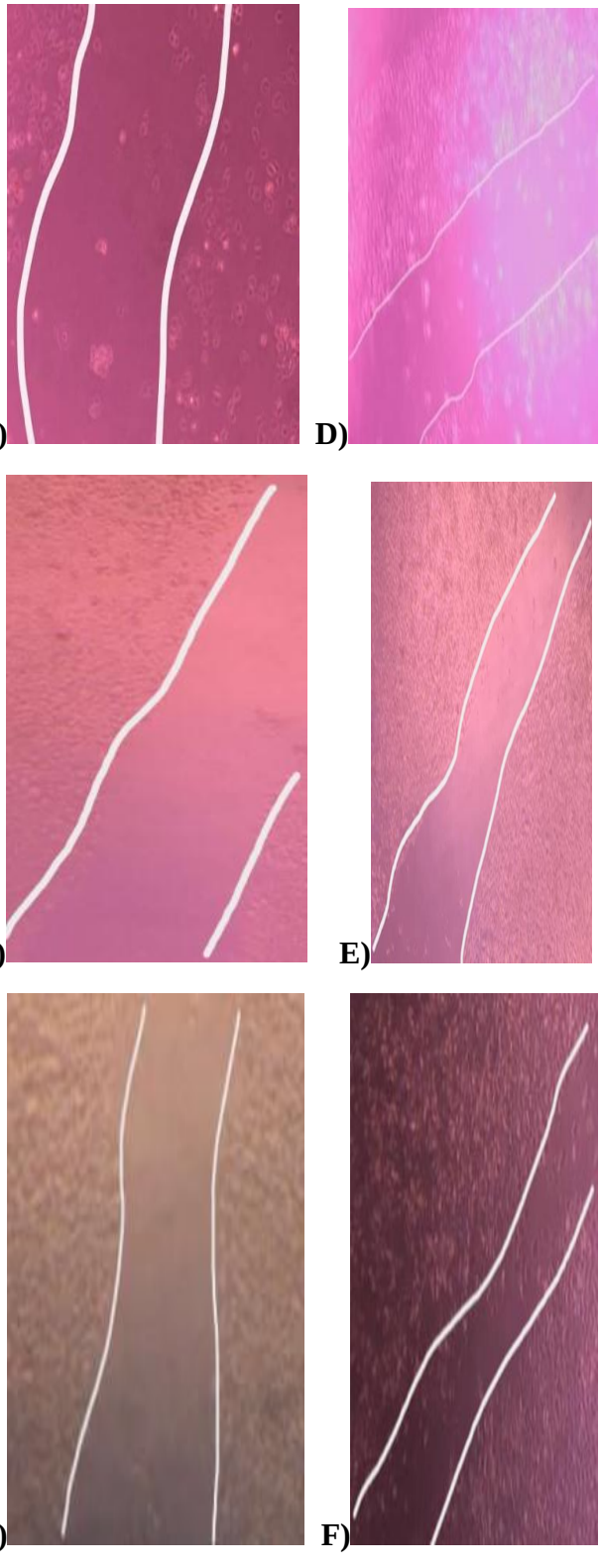
B)  E) 

)  F) 

MULTIFLORAL ANTIMIGRATORY CAPACITY

Fig. 8. Comparison of wound after gap creation (A) and after 24 h (B), after 48 h(C). Scratched cell monolayer created by means of pipette tip. Gaps created had showed variation in the number of migratory cells. Lines show edges of the cells at the scratched edges. Reduced migration after 24 h of MDA using scratching monolayer method. MULTIFLORAL honey sample don't show delays migration capacity of scratched MDA. The cancer cells migrate freely through the scratch at three-time levels.

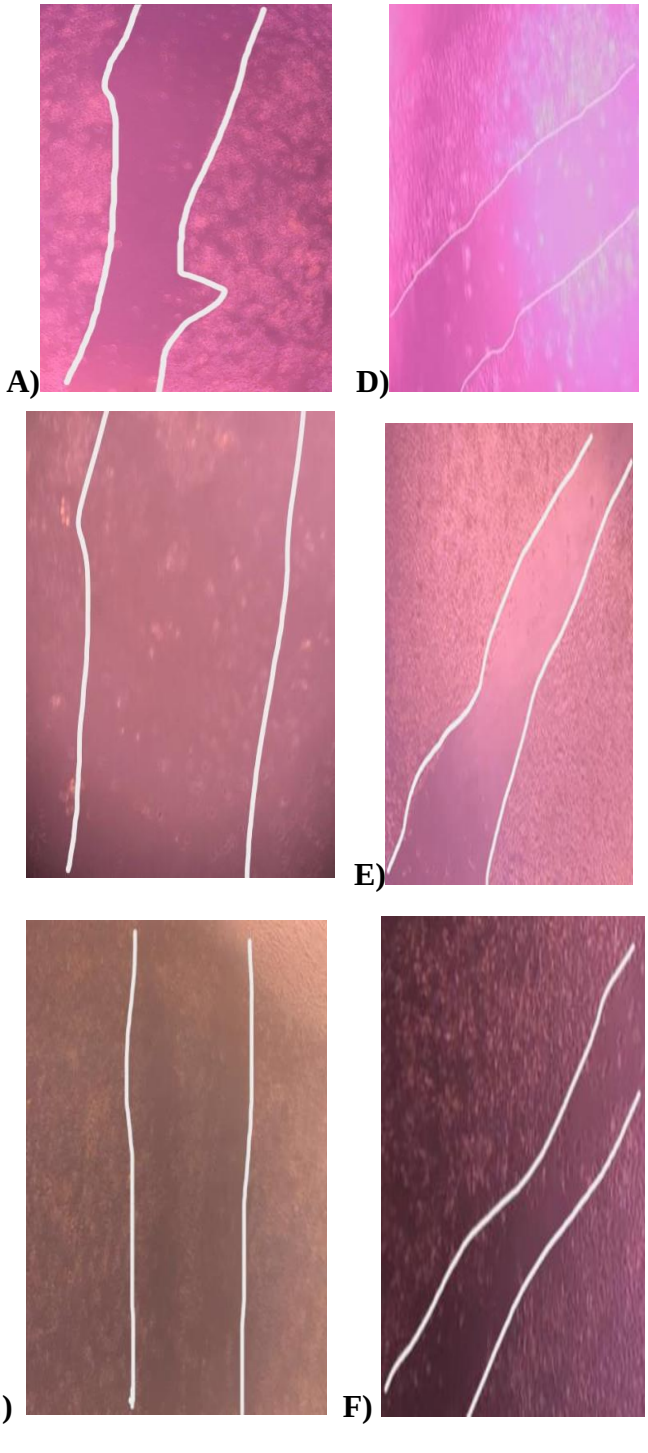
9)Rabat **Control**



RABAT ANTIMIGRATORY CAPACITY

Fig. 9. Comparison of wound after gap creation (A) and after 24 h (B), after 48 h(C). Scratched cell monolayer created by means of pipette tip. Gaps created had showed variation in the number of migratory cells. Lines show edges of the cells at the scratched edges. Reduced migration after 24 h of MDA using scratching monolayer method. RABAT honey sample don't show delays migration capacity of scratched MDA. The cancer cells migrate freely through the scratch at three-time levels.

10)Tebeyah **Control**



TEBBEYYAH ANTIMIGRATORY CAPACITY

Fig. 10. Comparison of wound after gap creation (A) and after 24 h (B), after 48 h(C). Scratched cell monolayer created by means of pipette tip. Gaps created had showed variation in the number of migratory cells. Lines show edges of the cells at the scratched edges. Reduced migration after 24 h of MDA using scratching monolayer method. TEBBEYYAH honey sample don't show delays migration capacity of scratched MDA. The cancer cells migrate freely through the scratch at three-time levels.

Figure 3 (1-.10): Revealing the percentage of migratory cells under the effect of each honey extract.

DPPH:

DPPH an organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. It is a dark, purple-colored crystalline powder composed of stable free-radical molecules. DPPH has two major applications, both in laboratory research: one is a monitor of chemical reactions involving radicals and another is a standard of the position and intensity of electron paramagnetic resonance signals.

DPPH is a well-known radical and a trap ("scavenger") for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about 520 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm or in the EPR signal of the DPPH (Munteanu et al., 2021)

The analyzed samples' capacity to neutralize DPPH free radicals was assessed and is represented as IC50 μg/mL. PH 3 and PH 5 samples showed the best results in neutralizing free radicals with DPPH; their respective IC50 values were 2.87 μg/mL and 0.7 μg/mL. PH 9 produced the lowest IC50 value (208 μg /mL). Honey is known for its powerful antioxidant qualities, which are attributed to its rich content of various phytochemicals, especially flavonoids and phenolics. Several investigations have indicated a robust association between honey's antioxidant potential and thequantity of phenolic components it contains (Baliyan et al., 2022), (Stagos et al., 2018), (Nicewicz et al., 2021). Numerous honey samples' antioxidant activity confirmed previous

research showing phenolic compounds are potent antioxidants. Notably, PH 5 was particularly effective and was found to have a high concentration of polyphenolic compounds among the examined samples of Palestinian honey. Compared to PH 5, PH 9 showed a minimal IC50 and was reported to have a much lower phenolic and flavonoid content.

According to IC50 values Sedr, Tebbeyah, Barsem and Khorfesh present the most effective concentration to neutralize the DPPH radical by 50% with $p<0.01$. Gabali and Kena have half maximum inhibitory concentration with $p<0.05$ with moderate evidence. Morar, Rabat and Multifloral worked but in weak evidence. Aghwar honey sample show in significant value indicating insufficient evidence in results. This is consistent with previous research, emphasizing the importance of its content of phytochemicals aligning with its precedent from the results previously discussed during the research narrative.

Table 2. Commercial Palestine Honey types and antioxidant activity. The asterisk indicates statistical significance compared to the untreated control, with significance recognized when p-value < 0.05. * p0.05, ** p0.01, ***p0.001 as considered significant compared to the control group one-way ANOVA followed by Dunnett's test.

Kind of Honey	**IC50 (w/w%)**
PH1	0.30
PH2	1.29
PH3	0.35
PH4	0.8
PH5	0.144
PH6	0.85
PH7	0.19
PH8	5
PH9	10

Effects on nitric oxide production:

Nitric oxide is one of the mediators in the inflammatory process that leads to vasodilation and the ensuing rise in blood flow. It is expected that the induction of LPS causes a significant increase in both NO production and the inflammatory response. Figure (4) illustrates how, in a dose-dependent manner, honey extracts both inhibit the LPS-mediated release of NO release by cultured THP-1 cells in a dose-dependent manner at a concentration of 1-2 mg/mL, reaching levels comparable to untreated cells.

M1-type macrophages are typically induced by the combination of IFN-γ and bacterial lipopolysaccharide (LPS), which leads to the secretion of pro-inflammatory factors, such as tumor necrosis factor (TNF)-α, IL-1β, IL-6, nitric oxide synthase (iNOS), chemokines, and an upregulation of some cell surface markers like CD40, CD80, CD86, and major histocompatibility complex class II receptor (MHC-IIR) (Xia et al., 2023).

The production levels of pro-inflammatory mediators (nitric oxide) were measured in lipopolysaccharide (LPS)-activated Human monocytic cell line THP-1- derived macrophages in the absence and presence of increasing concentrations (1-2mg/ml) of the ten honey extracts.

THP-1-derived macrophage cells were used because the cells can be activated by LPS to produce significant amount of nitric oxide (NO). The levels of NO were measured 72 h after treatment using a Griess assay. Plant extracts inhibited the production of NO in LPS-activated THP-1-derived macrophages in a dose-dependent manner at nontoxic concentration. NO release was inhibited from 194 μM to 34 μM and 14 μM after treatment with honey extracts at 1 mg/mL, respectively. The results show that Khorfesh, Sedr, Kena, Gabali and Aghwar decreased the NO production rate in significant value. While Barsem, Tebbeyah, Kena, Rabbat they worked, but with

less important considerations and significant values. By Multifloral there was no significant value in reducing NO production rate.

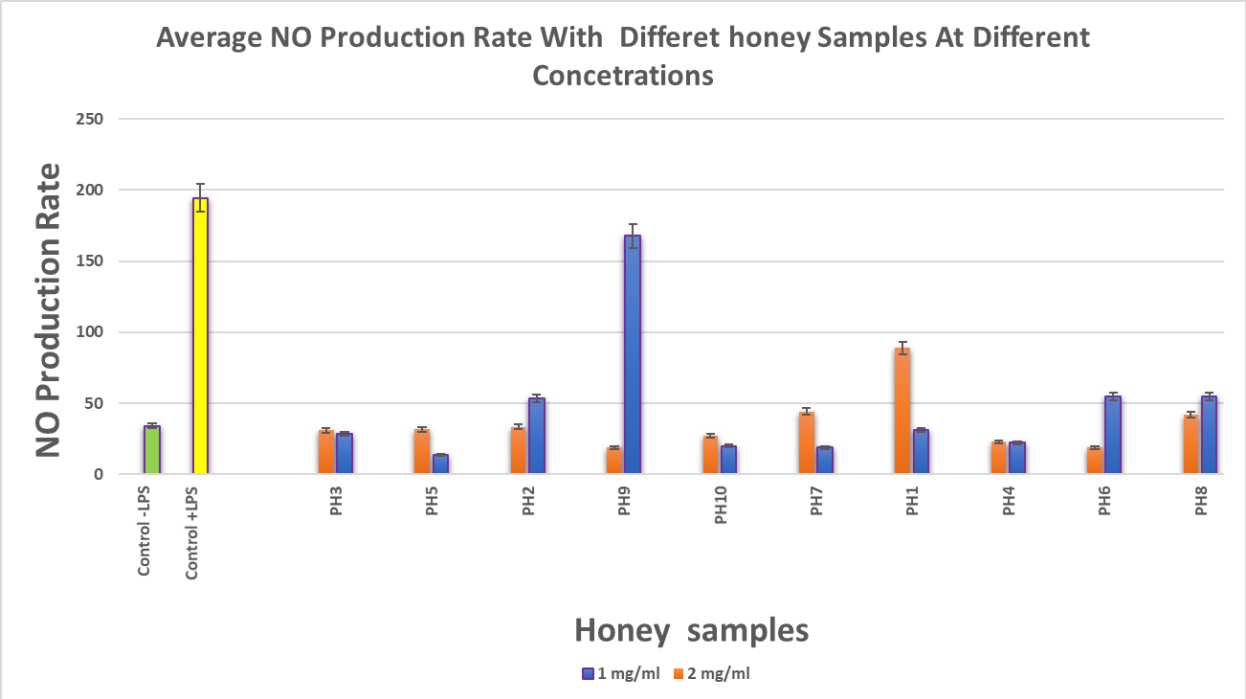


Figure 4. NO production by THP-1-derived macrophages after treatment with of increased concentrations of ten honey samples as measured with Griess assay. Values represent the mean ± SD of 3 independent experiments applied in triplicates. NO values of LPS treated cells were taken as 100%. with significance recognized when p-value < 0.05. The asterisk indicates statistical significance compared to the untreated control, with significance recognized when p-value < 0.05. * p0.05, ** p0.01, ***p0.001 as considered significant compared to the control group one-way ANOVA followed by Dunnett's test.

The honey extracts exhibited notably greater inhibitory effects at 1 mg/ml than the 2mg/ml concentration. This outcome may be explained by the fact that ethanolic extracts of honey

contained higher concentrations of total phenols, flavonoids, tannins, alkaloids, and steroids. Comparable outcomes were noted when using phytochemicals (like flavonoids) and crude extracts from medicinal plants (Kmail et al., 2015), (Saad et al., 2016), (Kianmehr, et al., 2023), (Park et al., 2023).

Chapter4: Conclusion

According to the research, honey's anticancer qualities vary depending on the kind and origin of the plant and the season in which it is collected depending on the floral origin of the honey. This result lead to examination of the migration and immunomodulatory abilities in decreasing the NO production rate, moreover the antioxidant properties of ten honey samples (PH1 to PH9) as well as their potential for therapeutic use on the MDA human breast cancer cell line. There was not a single cytotoxic sample. Cytostatic activity was demonstrated by samples PH2, PH3, PH5, and PH6, with PH2 and PH3 reducing cell viability by up to 43% at a concentration of 4 mg/mL. Furthermore, MDA cell migration was dramatically decreased by PH2, PH5, and PH6 treatments by 90%, 80%, and 72%, respectively. Moreover, the DPPH free radical neutralization IC50 values, which vary from 0.7 µg/mlmL to 2.87 µg/mlmL, were observed. These findings support honey's well-known qualities and raise the possibility that its benefits for cancer prevention may also be attributed to its cytostatic and antimigration qualities and immunomodulatory action.

References:

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *71*(3), 209-249.

Parsa, N. (2012). Environmental factors inducing human cancers. *Iranian journal of public health*, *41*(11), 1.

Cisse, O., Quraishi, M., Gulluni, F., Guffanti, F., Mavrommati, I., Suthanthirakumaran, M., ... & Maffucci, T. (2019). Downregulation of class II phosphoinositide 3-kinase PI3K-C2β delays cell division and potentiates the effect of docetaxel on cancer cell growth. *Journal of Experimental & Clinical Cancer Research*, *38*(1), 1-19.

Madu, C. O., Wang, S., Madu, C. O., & Lu, Y. (2020). Angiogenesis in breast cancer progression, diagnosis, and treatment. Journal of Cancer, 11(15), 4474.

Ji, P., Gong, Y., Jin, M.-L., Hu, X., Di, G.-H., & Shao, Z.-M. (2020). The Burden and Trends of Breast Cancer From 1990 to 2017 at the Global, Regional, and National Levels: Results From the Global Burden of Disease Study 2017. *Frontiers in Oncology*, *10*, 650.

Clemenceau, A., Michou, L., Diorio, C., & Durocher, F. (2020). Breast cancer and microcalcifications: an osteoimmunological disorder?. *International Journal of Molecular Sciences*, *21*(22), 8613.

Watkins, E. J. (2019). Overview of breast cancer. *Journal of the American Academy of PAs*, *32*(10), 13-17.

Burguin, A., Diorio, C., & Durocher, F. (2021). Breast cancer treatments: updates and new challenges. *Journal of personalized medicine*, *11*(8), 808.

Welsh, J. (2013). Animal models for studying prevention and treatment of breast cancer. In *Animal models for the study of human disease* (pp. 997-1018). Academic Press.

Howell, A., Anderson, A. S., Clarke, R. B., Duffy, S. W., Evans, D. G., Garcia-Closas, M., ... & Harvie, M. N. (2014). Risk determination and prevention of breast cancer. Breast Cancer Research, 16(5), 1-19.

Mohammad, R. M., Muqbil, I., Lowe, L., Yedjou, C., Hsu, H. Y., Lin, L. T., ... & Azmi, A. S. (2015, December). Broad targeting of resistance to apoptosis in cancer. In *Seminars in cancer biology* (Vol. 35, pp. S78-S103). Academic Press.

Fouqué, A., Lepvrier, E., Debure, L., Gouriou, Y., Malleter, M., Delcroix, V., ... & Legembre, P. (2016). The apoptotic members CD95, BclxL, and Bcl-2 cooperate to promote cell migration by inducing Ca 2+ flux from the endoplasmic reticulum to mitochondria. Cell Death & Differentiation, 23(10), 1702-1716.

Peterson, Q. P., Goode, D. R., West, D. C., Botham, R. C., & Hergenrother, P. J. (2010). Preparation of the caspase-3/7 substrate Ac-DEVD-pNA by solution-phase peptide synthesis. nature protocols, 5(2), 294-302.

Chmurska, A., Matczak, K., & Marczak, A. (2021). Two Faces of Autophagy in the Struggle against Cancer. International Journal of Molecular Sciences, 22(6), 2981.

Mohammad, R. M., Muqbil, I., Lowe, L., Yedjou, C., Hsu, H. Y., Lin, L. T., ... & Azmi, A. S. (2015, December). Broad targeting of resistance to apoptosis in cancer. In Seminars in cancer biology (Vol. 35, pp. S78-S103). Academic Press.

Feng, M., Jiang, W., Kim, B. Y., Zhang, C. C., Fu, Y. X., & Weissman, I. L. (2019). Phagocytosis checkpoints as new targets for cancer immunotherapy. Nature Reviews Cancer, 19(10), 568-586.

Rahman, M. M., Sarker, M. T., Alam Tumpa, M. A., Yamin, M., Islam, T., Park, M. N., ... & Kim, B. (2022). Exploring the recent trends in perturbing the cellular signaling pathways in cancer by natural products. *Frontiers in Pharmacology*, *13*, 950109.

Aslam, M. S., Naveed, S., Ahmed, A., Abbas, Z., Gull, I., & Athar, M. A. (2014). Side effects of chemotherapy in cancer patients and evaluation of patients opinion about starvation based differential chemotherapy. Journal of Cancer Therapy, 2014.

Zaid, H., Silbermann, M., Ben-Arye, E., & Saad, B. (2012). Greco-Arab and Islamic herbal-derived anticancer modalities: from tradition to molecular mechanisms. Evidence-Based Complementary and Alternative Medicine, 2012.

Varijakzhan, D., Chong, C. M., Abushelaibi, A., Lai, K. S., & Lim, S. H. E. (2020). Middle Eastern plant extracts: An alternative to modern medicine problems. Molecules, 25(5), 1126.

Barreca, D., Gattuso, G., Bellocco, E., Calderaro, A., Trombetta, D., Smeriglio, A., ... & Nabavi, S. M. (2017). Flavanones: Citrus phytochemical with health-promoting properties. BioFactors, 43(4), 495-506.

Eteraf-Oskouei, T., & Najafi, M. (2022). Uses of natural honey in cancer: An updated review. *Advanced Pharmaceutical Bulletin*, *12*(2), 248.

Miguel, M. G., Antunes, M. D., & Faleiro, M. L. (2017). Honey as a complementary medicine. *Integrative medicine insights*, *12*, 1178633717702869.

Lazarević, K. B., Jovetić, M. S., & Tešić, Ž. L. (2017). Physicochemical parameters as a tool for the assessment of origin of honey. Journal of AOAC International, 100(4), 840-851.

Dżugan, M., Tomczyk, M., Sowa, P., & Grabek-Lejko, D. (2018). Antioxidant activity as biomarker of honey variety. Molecules, 23(8), 2069.

Hadidi, M., Palacios, J. C. O., McClements, D. J., Mahfouzi, M., & Moreno, A. (2023). Alfalfa as a sustainable source of plant-based food proteins. *Trends in Food Science & Technology*.

Dimkić, I., Petrović, M., Gavrilović, M., Gašić, U., Ristivojević, P., Stanković, S., & Janaćković, P. (2020). New perspectives of purple starthistle (Centaurea calcitrapa) leaf extracts: Phytochemical analysis, cytotoxicity and antimicrobial activity. *AMB Express*, *10*(1), 1-21.

Aboul-Soud, M. A., Ennaji, H., Kumar, A., Alfhili, M. A., Bari, A., Ahamed, M., ... & Giesy, J. P. (2022). Antioxidant, anti-proliferative activity and chemical fingerprinting of centaurea calcitrapa against breast cancer cells and molecular docking of caspase-3. *Antioxidants*, *11*(8), 1514.

Abenavoli, L., Izzo, A. A., Milić, N., Cicala, C., Santini, A., & Capasso, R. (2018). Milk thistle (Silybum marianum): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. *Phytotherapy Research*, *32*(11), 2202-2213.

Debbabi, H., El Mokni, R., Chaieb, I., Nardoni, S., Maggi, F., Caprioli, G., & Hammami, S. (2020). Chemical Composition, Antifungal and Insecticidal Activities of the Essential Oils from Tunisian Clinopodium nepeta subsp. nepeta and Clinopodium nepeta subsp. glandulosum. *Molecules*, *25*(9), 2137.

Sakna, S. T., Maghraby, Y. R., Abdelfattah, M. S., & Farag, M. A. (2023). Phytochemical diversity and pharmacological effects of triterpenes from genus Ziziphus: a comprehensive review. *Phytochemistry Reviews*, *22*(6), 1611-1636.

Jaradat, N., Al-Maharik, N., Hawash, M., Qadi, M., Issa, L., Anaya, R., ... & Aboturabi, R. A. (2023). Eucalyptus camaldulensis Dehnh Leaf Essential Oil from Palestine Exhibits Antimicrobial and Antioxidant Activity but No Effect on Porcine Pancreatic Lipase and α-Amylase. *Plants*, *12*(22), 3805.

Shah, N. Z., Muhammad, N., Khan, A. Z., Samie, M., Khan, H., Uddin, G., & Rauf, A. (2013). Phytochemical analysis and antioxidant studies of Conyza bonarensis. *Acad J Plant Sci*, *6*(3), 109-12.

Kaspary, T. E., Waller Barcena, M. E., García, M. A., Cabrera, M., & Hill, S. M. (2023). Conyza bonariensis' Resistance to Glyphosate, Diclosulam, and Chlorimuron: Confirmation and Alternative Control for the First Case of Multiple and Cross-Resistance in Uruguay. *Agronomy*, *14*(1), 79.

Jaafar, N. S., & Jaafar, I. S. (2019). Eruca sativa Linn.: Pharmacognostical and pharmacological properties and pharmaceutical prepara-tions. Asian J Pharm Clin Res, 12(3), 39-45.

Dosoky, N. S., & Setzer, W. N. (2018). Chemical composition and biological activities of essential oils of Curcuma species. Nutrients, 10(9), 1196.

Chenoweth, D. M. (2020). *Chemical Tools for Imaging, Manipulating, and Tracking Biological Systems: Diverse Methods for Prokaryotic and Eukaryotic Systems*. Academic Press.

Mirhosseini, S., Nasiri, A. F., Khatami, F., Mirzaei, A., Aghamir, S. M. K., & Kolahdouz, M. (2024). A digital image colorimetry system based on smart devices for immediate and simultaneous determination of enzyme-linked immunosorbent assays. *Scientific Reports*, *14*(1), 2587.

Kmail, A., Lyoussi, B., Zaid, H., & Saad, B. (2015). In vitro assessments of cytotoxic and cytostatic effects of Asparagus aphyllus, Crataegus aronia, and Ephedra alata in monocultures and

co-cultures of Hepg2 and THP-1-derived macrophages. Pharmacognosy Communications, 5(3), 165.

Battino, M., Giampieri, F., Cianciosi, D., Ansary, J., Chen, X., Zhang, D., ... & Forbes-Hernández, T. (2021). The roles of strawberry and honey phytochemicals on human health: A possible clue on the molecular mechanisms involved in the prevention of oxidative stress and inflammation. *Phytomedicine*, *86*, 153170.

T. Chen, W. Luo, G. Wu, L. Wu, S. Huang, J. Li, J. Wang, X. Hu, W. Huang, G. Liang A novel MyD88 inhibitor LM9 prevents atherosclerosis by regulating inflammatory responses and oxidative stress in macrophages Toxicol. Appl. Pharmacol., 370 (2019), pp. 44-55

Ahmed, S., Sulaiman, S. A., Baig, A. A., Ibrahim, M., Liaqat, S., Fatima, S., ... & Othman, N. H. (2018). Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. *Oxidative medicine and cellular longevity*, *2018*.

Xia, T., Fu, S., Yang, R., Yang, K., Lei, W., Yang, Y., ... & Zhang, T. (2023). Advances in the study of macrophage polarization in inflammatory immune skin diseases.

Mahajna, S., Kadan, S., Tietel, Z., Saad, B., Khasib, S., Tumeh, A., ... & Zaid, H. (2019). In vitro evaluation of chemically analyzed hypericum triquetrifolium extract efficacy in apoptosis induction and cell cycle arrest of the hct-116 colon cancer cell line. Molecules, 24(22), 4139.

Peterson, Q. P., Goode, D. R., West, D. C., Botham, R. C., & Hergenrother, P. J. (2010). Preparation of the caspase-3/7 substrate Ac-DEVD-pNA by solution-phase peptide synthesis. Nature000 protocols, 5(2), 294-302.

Katt, M. E., Placone, A. L., Wong, A. D., Xu, Z. S., & Searson, P. C. (2016). In vitro tumor models: advantages, disadvantages, variables, and selecting the right platform. Frontiers in bioengineering and biotechnology, 4, 12.

Touzani, S., Embaslat, W., Imtara, H., Kmail, A., Kadan, S., Zaid, H., ... & Saad, B. (2019). In vitro evaluation of the potential use of propolis as a multitarget therapeutic product: physicochemical properties, chemical composition, and immunomodulatory, antibacterial, and anticancer properties. BioMed research international, 2019.

Erejuwa, O. O., Sulaiman, S. A., & Wahab, M. S. A. (2014). Effects of honey and its mechanisms of action on the development and progression of cancer. *Molecules*, *19*(2), 2497-2522.

López, E. P. F., García, F. G., & Jornet, P. L. (2019). Combination of 5-Florouracil and polyphenol EGCG exerts suppressive effects on oral cancer cells exposed to radiation. *Archives of oral biology*, *101*, 8-12.

Chung, S. S., Dutta, P., Austin, D., Wang, P., Awad, A., & Vadgama, J. V. (2018). 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*, *9*(68), 32943.

Riahi-Chebbi, I., Souid, S., Othman, H., Haoues, M., Karoui, H., Morel, A., ... & Essafi-Benkhadir, K. (2019). The Phenolic compound Kaempferol overcomes 5-fluorouracil resistance in human resistant LS174 colon cancer cells. Scientific Reports, 9(1), 195.

Martin, T. A., Ye, L., Sanders, A. J., Lane, J., & Jiang, W. G. (2013). Cancer invasion and metastasis: molecular and cellular perspective. In *Madame Curie Bioscience Database [Internet]*. Landes Bioscience.

Thompson, E. W., & Price, J. T. (2002). Mechanisms of tumour invasion and metastasis: emerging targets for therapy. *Expert opinion on therapeutic targets*, *6*(2), 217-233.

Orsolic, N., & Basic, I. (2004). ANTIMETASTATIC EFFECT OF HONEY. *Mellifera*, *4*(7).

Reddy, R. A., & Kumar, R. S. (2023). Matrix Metalloproteinase-2 (MMP-2): As an Essential Factor in Cancer Progression. *Recent Patents on Anti-cancer Drug Discovery*.

Ho, H. H., Chang, C. S., Ho, W. C., Liao, S. Y., Wu, C. H., & Wang, C. J. (2010). Anti-metastasis effects of gallic acid on gastric cancer cells involves inhibition of NF-κB activity and downregulation of PI3K/AKT/small GTPase signals. *Food and Chemical Toxicology*, *48*(8-9), 2508-2516.

Batumalaie, K., Zaman Safi, S., Mohd Yusof, K., Shah Ismail, I., Devi Sekaran, S., & Qvist, R. (2013). Effect of gelam honey on the oxidative stress-induced signaling pathways in pancreatic hamster cells. *International Journal of Endocrinology*, *2013*.

Hussein, S. Z., Mohd Yusoff, K., Makpol, S., & Mohd Yusof, Y. A. (2013). Gelam honey attenuates carrageenan-induced rat paw inflammation via NF-κB pathway. *PLoS One*, *8*(8), e72365.

Moskwa, J., Borawska, M. H., Markiewicz-Zukowska, R., Puscion-Jakubik, A., Naliwajko, S. K., Socha, K., & Soroczynska, J. (2014). Polish natural bee honeys are anti-proliferative and anti-metastatic agents in human glioblastoma multiforme U87MG cell line. *PloS one*, *9*(3), e90533.

Majtan, J., Bohova, J., Garcia-Villalba, R., Tomas-Barberan, F. A., Madakova, Z., Majtan, T., ... & Klaudiny, J. (2013). Fir honeydew honey flavonoids inhibit TNF-α-induced MMP-9 expression in human keratinocytes: a new action of honey in wound healing. *Archives of dermatological research*, *305*, 619-627.

Vijayababu, M. R., Arunkumar, A., Kanagaraj, P., Venkataraman, P., Krishnamoorthy, G., & Arunakaran, J. (2006). Quercetin downregulates matrix metalloproteinases 2 and 9 proteins expression in prostate cancer cells (PC-3). *Molecular and cellular biochemistry, 287*, 109-116.

Jaganathan, S. K., & Mandal, M. J. J. A. (2009). Honey constituents and their apoptotic effect in colon cancer cells. *Journal of Apiproduct and Apimedical Science*, *1*(2), 29-36.

Samarghandian, S., Farkhondeh, T., & Samini, F. (2018). Honey and health: A review of recent clinical research. Pharmacognosy Research, 9 (2), 121–127.

Eteraf-Oskouei, T., & Najafi, M. (2022). Uses of natural honey in cancer: An updated review. *Advanced Pharmaceutical Bulletin*, *12*(2), 248.

Masad, R. J., Haneefa, S. M., Mohamed, Y. A., Sbiei, A. A., Bashir, G., & Cabezudo, M. J. The immunomodulatory effects of honey and associated flavonoids in cancer. Nutrients. 2021; 13 (4): 1269.

Hapach, L. A., Wang, W., Schwager, S. C., Pokhriyal, D., Fabiano, E. D., & Reinhart-King, C. A. (2023). Phenotypically sorted highly and weakly migratory triple negative breast cancer cells exhibit migratory and metastatic commensalism. Breast Cancer Research, 25(1), 102.

Radstake, W. E., Gautam, K., Van Rompay, C., Vermeesen, R., Tabury, K., Verslegers, M., ... & Baselet, B. (2023). Comparison of in vitro scratch wound assay experimental procedures. Biochemistry and Biophysics Reports, 33, 101423.

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, *22*(7), 3380.

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. *Molecules*, *27*(4), 1326.

Nicewicz, A. W., Nicewicz, Ł., & Pawłowska, P. (2021). Antioxidant capacity of honey from the urban apiary: A comparison with honey from the rural apiary. *Scientific Reports*, *11*(1), 9695.

Stagos, D., Soulitsiotis, N., Tsadila, C., Papaeconomou, S., Arvanitis, C., Ntontos, A., ... & Mossialos, D. (2018). Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. *International journal of molecular medicine*, *42*(2), 726-734.

Saad, B., Embaslat, W. H., Abu-Farich, B., Mahajna, S., & Azab, M. (2016). Hypericum triquetrifolium extracts modulate IL-6, IL-10 and TNF-α protein and mRNA expression in LPS-

activated human peripheral blood mononuclear cells and THP-1-derived macrophages. *Med. Aromat. Plants S, 3*, 2167-0412.

Kianmehr, M., Behdadfard, M., Hedayati-Moghadam, M., & Khazdair, M. R. (2023). Effects of Herbs and Derived Natural Products on Lipopolysaccharide-Induced Toxicity: A Literature Review. *Oxidative Medicine and Cellular Longevity*, *2023*.

Park, J. H., Hwang, J. W., Lee, H. J., Jang, G. M., Jeong, Y. J., Cho, J., ... & Hoe, H. S. (2023). Lomerizine inhibits LPS-mediated neuroinflammation and tau hyperphosphorylation by modulating NLRP3, DYRK1A, and GSK3α/β. *Frontiers in Immunology*, *14*.

ملخص

السرطان مرض معقد ينتشر بشكل كبير في جميع أنحاء العالم، ويعتبر سرطان الثدي السبب الرئيسي للوفاة بين جميع أنواع السرطان الأخرى. يتم استخدام العديد من العلاجات حاليًا في إدارة هذا المرض، ولكنها ترتبط بآثار جانبية خطيرة متعددة. ومن ثم، فإن البحث عن الطب البديل المعتمد على الأعشاب كمصدر للمركبات النشطة بيولوجيًا ذات القيمة العالية مع الحد الأدنى من الآثار الجانبية هو موضع تقدير كبير. استنادا إلى الأدبيات المنشورة، تم تقييم آثار عينات العسل الأزهار (المسمى PH1 إلى PH9) في هذه الدراسة المقترحة في المختبر. باستخدام خط خلايا سرطان الثدي البشري MDA، تبحث الدراسة في التأثيرات المحتملة المضادة للسرطان لعينات العسل. تم استخدام اختبار MTT لتقييم آثارها السامة للخلايا وتثبيط الخلايا. وعلاوة على ذلك، تم تقييم التأثير المضاد للهجرة باستخدام مقايسة الخدش. ولم يلاحظ أي تأثيرات سامة للخلايا في أي من العينات التي تم اختبارها في جميع التراكيز. وكما أظهرت أنواع العسل التي تم تحديدها على أنها PH2 (مورار)، وPH3 (خورفش)، وPH5 (سدر)، وPH6 (كينا) نشاطًا مثبطًا للخلايا على خلايا MDA، مما أدى إلى انخفاض في قابلية الخلية للنمو بنسبة تصل إلى 43٪ عند تركيز 4 ملجم / مل. مقارنة بخلايا التحكم غير المعالجة. علاوة على ذلك، تشير البيانات إلى أن معدل هجرة خلايا MDA ينخفض بشكل ملحوظ بعد العلاج بـ PH2 وPH3 وPH5 وPH6 وPH7 مقارنة بالخلايا غير المعالجة ($P < 0.05$). على وجه التحديد، خفضت PH2 وPH5 وPH6 هجرة خلايا MDA بنسبة 90% و80% و72% على التوالي، مقارنة بخلايا التحكم. بالإضافة إلى ذلك، تم اختبار تقييم التأثيرات المضادة للالتهابات للعينات عن طريق قياس إفراز أكسيد النيتريك (NO) من البلاعم المشتقة من THP-1 المنشط بـ LPS. أظهرت النتائج أن خورفش والسدر والكينا جبلي والأغوار خفضت معدل إنتاج أكسيد النيتروجين بشكل ملحوظ. ويركز

التقييم أيضًا على قياس مضادات الأكسدة (نشاط الكسح القائم على DPPH). تراوحت قيم IC50 لمعادلة الجذور الحرة DPPH من 0.7 ميكروجرام/مل إلى 2.87 ميكروجرام/مل. تشير النتائج التي تم الحصول عليها إلى أن التأثيرات المثبطة للخلايا والمضادة للهجرة قد تساهم في فوائد مضادة للسرطان. تعتبر التحقيقات المستقبلية الإضافية ضرورية للكشف عن الآليات الجزيئية ودور المواد الكيميائية النباتية المعزولة في النتائج التي تم الحصول عليها. هذه خطوة مهمة في تطوير الأدوية المضادة للسرطان المعتمدة على الأعشاب.