scientific reports

Check for updates

OPEN

Novel antiluminal breast cancer and immunological activities of Sidr and SidrZamZam honeys and their biogenic AgNPs

Hamed A. Ghramh[1,2,3], Essam H. Ibrahim[2,3,4], Amr Ahmed El-Arabey[2,5,6]✉ & Khalid Ali Khan[2,5]

Honey and its derivatives are extensively acknowledged for their diverse biological attributes. Sidr honey, a specific type of monofloral honey, is obtained from the nectar of the Sidr tree (*Ziziphus spina-christi*). Muslims believe that water from the Zamzam well has the potential to treat a wide range of diseases. Across the globe, millions utilize Zamzam water, which is characterized by its naturally alkaline properties. SidrZamZam honey represents an innovative variant of honey, fundamentally akin to Sidr honey, with the notable difference being that bees are supplied with Zamzam water as their hydration source. The objective of this research was to evaluate the capacity of two distinct varieties of honey to produce silver nanoparticles (AgNPs), examine their antibacterial properties, investigate their impact on immune cells, and assess their anticancer efficacy against luminal A breast cancer (MCF-7). The findings indicated that both Sidr and SidrZamZam honeys possessed the ability to synthesize varying sizes of silver nanoparticles (AgNPs). In this study, AgNPs synthesized by both honeys and incorporated within them demonstrated significant antibacterial properties. In addition, the two varieties of honey, when evaluated independently, displayed anticancer effects against the MCF-7 cancer cell line; however, this was not the case when these honeys were combined with AgNPs. Additionally, both varieties of honey facilitated the proliferation of immune cells. In this context, our bioinformatic analyses demonstrate that several immune cell types exert a clinically significant influence on patients with luminal-A breast cancer. These immune cell types include $CD8^+$ T cells, regulatory T cells (T_{regs}), neutrophils, M0 macrophages, M1 macrophages, M2 macrophages, resting myeloid dendritic cells, resting mast cells, and follicular helper T cells. Therefore, these immune cell subsets represent promising targets for the treatment of luminal-A breast cancer utilizing both varieties of honey. In conclusion, Sidr and SidrZamZam honeys may serve as a source for the environmentally-friendly synthesis of AgNPs. Furthermore, both honey types have the potential to enhance immune cell activity and can function as immunomodulators. Besides, both honeys may hold promise in the combat against breast cancer, pending further research.

Keywords ZamZam, Honey, Sidr, MCF-7, Silver nanoparticles, Immune cells

Sidr honey has been valued in traditional medicine for its therapeutic properties, containing various chemical constituents like sugars, enzymes, acids, proteins, flavonoids, and minerals[1]. Its composition varies based on floral origin, geography, and honeybee species[2]. Saudi Arabia, a notable source of monofloral honey, produces *ziziphus* honey, also known as Sidr honey[3]. Derived from *Ziziphus nummularia*, *Z. mucronata*, and *Z. spina-christi*[4], this honey exhibits medicinal properties[5]. Research has linked its bioactive compounds to antioxidant, anti-inflammatory, and antitumor effects, particularly through polyphenols and flavonoids that inhibit tumorigenesis[6]. Specifically, compounds like carvacrol and thymol contribute to its anticancer potential[7].

[1]Central Labs, King Khalid University, AlQura'a, P.O. Box 960, Abha, Saudi Arabia. [2]Center of Bee Research and Its Products, King Khalid University, P.O. Box 9004, 61413 Abha, Saudi Arabia. [3]Biology Department, Faculty of Science, King Khalid University, P.O. Box 9004, 61413 Abha, Saudi Arabia. [4]Blood Products Quality Control and Research Department, National Organization for Research and Control of Biologicals, Cairo, Egypt. [5]Applied College, King Khalid University, P.O. Box 9004, 61413 Abha, Saudi Arabia. [6]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University, Cairo 11751, Egypt. ✉email: ph.amrcapa@gmail.com

The importance of water in living organisms is critical, and honey typically contains 16–20% moisture. Zamzam water, sourced from a well in Makkah, Saudi Arabia, is believed to have unique curative properties and has been in use for around 4000 years[8]. This water's mineral composition, including calcium, sodium, potassium, and magnesium, differs significantly from other waters, and it is safe for consumption as toxic elements are below harmful levels.

The application of nanotechnology in food safety and biomedicine has increased[9]. Nanoparticles, especially silver nanoparticles, are notable for their applications in healthcare. They aid in diagnostics, drug delivery, and anticancer therapies[10]. Green synthesis using natural products for nanoparticle production is gaining popularity, as it is more cost-effective and sustainable[11]. Honey has shown synergistic effects with silver nanoparticles against infections, encouraging combined use[12].

Globally, cancer remains a pressing healthcare challenge, with breast cancer as the most common malignancy and a leading cause of cancer death among women. In Saudi Arabia, it constitutes 19.8% of cancer cases, predominantly the luminal A subtype[13,14]. Despite advancements in prevention and treatment, challenges remain in eradicating the disease, influenced by the tumor microenvironment, which includes various cell types crucial for tumor progression[15].

Exploring complementary and alternative medicines could unveil novel therapeutic molecules. Honey, a prominent source of bioactive phytochemicals, shows promise in cancer therapy[16].

This study focused on comparing the biological activities of two honey types and their synthesized silver nanoparticles. *Ziziphus* honey samples and SidrZamZam honey samples (where honeybees were fed with Zamzam water) were sourced from King Khalid University. Further, bioinformatic analyses were performed on immune cells within the tumour microenvironment of luminal-A breast cancer patients to identify prospective treatment targets.

Results

Production and characterization of nano silver

The assessment of nano silver synthesis was performed by monitoring the color changes in the mixtures of $AgNO_3$ with Sidr honey (Fig. 1A) and $AgNO_3$ with SidrZamZam honey (Fig. 1C). Following the observation of these color changes, spectrophotometric analysis was executed to evaluate the formation of AgNPs. The results from the reaction involving $AgNO_3$ and Sidr honey produced significant observations, indicating the presence of a distinct peak region indicative of AgNPs (Fig. 1B). This peak region was identified within the wavelength range of 380–450 nm, with the peak intensity reaching its maximum at 408 nm. Conversely, the analysis of the $AgNO_3$/SidrZamZam honey mixture also revealed a distinct peak associated with AgNPs (Fig. 1D), observed within the wavelength range of 360–460 nm, with the peak intensity occurring at 407 nm.

Furthermore, investigations conducted using scanning electron microscopy (SEM) demonstrated that the AgNPs derived from both Sidr honey and SidrZamZam honey exhibited a nearly spherical morphology (Fig. 2). The average particle size of the AgNPs was determined to be 72 nm for Sidr honey (Fig. 2A) and 82 nm for SidrZamZam honey (Fig. 2B). The SEM images further indicated the presence of agglomerations of AgNPs.

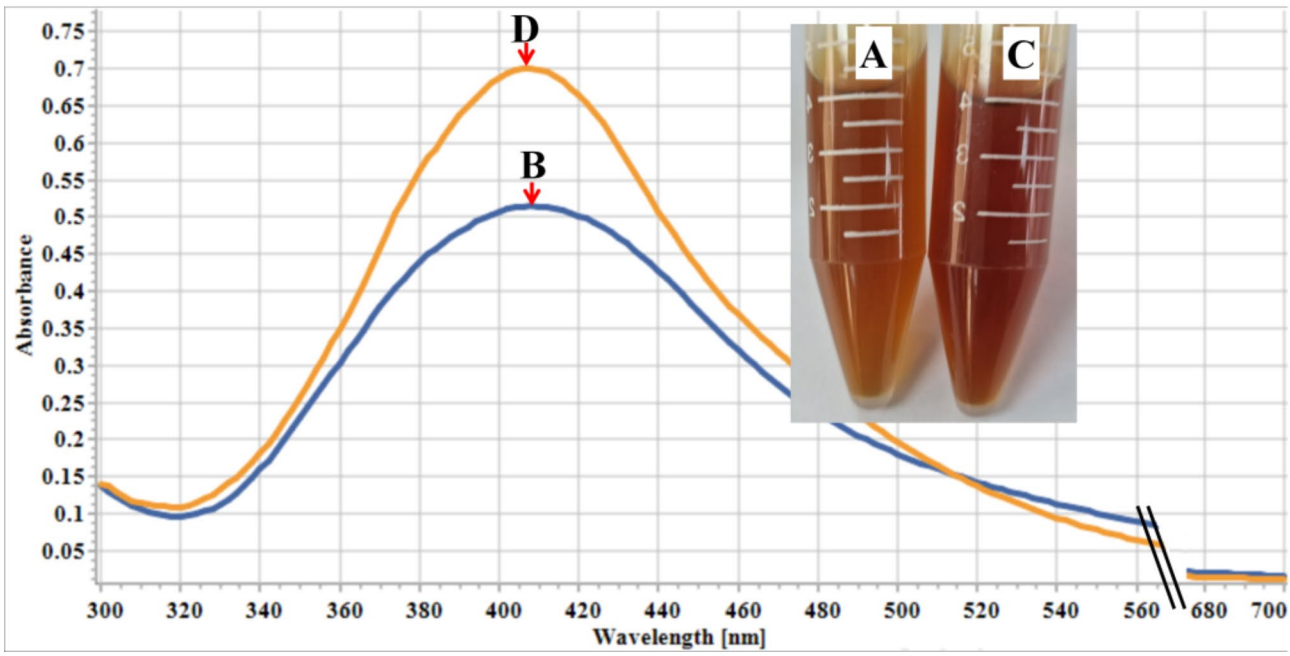

Fig. 1. Monitoring of AgNPs synthesis. (**A**) Change in color of 40% Sidr honey; (**B**) light absorbance of 40% Sidr honey containing AgNPs; (**C**) Change in color of 40% SidrZamZam honey; (**D**) light absorbance of 40% SidrZamZam honey containing AgNPs.

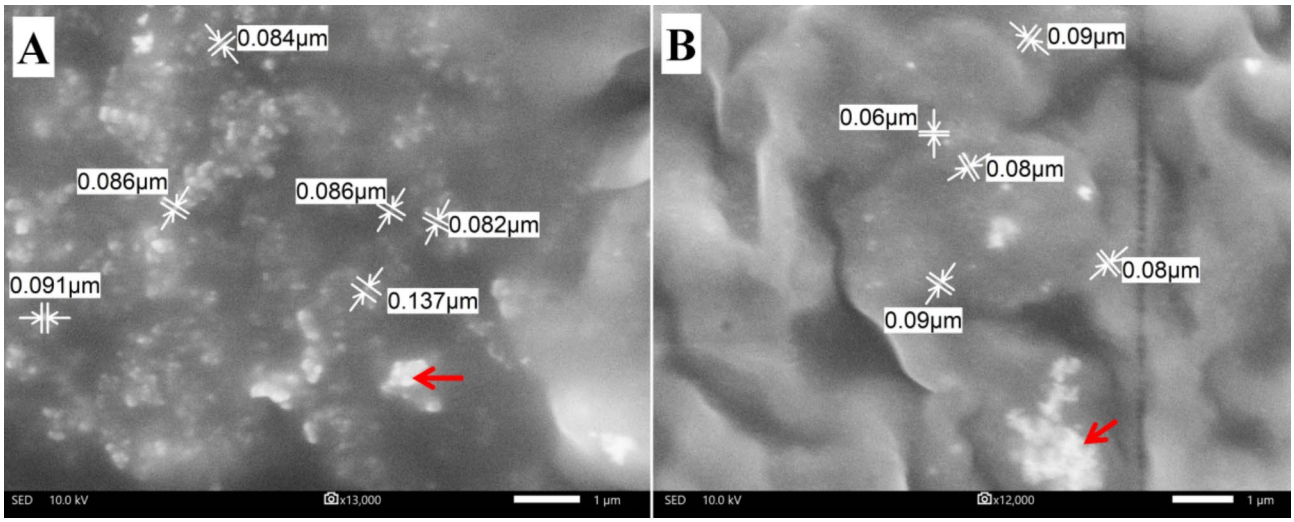

Fig. 2. SEM analysis of AgNPs. (**A**) AgNPs synthesized by Sidr honey; (**B**) AgNPs synthesized by SidrZamZam honey; and Red arrows indicate AgNPs agglomerations.

	Clearance zone (mm^2)													
	Sidr-NPs (%)						SidrZam-NPs						Positive control	
	40	20	10	5	2.5	1.25	40	20	10	5	2.5	1.25	Cefalexin	Amoxicillin
P. aeruginosa	11^a	10^a	7^b	6^b	0	0	21	17	11	0	0	0	20	25
	Clearance zone (mm^2)													
	Sidr-NPs (%)						SidrZam-NPs						Positive control	
	40	20	10	5	2.5	1.25	40	20	10	5	2.5	1.25	Cefalexin	Amoxicillin
E. Coli	10^c	11^c	12^c	9^c	0	0	22	17	12^c	8^d	7^d	0	22	18
	Clearance zone (mm^2)													
	Sidr-NPs (%)						SidrZam-NPs						Positive control	
	40	20	10	5	2.5	1.25	40	20	10	5	2.5	1.25	Cefalexin	Amoxicillin
St. aureus	26^e	17	14	7f	0	0	15	10	9	7f	0	0	25	14
	Clearance zone (mm^2)													
	Sidr-NPs (%)						SidrZam-NPs						Positive control	
	40	20	10	5	2.5	1.25	40	20	10	5	2.5	1.25	Cefalexin	Amoxicillin
B. subtilis	23^g	12^h	11^h	10^h	11^h	0	10^h	11^h	8	0	0	0	27	12

Table 1. Antibacterial potential of different dilution of Sidr + AgNPs and SidrZamZam + AgNPs. NB: All organisms were treated with different concentrations of the two honey types, significant differences were calculated for each organism separately.

Antimicrobial potentiality

The current investigation sought to assess the antimicrobial effectiveness of Sidr honey and SidrZamZam honey either alone or in combination with AgNPs, against four bacterial strains, specifically *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus subtilis*. The inhibition zones generated by both honey varieties were measured in millimeters, as presented in Table 1.

The honey samples consisted of six distinct concentrations, utilized either independently or in conjunction with AgNPs. Neither type of honey alone demonstrated antibacterial properties; however, both exhibited antibacterial activity when augmented with AgNPs. The two antibiotics used as positive controls, Cefalexin and Amoxicillin, exhibited notable antibacterial efficacy. The results revealed that Sidr honey containing AgNPs displayed a significant antibacterial effect, particularly against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, in contrast to Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli*. Conversely, SidrZamZam honey, when combined with AgNPs, exhibited a stronger antibacterial response against Gram-negative bacteria than against Gram-positive bacteria. The antibacterial properties demonstrated by both honey types were analogous to those observed in the positive control samples.

Effects on immune cells

Here, the objective of the study was to evaluate the efficacy of Sidr honey, Sidr honey + AgNPs, SidrZamZam honey, and SidrZamZam honey + AgNPs in modulating the division of splenic cells. Splenic cells were utilized to represent two distinct functionalities: the first being as a control for normal cells, and the second as rapidly dividing cells. The assessment was conducted by determining the average percentage of cell stimulation or inhibition. Cell stimulation serves as a measurable parameter that reflects the ability of a substance to promote the proliferation and division of spleen-derived cells in a controlled laboratory setting. An increase in cellular stimulation is indicative of a stronger stimulatory effect, whereas a decline suggests either a reduced stimulatory response or the potential for cytotoxic effects. The results revealed variability in mean cell stimulation, which was influenced by the concentration and type of honey sample utilized (Fig. 3).

All honey preparations demonstrated stimulatory effects on immune cells harvested from rat spleens. Sidr honey (Fig. 3A) showed a significant ($p < 0.001$) dose response effects on normal splenic cells, where the growth stimulation effect decreased when the honey concentration decreased. The same trend was clear in Sidr honey containing AgNPs (Fig. 3A). At each honey concentration, Sidr honey containing AgNPs showed a significantly ($p < 0.001$) higher cell growth stimulation than Sidr honey alone. Also, SidrZamZam honey and SidrZamZam honey + AgNPs showed a significant ($p < 0.001$) dose response effects on growth of normal splenic cells (Fig. 3B). Notably, the SidrZamZam honey solution containing AgNPs exhibited a significant ($p < 0.001$) capacity to stimulate cell activity at 40% concentration than that SidrZamZam honey alone (Fig. 4).

Inter-group comparisons showed a significant variability among different splenic cells treatment as indicated in Fig. 4. However, it is critical to acknowledge that the 5% dilution of SidrZamZam honey did not exhibit any observable effects on splenic cells, lacking both stimulating and inhibiting activities. We conducted pathway and cell type enrichment analyses against proteins that were significantly expressed within splenic tissue by employing a curated tissue protein expression dataset. Our findings from the Elsevier pathway collection revealed that these proteins were significantly integrated into numerous essential pathways associated with immunity, inflammation, and oncogenesis (see Table 2).

Next, we performed cell type enrichment analysis using PanglaoDB's upgraded databases, which revealed significant similarities between these proteins and numerous immune cell types such as macrophages, B cells, dendritic cells, and monocytes (see Table 3).

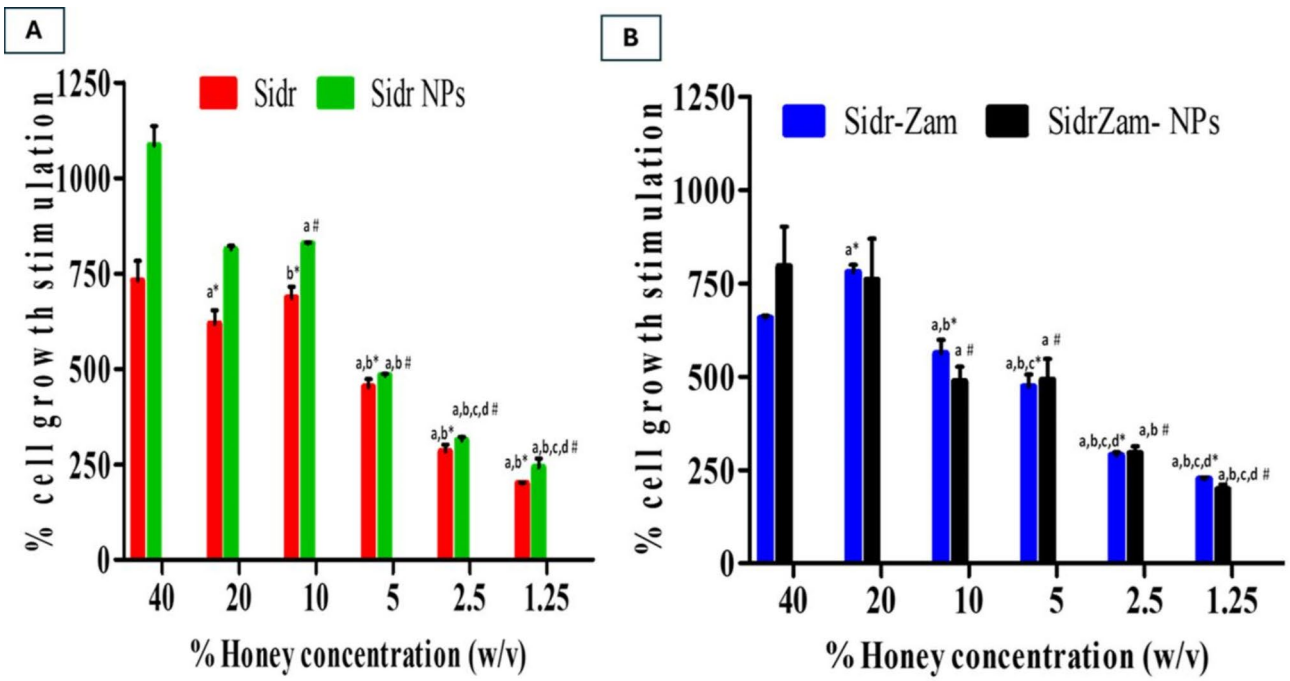

Fig. 3. Mean cell stimulation (%) of normal splenic cells treated with different concentrations of honey samples. Where Sidr: Sidr (*Ziziphus spina-christi*) honey; Sidr NPs: Sidr honey containing AgNPs; SidrZam: SidrZamZam honey; SidrZam NPs: SidrZamZam honey containing AgNPs. Different letters (a,b,c,d) above the columns indicate significant differences between the different concentrations in each group: a significance versus concentration 40, b significance versus concentration 20, c significance versus concentration 10, d significance versus concentration 5, *significance within Sidr group or Sidr-Zam group, # significance within Sidr NPs group or Sidr-Zam-NPs group. Letters (a,b,c,d) indicate significant differences at $p < 0.05$.

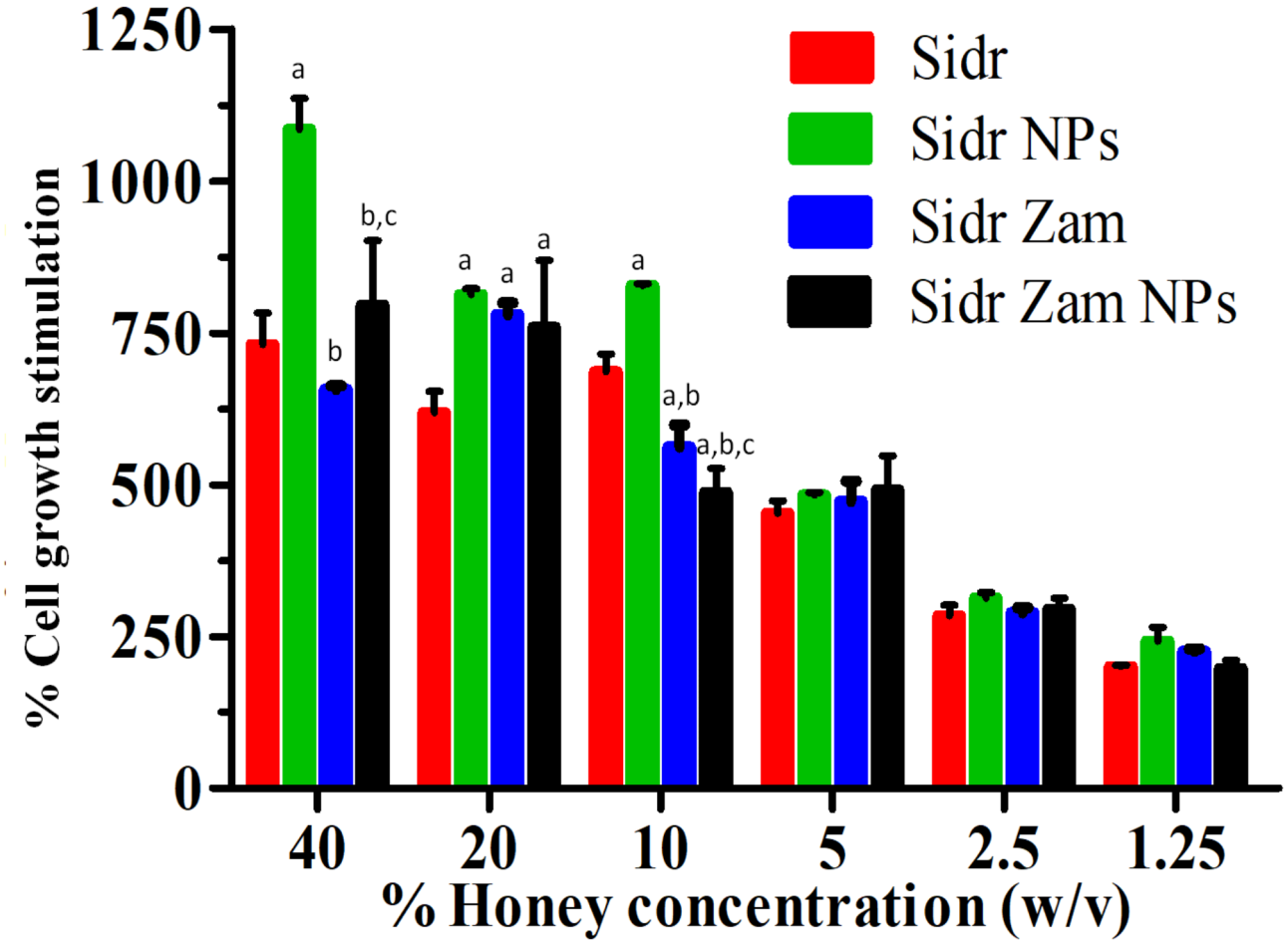

Fig. 4. Mean cell stimulation (%) of normal splenic cells treated with different concentrations of honey samples. Where Sidr: Sidr (*Ziziphus spina-christi*) honey; Sidr NPs: Sidr honey containing AgNPs; SidrZam: SidrZamZam honey; SidrZam NPs: SidrZamZam honey containing AgNPs. Error bars represent standard deviation. The letters (a,b,c) above the columns denote significant changes within each concentration: a versus Sidr; b versus Sidr NPs; c versus Sidr Zam. Letters (a,b,c) indicate significant differences at $p < 0.05$.

Index	Name	*P*-value	Adjusted p-value
1	T-Cell Independent B-Cell Activation	7.295E−10	5.618E−7
2	NFKB Canonical Signaling Activation in Cancer	9.194E−10	5.618E−7
3	NF-kB Canonical Signaling	2.509E−9	0.000001022
4	NFKB Signaling Activation by Blocking of Tumor Suppressors	5.156E−8	0.00001575
5	TLR4—> IRF Signaling	1.041E−7	0.00002543
6	B-Cell Chronic Lymphocytic Leukemia	2.095E−7	0.00004267
7	Diffuse Large-B-Cell Lymphoma	7.691E−7	0.0001214
8	Mucosa-Associated Lymphoid Tissue (MALT) Lymphoma	7.947E−7	0.0001214
9	mTOR/NF-kB/BCR Signaling Disregulation in Mantle Cell Lymphoma	9.648E−7	0.0001310
10	Toll-like Receptors in Sterile Inflammation	0.000001168	0.0001427

Table 2. Pathway enrichment study of spleen protein expression using the Elsevier pathway collection. * The symbol "E" represents "*10^ ". The adjusted p value is computed using the Benjamini–Hochberg method for correction for multiple hypotheses testing.

Effects on cancer cell lines

The cytotoxic effects of Sidr honey, Sidr honey combined with silver AgNPs, SidrZamZam honey, and SidrZamZam honey combined with AgNPs displayed variability contingent upon the concentration, as illustrated in Fig. 5.

Index	Name	*P* value	Adjusted *p* value
1	Macrophages	5.540E−25	5.462E−23
2	B Cells Naive	6.661E−25	5.462E−23
3	B Cells Memory	2.306E−24	1.261E−22
4	Dendritic Cells	1.312E−23	5.379E−22
5	B Cells	1.505E−21	4.937E−20
6	Kupffer Cells	1.536E−18	4.198E−17
7	Microglia	2.496E−18	5.847E−17
8	Langerhans Cells	4.145E−18	8.498E−17
9	Monocytes	9.280E−17	1.691E−15
10	Plasmacytoid Dendritic Cells	1.745E−15	2.862E−14

Table 3. Cell types enrichment study of spleen protein expression using the PanglaoDB upgraded databases: *The symbol "E" represents "*10^ ". The adjusted *p* value is computed using the Benjamini–Hochberg method for correction for multiple hypotheses testing.

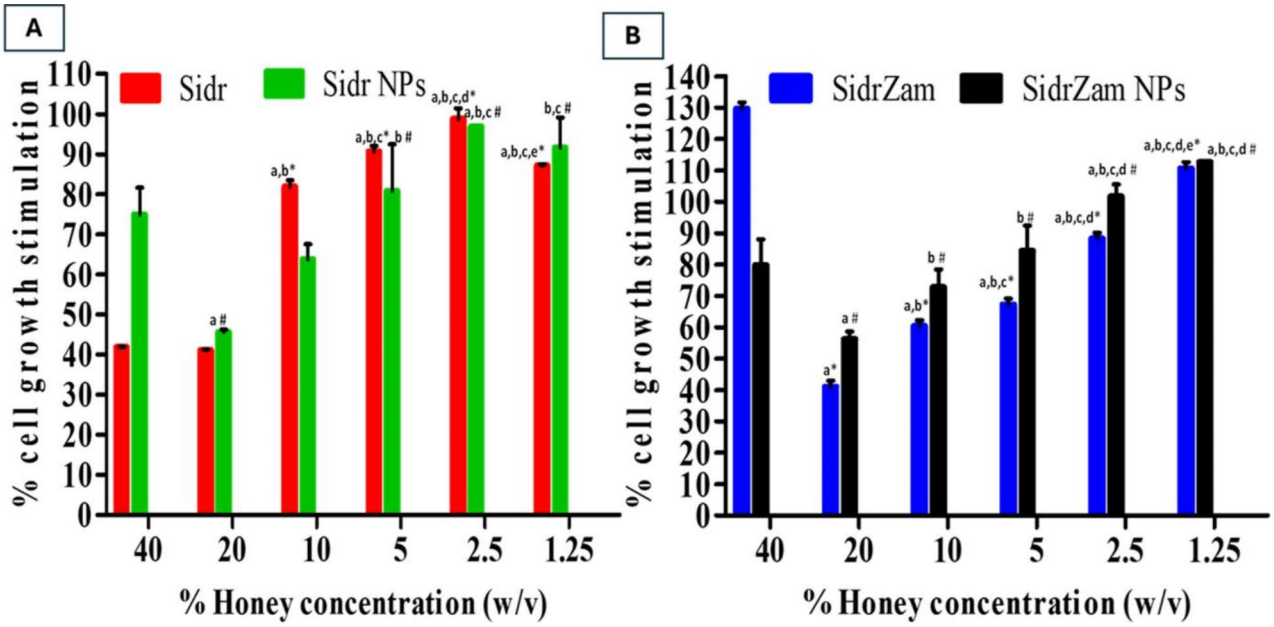

Fig. 5. Mean cell growth stimulation (%) of MCF-7 cells treated with different concentrations of honey samples. Where Sidr: Sidr (*Ziziphus spina-christi*) honey; Sidr NPs: Sidr honey containing AgNPs; SidrZam: SidrZamZam honey; SidrZam NPs: SidrZamZam honey containing AgNPs. Error bars represent standard deviation. The letters (a,b,c,d,e) above the columns show significant differences between the varied concentrations in each group: a Significance versus concentration 40, b Significance versus concentration 20, c Significance versus concentration 10, d Significance versus concentration 5, e Significance versus concentration 2.5, * Significance within the Sidr or Sidr-Zam groups, # Significance within the Sidr NPs or Sidr-Zam-NPs groups. Letters (a,b,c,d,e) indicate significant differences at $p < 0.05$.

Sidr honey exhibited an inhibitory effect on the proliferation of MCF-7 cells, which was found to be significantly reduced ($p < 0.001$) in correlation with a decrease in the concentration of Sidr honey (see Fig. 5A). Inclusion of AgNPs in Sidr honey significantly ($p < 0.001$) reduced its growth-inhibitory effect at a concentration of 40%. Both Sidr honey and the combination of Sidr honey with AgNPs displayed diminished inhibitory effects on cell growth as the concentration of honey decreased. When comparing the inhibitory effects of Sidr honey and Sidr combined with AgNPs at concentrations of 20–1.25%, their effects were found to be comparable (see Fig. 6).

SidrZamZam honey showed cell growth stimulatory effect on MCF-7 cells (Fig. 5B) at 40% concentration, but upon dilution to 20% concentration, SidrZamZam honey turned into MCF-7 cell growth inhibitory factor. This cell growth inhibitory effect decreased with the decrease of honey concentration. In contrast, the application of SidrZamZam honey combined with AgNPs at a 40% concentration resulted in a noteworthy inhibition in cellular division (Fig. 5B), which increased upon honey dilution to 20%. The inhibitory cell division effect of SidrZamZam honey + AgNPs decreased upon further dilution.

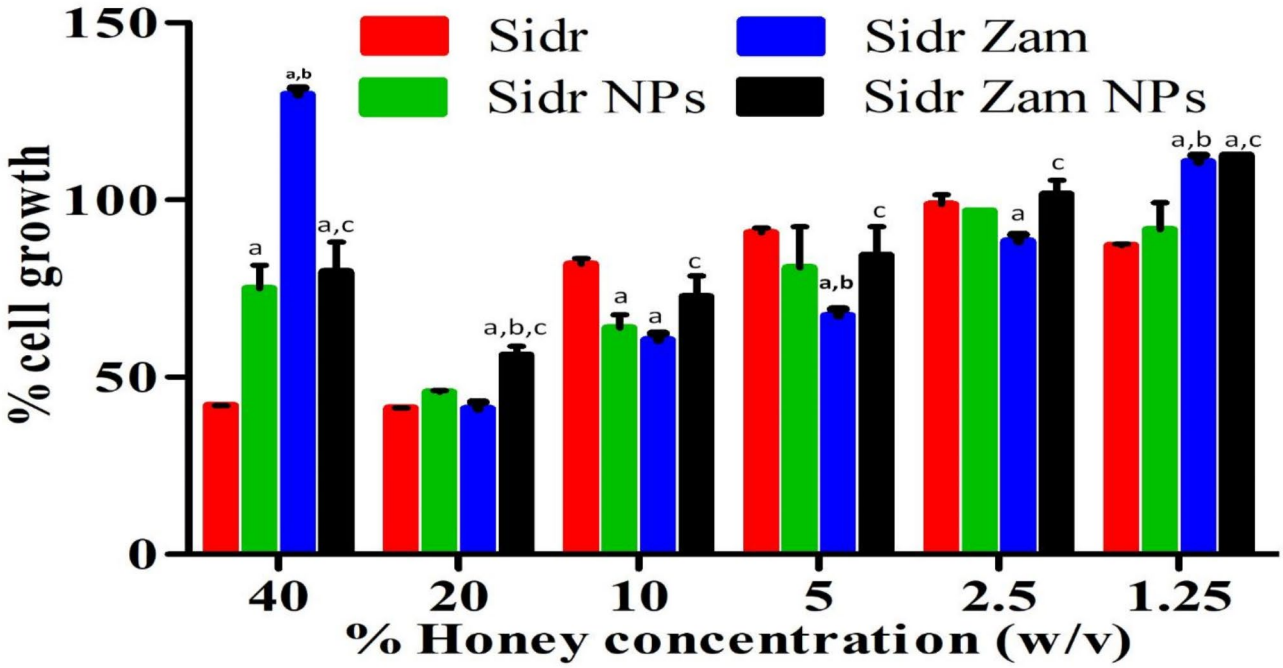

Fig. 6. Mean cell growth stimulation (%) of MCF-7 cells treated with different concentrations of honey samples. Where Sidr: Sidr (*Ziziphus spina-christi*) honey; Sidr NPs: Sidr honey containing AgNPs; SidrZam: SidrZamZam honey; SidrZam NPs: SidrZamZam honey containing AgNPs. Error bars represent standard deviation. The initials (a,b,c) above the columns represent significant differences within each concentration: a versus Sidr; b versus Sidr NPs; and c versus Sidr Zam. Letters (a,b,c) indicate significant differences at $p < 0.05$.

The LogIC50 for Sidr honey was estimated to be approximately 0.09506 (IC50 = 13.12 to 44.22), the LogIC50 for Sidr honey + AgNPs was estimated to be approximately 0.2343 (IC50 = 0.4890 to 9.772). The LogIC50 for SidrZamZam was approximately 3.738 (IC50 = 1.004e−010 to 5.674e+010) and the LogIC50 for the SidrZamZam combined with AgNPs was approximately 1.985 (IC50 = 8.058 to 8.482e+011).

Our bioinformatic analysis of The Cancer Genome Atlas (TCGA) data utilizing TIMER2.0 elucidated the clinical significance of various immune cell subsets within the tumor microenvironment of luminal-A breast cancer. This analysis encompassed CD8 + T cells, regulatory T cells (Tregs), neutrophils, and three macrophage types (M0, M1, and M2), along with resting myeloid dendritic cells, resting mast cells, and T follicular helper cells (refer to Fig. 7A–I). Likewise, when incorporating race as a clinical factor, our findings indicated that these same immune cell types maintained a clinically significant impact, albeit with varying p values (see Fig. 8A–I).

Conversely, when examining age as a clinical variable, our results indicated that Tregs, neutrophils, M0 macrophages, M1 macrophages, resting mast cells, and T follicular helper cells exhibited a substantial clinical impact on the survival of patients with luminal A breast cancer (refer to Fig. 9A–F). With respect to clinical staging, only five immune cell types were found to exert a clinical influence on the survival outcomes of luminal A breast cancer patients (see Fig. 10A–E). Ultimately, our investigation identified four immune subsets that demonstrated a clinically relevant impact on the purity of luminal A breast cancer patients: CD8 + T cells, neutrophils, resting myeloid dendritic cells, and T follicular helper cells (refer to Fig. 11A–D).

In contrast, M2 macrophage-like mast cells exhibited no clinical influence concerning age or purity, and M1 macrophages showed minimal clinical relevance with respect to stage or purity. Furthermore, in terms of purity, M0 macrophage-like Tregs demonstrated no significant therapeutic advantage, and T follicular helper cells did not display clinically relevant influence regarding stage (see Figs. 12 and 13).

Honey's sugars content

In Sidr honey, the concentrations of fructose and glucose were identified as 38% and 28%, respectively, while the levels of sucrose and maltose were observed to be comparatively low, at 4% and 1%, respectively, as presented in Table 4.

In SidrZamZam honey, the concentrations of fructose and glucose were determined to be 37.88% and 36.25%, respectively. Conversely, the proportions of sucrose and maltose were found to be relatively low, recorded at 5.03% and 0% respectively, as illustrated in Table 4. Glucose concentration was significantly (< 0.001) higher in SidrZamZam honey than Sidr honey. Maltose sugar was absent in SidrZamZam honey.

Discussion

In the present investigation, two distinct types of honey were employed in the production of AgNPs. The synthesis procedure was visually observed through changes in color, and optically monitored by doing UV/Vis scanning[17].

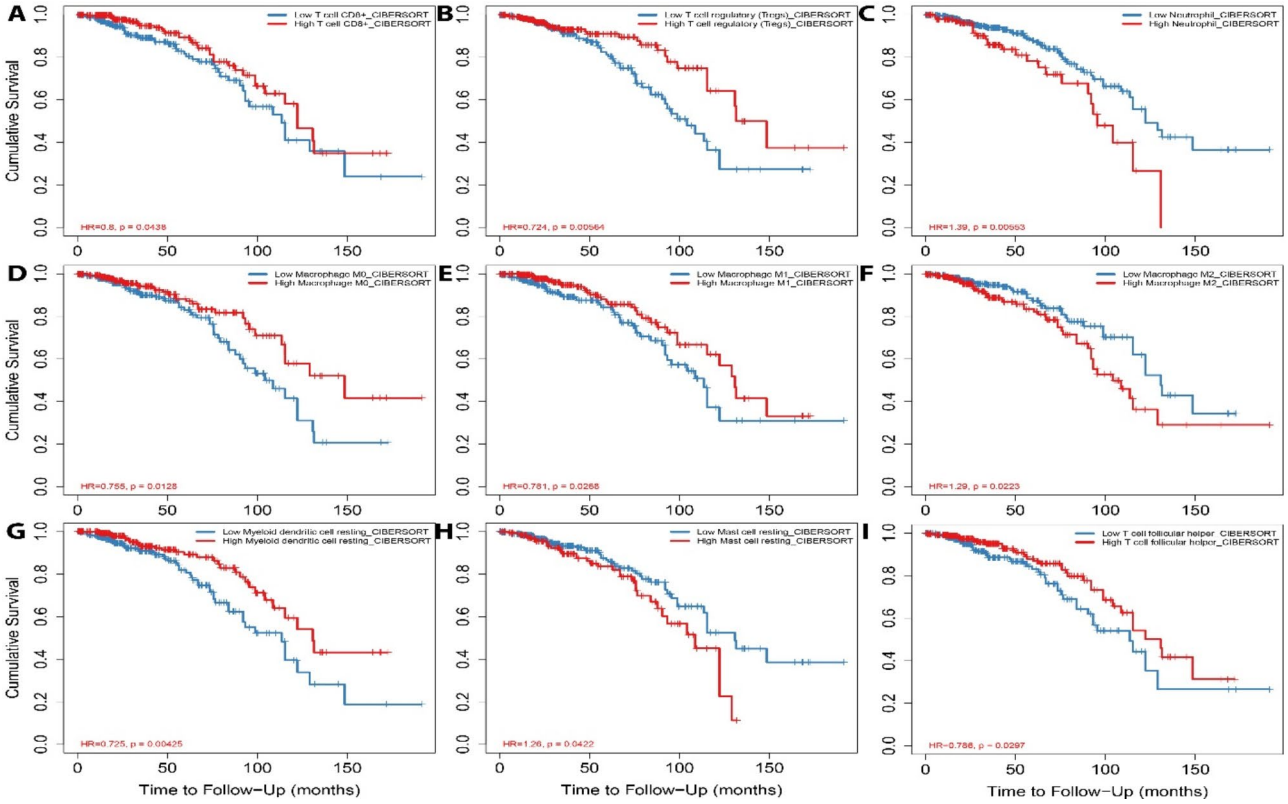

Fig. 7. Bioinformatic analysis of TCGA data from TIMER 2.0: The clinical significance of immune subsets inside the tumor microenvironment of luminal A breast cancer, including $CD8^+$ T cells ($p = 0.04$) (**A**), T cells regulatory (T_{regs}) ($p = 0.005$) (**B**), neutrophils ($p = 0.005$) (**C**), M0 macrophages ($p = 0.012$) (**D**), M1 macrophages ($p = 0.026$) (**E**), M2 macrophages ($p = 0.023$) (**F**), myeloid dendritic cell resting ($p = 0.004$) (**G**), mast cell resting ($p = 0.042$) (**H**), and T cell follicular helper ($p = 0.029$) (**I**).

The variation in coloration acted as an indicator of the synthesis of silver nanoparticles (AgNPs) as referenced by Verma and Mehata[18]. The spectroscopic analysis conducted via scanning revealed that the AgNPs produced from both honey samples were situated within the characteristic peak absorption region specific to AgNPs. Notably, the spectral peak absorption range for AgNPs was identified within the wavelength spectrum of 390–460 nm. The absorbance peaks observed in the AgNPs synthesized from both honey types exhibited an extensive area under the curve, which implies the occurrence of agglomeration, or a variety of sizes, in the synthesized AgNPs. Numerous chemical and physical methodologies exist for the production of metal nanoparticles, with an intention to attain specific size and morphology characteristics. However, it is essential to recognize that a considerable number of these techniques may lack economic feasibility or environmental sustainability. Consequently, in this research endeavor, we opted for the environmentally friendly and cost-efficient strategy of green synthesis to generate AgNPs, as noted by Chirumamilla et al.[19]. A multitude of studies has substantiated the considerable antibacterial attributes of honey, demonstrating its effectiveness against a diverse range of microorganisms[20]. In the current investigation, both honey varieties exhibited no antibacterial efficacy; however, when combined with AgNPs, their antibacterial properties were significantly enhanced. The observed absence of antibacterial activity in the two honey types when used independently may be attributed to their inability to diffuse effectively through the agar or to interact with the bacterial media. All tested dilutions of the two honey types containing AgNPs were capable of inhibiting bacterial biofilm growth at concentrations of up to 5% in most instances, with this activity declining at reduced concentrations of honey + AgNPs. Recent advancements in treatment alternatives for antibiotic-resistant bacteria have led to the approval of several novel agents based on their in vitro and in vivo efficacy. Additionally, antibiotics may sometimes be co-utilized alongside these substances to enhance their effectiveness. A substantial number of these agents have been discovered to exhibit comparable inhibitory effects and mechanisms to conventional antibiotics, influencing the bacterial cell wall and influencing microbial protein synthesis[21]. Several studies have indicated that honey possesses a significant concentration of phenolic compounds, hence contributing to its antibacterial properties[20]. The antibacterial mechanism of honey has been attributed by certain researchers primarily to the presence of hydrogen peroxide, albeit at minimal concentrations[22]. Furthermore, various studies have identified heightened sugar concentration, acidic pH, bee defensin-1, methylglyoxal, and hydrogen peroxide as widely acknowledged antimicrobial components within honey[23]. Previous research has documented the antibacterial activities of silver nanoparticles[24]. The synergistic action of the antimicrobial compounds embedded in honey and AgNPs demonstrated noteworthy antibacterial efficacy. The antibacterial properties intrinsic to AgNPs are contingent upon their interactions with thiol groups present in bacterial cells, leading to the inhibition of respiratory functions as well as transcription and replication processes

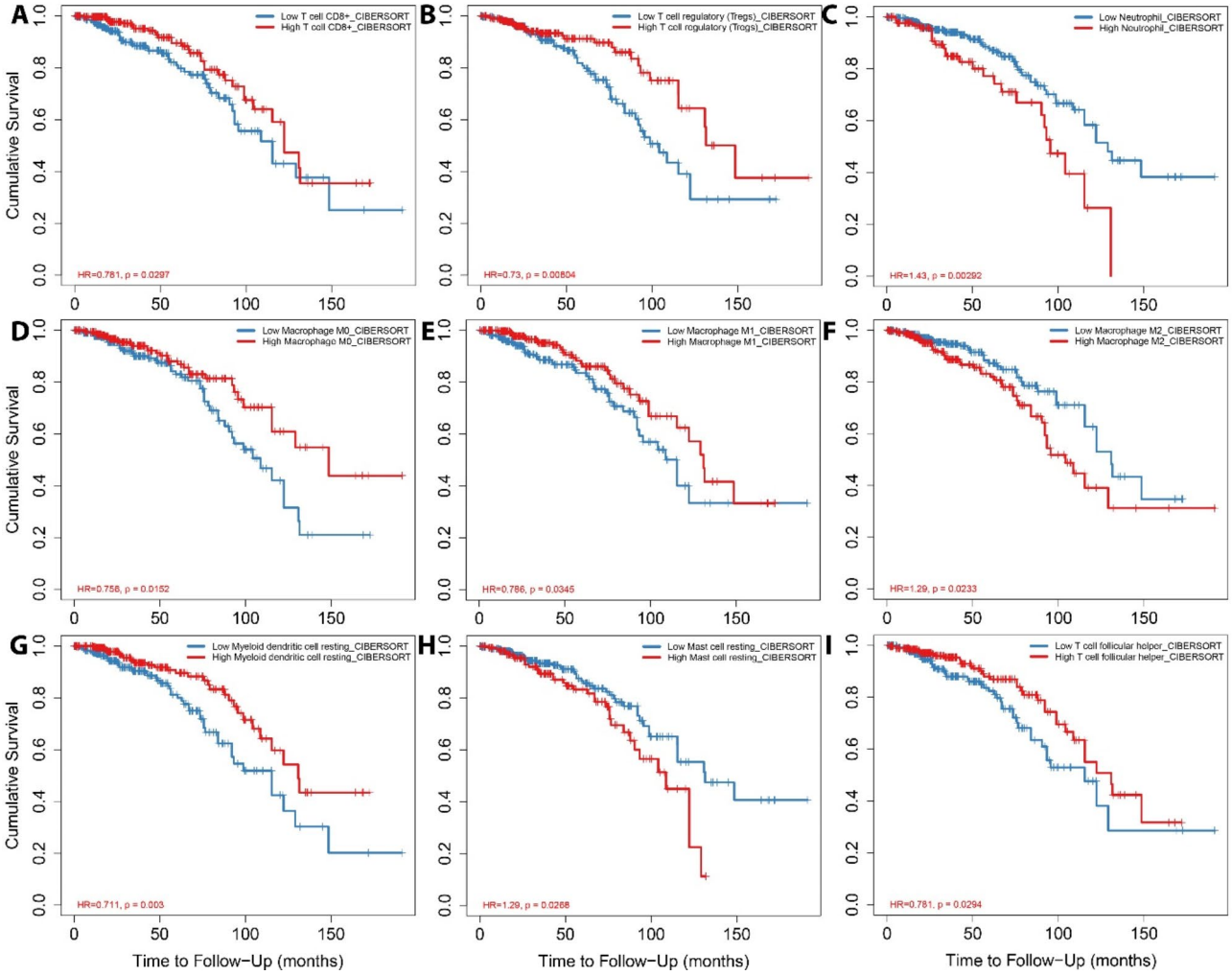

Fig. 8. Bioinformatic analysis of TCGA data from TIMER 2.0: The clinical significance of immune subsets inside the tumor microenvironment of luminal A breast cancer dependent on race as a clinical determinant, including $CD8^+$ T cells ($p = 0.029$) (**A**), T cells regulatory (T_{regs}) ($p = 0.008$) (**B**), neutrophils ($p = 0.002$) (**C**), M0 macrophages ($p = 0.015$) (**D**), M1 macrophages ($p = 0.034$) (**E**), M2 macrophages ($p = 0.023$) (**F**), myeloid dendritic cell resting ($p = 0.003$) (**G**), mast cell resting ($p = 0.026$) (**H**), and T cell follicular helper ($p = 0.029$) (**I**).

involving RNA and DNA. Consequently, bacterial survival is compromised, resulting in their eventual death[25]. Both Sidr honey and SidrZamZam honey displayed significant enhancements in splenic cell proliferation across all tested concentrations, indicating their potential as immunostimulants or agents that bolster the immune system. Emerging evidence supports the assertion that certain types of honey can stimulate the production of essential cytokines such as tumor necrosis factor-α, IL-1β, and IL-6. Past research has illustrated honey's capacity to augment the generation of various immune cells, encompassing T and B lymphocytes, eosinophils, neutrophils, monocytes, and natural killer cells, alongside promoting antibody production during both primary and secondary immune responses in tissue culture[26]. These characteristics may account for the significant elevation in splenic cell numbers observed following in vitro treatment with the two honey types. The presence of AgNPs in various concentrations within the two honey types resulted in a marked decrease in cell proliferation. In this context, nanoplatforms may be employed alongside established antitumor strategies, including radiation, immunotherapy, phototherapy, and chemotherapy, to enhance clinical outcomes in solid tumors[27]. There exists evidence suggesting that AgNPs can traverse multiple pathways into the human body, subsequently accumulating in various organs, including the spleen[28]. Honey's major flavonoid, chrysin, has been demonstrated to decrease IL-2, IL-1β, IL-12, IFN-γ, and TNF-α production from the spleen[29]. It is not surprising that an increase in cellular activation of the spleen indicates a more potent stimulatory action of Sidr honey and SidrZamZam honey.

Clinical trials are examining the potential of combining immunotherapy with conventional treatments, such as hormone therapy or chemotherapy, to ascertain whether this strategy can enhance therapeutic outcomes for individuals with this subtype of cancer. Furthermore, ongoing studies aim to identify biomarkers that might predict which luminal A breast cancer patients would derive the greatest benefit from immunotherapy[30]. The luminal A subtype of breast cancer is characterized by its heterogeneity, necessitating a deeper understanding of its molecular mechanisms and immunological diversity to improve treatment strategies[31]. Thus, we propose an exploration of the

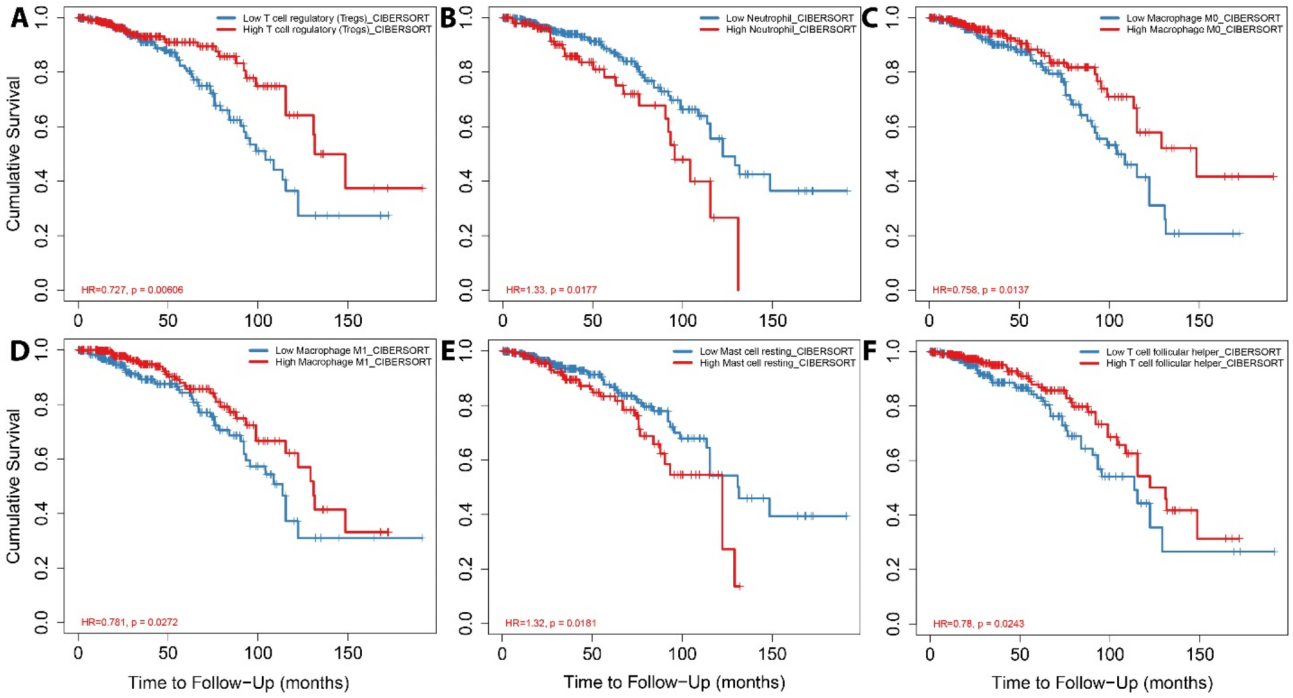

Fig. 9. Bioinformatic analyses of TCGA data of TIMER2.0: The clinical impact of immune subsets within the tumor microenvironment of luminal A breast cancer based on age as clinical condition, includes T cells regulatory (T_{regs}) ($p = 0.006$) (**A**), neutrophils ($p = 0.017$) (**B**), M0 macrophages ($p = 0.013$) (**C**), M1 macrophages ($p = 0.027$) (**D**), mast cell resting ($p = 0.018$) (**E**), and T cell follicular helper ($p = 0.024$) (**F**).

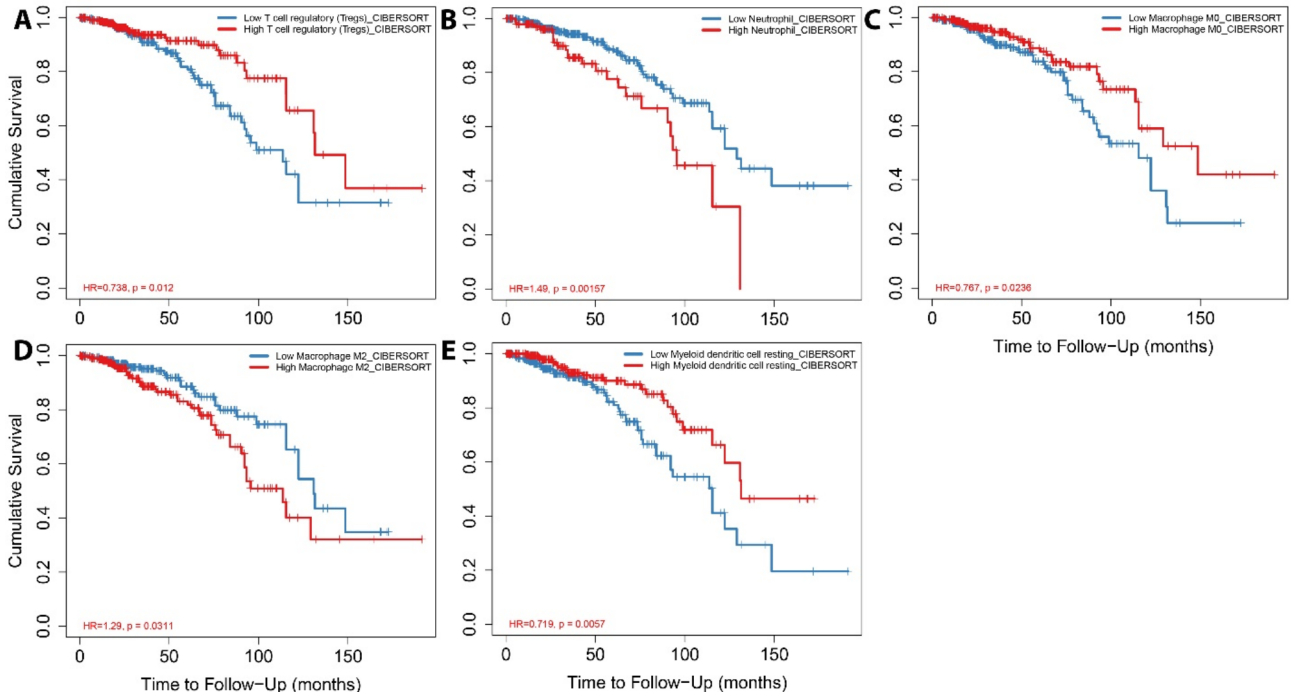

Fig. 10. Bioinformatic Analysis of TCGA Data from TIMER 2.0: The therapeutic importance of immune subsets within the tumor microenvironment of luminal A breast cancer depends on stage as a clinical factor, including T cells regulatory (T_{regs}) ($p = 0.012$) (**A**), neutrophils ($p = 0.001$) (**B**), M0 macrophages ($p = 0.023$) (**C**), M2 macrophages ($p = 0.031$) (**D**) and myeloid dendritic cell resting ($p = 0.005$) (**E**).

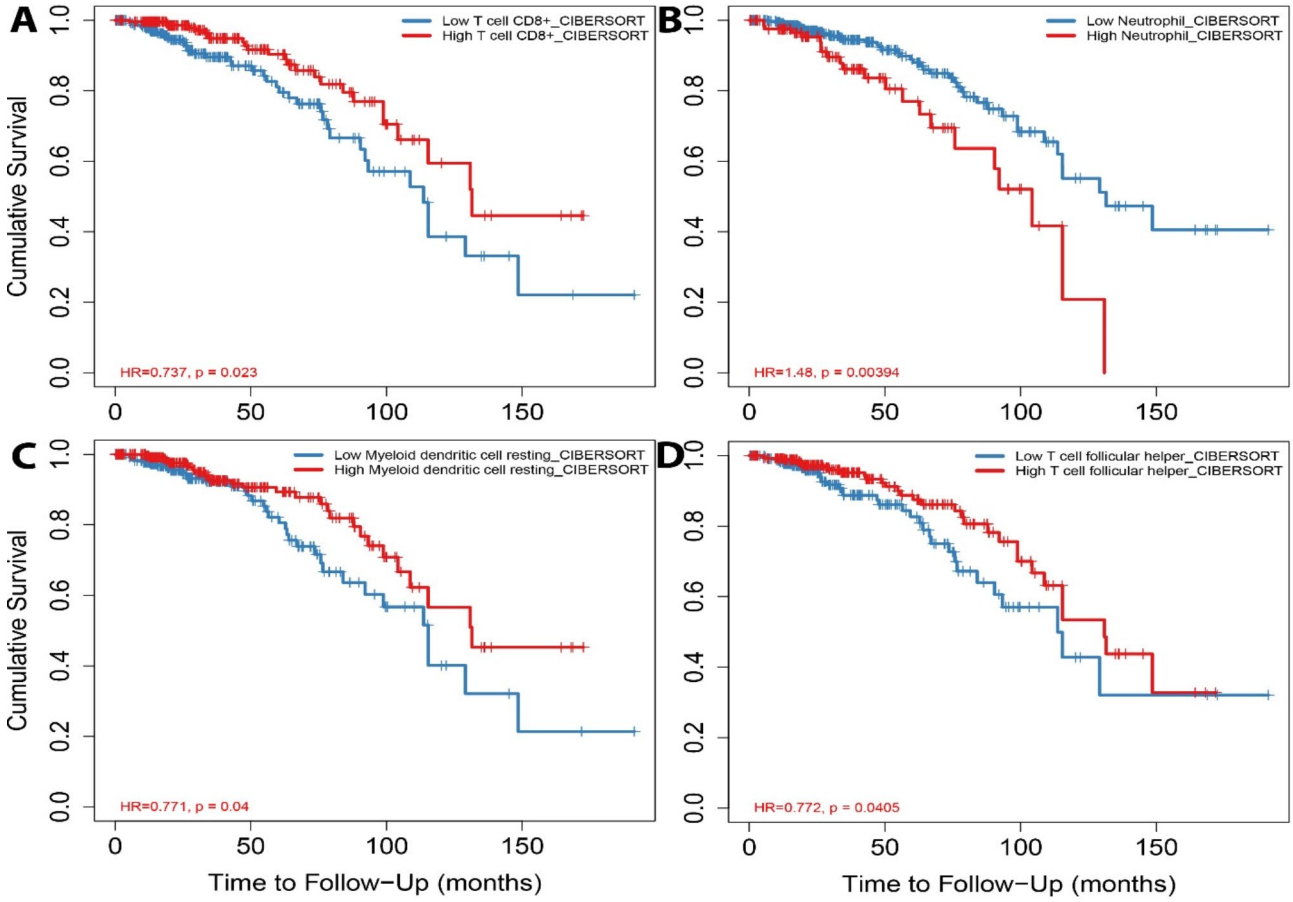

Fig. 11. Bioinformatics Analysis of TCGA Data from TIMER 2.0: The therapeutic significance of immune subsets within the tumor microenvironment of luminal A breast cancer is determined by purity as a clinical state, including $CD8^+$ T cells ($p = 0.023$) (**A**), neutrophils ($p = 0.003$) (**B**), myeloid dendritic cell resting ($p = 0.04$) (**C**) and T cell follicular helper ($p = 0.040$) (**D**).

intricate interplay among immune cells within the tumor microenvironment of luminal A breast cancer patients. Notably, our bioinformatics analyses from TCGA data established the clinical significance of nine immune cell subsets within the tumor microenvironment of luminal A breast cancer: $CD8^+$ T cells, regulatory T cells (T_{regs}), neutrophils, M_0 macrophages, M1 macrophages, M_2 macrophages, resting myeloid dendritic cells, resting mast cells, and follicular helper T cells. The comparative toxicity of AgNPs is markedly elevated relative to other metallic nanoparticles, a phenomenon attributed to the generation of higher levels of reactive oxygen species and the consequent release of the enzyme lactate dehydrogenase[32]. The cytotoxic effects imparted by AgNPs can be influenced by diverse factors, encompassing the physical and chemical properties of the nanoparticles, the local microenvironment, and the interactions between the particles and cellular entities[33]. The MCF-7 cell line, derived from luminal A breast cancer, is routinely used as a model for probing human breast cancer[34]. The current study revealed a reduction in the number of MCF-7 cells across various dilutions of both Sidr honey and SidrZamZam honey. However, it should be emphasized that the combination of SidrZamZam honey and AgNPs did not elicit this same effect, except at a dilution rate of 10%. This decrease in cell viability can be attributed to the apoptotic properties of the two types of honey in relation to MCF-7 cells. Hamadou and his colleagues[35] have documented the capability of honey to induce apoptosis in the MCF-7 cell line, initiating cell cycle arrest at the G_0/G1 phase. A recent investigation elucidated ATP-induced cell death within breast cancer cell lines[36], indicating that honey may serve as an energy source for cellular processes. Moreover, honey has been evidenced to promote the production of immune-modulatory cytokines such as TNF-α and IL-1β, facilitating the release of calcium ions from the endoplasmic reticulum. Previous research[37] has demonstrated that the activation of caspases-6 and -9 leads to the induction of apoptotic processes. Additionally, some studies have indicated honey's ability to induce cytotoxicity against MCF-7 cells, primarily associated with the phenolic content and antioxidant properties[38]. Bioactive compounds derived from honey are believed to stimulate p53-dependent apoptotic pathways[35].

Conclusions

This comprehensive study meticulously explored the potential of Sidr honey and SidrZamZam honey in the green synthesis of silver nanoparticles (AgNPs), encompassing their antimicrobial properties, effects on immune cell proliferation, and anticancer efficacy against luminal A breast cancer cell lines. The synthesis of silver nanoparticles

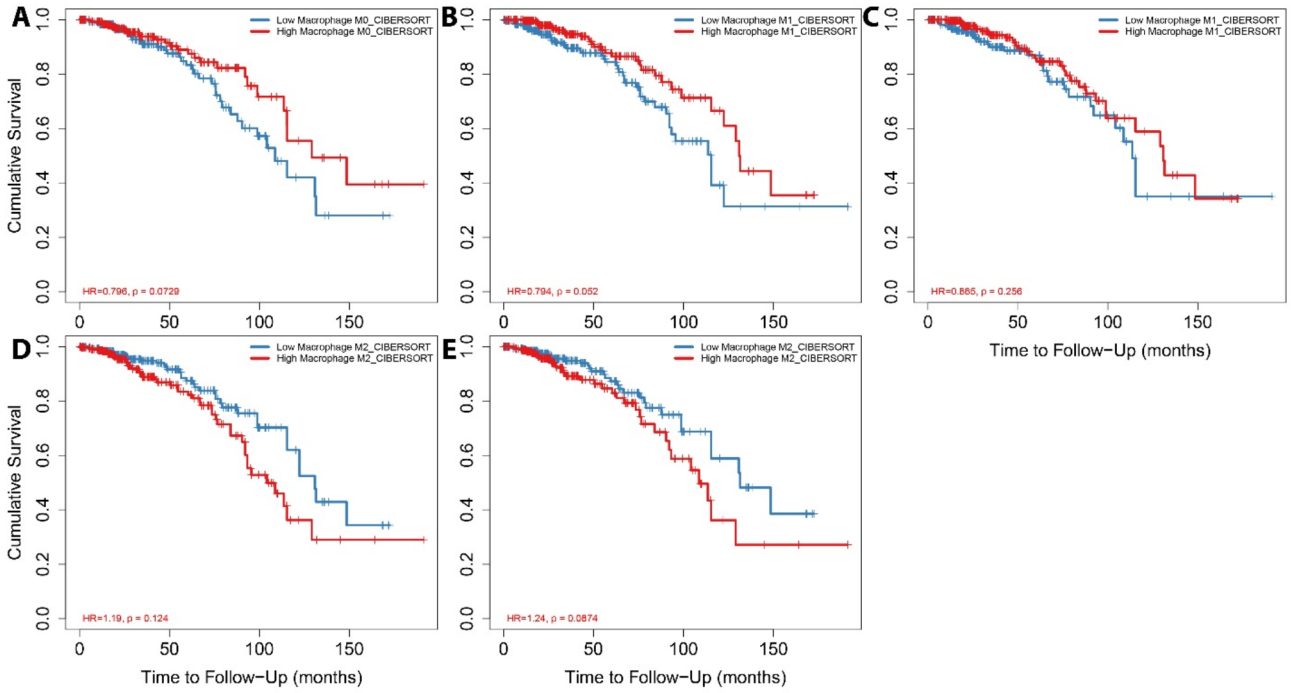

Fig. 12. Bioinformatics Analysis of TCGA Data from TIMER 2.0: The clinical importance of immune subgroups inside the tumour microenvironment of luminal Breast cancer depends on age and purity. M2 macrophages exhibited little clinical influence in terms of age or purity, while M0 macrophages had no significant therapeutic benefit. M1 macrophages have limited clinical value in terms of stage and purity.

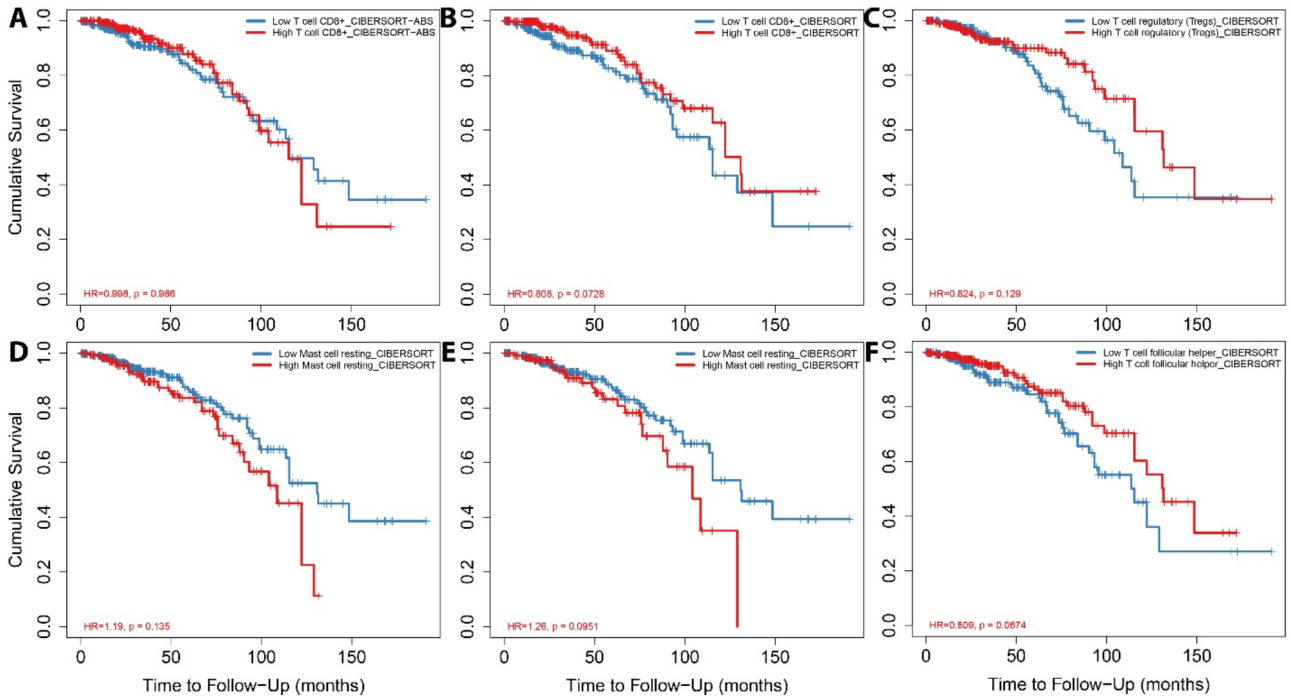

Fig. 13. Bioinformatics Analysis of TCGA Data from the TIMER 2.0: The clinical significance of immunological subsets within the tumour microenvironment of luminal Breast cancer varies. Mast cells have little clinical significance in terms of age or purity. T cell regulatory (Tregs) have demonstrated no significant therapeutic advantage. T cell follicular helper had no clinically significant influence on stage.

Sugar	Sidr honey (%)	SidrZamZam honey (%)
Fructose	38.02200[a]	37.87958[a]
Glucose	28.88848[b]	36.24784[a]
Sucrose	4.27660[c]	5.02549[c]
Maltose	1.01143[d]	0[d]

Table 4. Sugar content in Sidr and SidrZamZam honeys.

was achievable with both varieties of honey, which demonstrated antibacterial activities in tandem with AgNPs. Furthermore, this research underscored the immunostimulatory capacities of both Sidr honey and SidrZamZam honey. In terms of anticancer efficacy, both honey types exhibited a capability to diminish the viability of luminal A breast cancer MCF-7 cells. The bioinformatics analyses of data from The Cancer Genome Atlas further elucidated the clinical significance of targeting diverse immune cell subsets within the tumor microenvironment.

Materials and methods

Preparation of honey and synthesis of nanoparticles

Sidr honey and SidrZamZam honey were obtained from bee colonies maintained in the apiary of King Khalid University. These honeys were utilized to synthesize silver nanoparticles (AgNPs) following the methodologies outlined by Ibrahim et al.[39]. Sidr honey is characterized as a monofloral honey sourced from the Sidr tree, while SidrZamZam honey is equivalent to Sidr honey, with the distinction that the honeybees were provided with ZamZam water for consumption. Various concentrations of honey were prepared using double-distilled water, specifically at concentrations of 40%, 20%, 10%, 5%, 2.5%, and 1.25% (w/v). Prior to utilization, all honey solutions underwent filter sterilization using a filter with a pore size of 0.45 µm (Coaster). The honey solutions, ranging from 1.25 to 40%, were separately mixed with silver nitrate ($AgNO_3$) at a concentration of 0.01 M (filtered) in straightforward chemical reactions. The pH of each dilution was incrementally elevated using 0.1 M sodium hydroxide (NaOH) until a discernible color change was observed in the mixture[40]. The transition of the mixture solution to a brown hue indicated the successful formation of AgNPs. All honey preparations, whether or not containing AgNPs, were freshly prepared prior to their application.

Characterization of Sider and SidrZamZam-produced AgNPs

The synthesis of silver nanoparticles was characterized using a double-beam spectrophotometer (Lambda 25, PerkinElmer), with measurements taken at wavelengths spanning from 300 to 700 nm, as detailed by Ghramh et al.[41]. The morphology and dimensions of the synthesized AgNPs were examined using a scanning electron microscope (SEM, JEM-1011, JEOL, Tokyo, Japan).

Well-diffusion method for antibacterial susceptibility assay

The susceptibility of bacteria to Sidr and SidrZamZam honey samples, ranging from 1.25 to 40% concentration, was investigated. Additionally, the antibacterial properties of these honey samples containing AgNPs were examined. The growth of the bacterial strains, specifically *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Bacillus subtilis*, was monitored until they attained the logarithmic phase, defined as an optical density at 610 nm (OD_{610}) between 0.4 and 0.6. This proliferation was detected in Mueller Hinton (MH) broth. Following this, further dilution of the bacterial strains was performed in MH broth until reaching a theoretical optical density of 0.01 at OD_{610}. To evaluate the antibacterial efficacy of the two types of honey, either alone or containing AgNPs, the agar well-diffusion technique employed by Arora et al[42] was utilized. In this experimental setup, aseptic techniques were applied to create wells in a nutrient agar medium using a sterile syringe cap, with the wells measuring 6 mm in diameter. A sterile cotton swab, preloaded with a diluted culture, was employed to establish a grass culture on the agar medium. Subsequently, 20 µL aliquots from various dilutions of the two types of honey, either independently or combined with AgNPs, were placed in the wells of Petri dishes prepared in triplicate. These Petri dishes were then incubated under aerobic conditions at 37 °C for a period of 24 h. The diameter of the clear zone, indicative of bacterial growth inhibition, was measured in millimeters. Subsequently, 20 µL aliquots from various dilutions of the two types of honey, either independently or combined with AgNPs, were placed in the wells of Petri dishes prepared in triplicate. These Petri dishes were then incubated under aerobic conditions at 37 C for a period of 24 h. The diameter of the clear zone, indicative of bacterial growth inhibition, was measured in millimeters.

In vitro effects of the two types of honey alone and containing AgNPs on normal splenic cell proliferation

The experiment was executed in alignment with the methodologies established by Ibrahim et al.[43], albeit with certain modifications. A single-cell suspension from the spleen was prepared using healthy adult male Sprague Dawley rats, each averaging approximately 280 g in weight. The rats were generously supplied by the animal facility at King Khalid University. The cells were then suspended in a complete RPMI-1640 medium supplemented with 10% fetal calf serum, achieving a density of 0.05×10^6 cells per milliliter. The objective of this experiment was to assess the cytotoxicity and the capacity to promote normal cell division of Sider and SidrZamZam honeys, either independently or in conjunction with AgNPs, on splenic cells. Each honey solution was applied to distinct wells, each containing 5000 rat splenic cells, in triplicate. A control culture was established by introducing 5000 splenic cells in 200 µL of the culture media into 8 wells. The plates were incubated at a temperature of 37 °C within a

controlled, humidified atmosphere containing 5% carbon dioxide, utilizing a CO_2 incubator manufactured by Memmert GmbH, for a duration of 72 h. Cell viability assessments across all plates were performed employing the Vybrant® MTT cell proliferation test kit (Thermo Fisher Scientific), in accordance with the manufacturer's instructions. The evaluation of the percentage increase or decrease in cell number was carried out following the methodology delineated in the study conducted by Oves et al.[44].

Anticancer properties of Sider and SidrZamZam honeys

The American Type Culture Collection (ATCC) MCF-7 human breast cancer cell line served as the model for this investigation. In accordance with the methodologies established by Ibrahim et al.[39], MCF-7 cells were cultured in cell culture flasks and sustained in RPMI-1640 medium, which was supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin at concentrations of 100 U/mL and 100 µg/mL, respectively. The cells underwent incubation within a CO_2 incubator, as noted by Masad et al.[45]. Subsequently, MCF-7 cells were seeded into 96-well plates at a density of 1×10^5 cells per well and the plates were incubated for a duration of 24 h within a CO_2 incubator. Following this incubation period, the medium from all wells was removed, and the cells were treated with various concentrations of Sidr and SidrZamZam honey formulations, ranging from 1.25 to 40%. These treatments were applied either individually or in conjunction with AgNPs over a span of 72 h. Cell viability assessments were performed utilizing the MTT cell proliferation assay, as detailed above.

Honey's sugar content

Determination of sugar contents in Sidr and SidrZamZam honey samples was done using high performance liquid chromatography (HPLC, Agilent) using the method and material described by Ghramh et al.[46].

Bioinformatic analyses

We used many bioinformatics tools, such as the Harmonizome web portal, to explore the pathways and cell types of enrichment analysis by employing the highly expressed proteins in the spleen from the curated tissue protein expression dataset[47,48]. We evaluated the clinical significance of tumor immune cells using CIBERSORT deconvolution of TCGA data via the outcome module TIMER2.0, which allows for the correction of many covariates in a multivariable Cox proportional hazards model[49]. Furthermore, covariates may include clinical characteristics such as age, stage, race, and purity[50].

Statistical analysis

Outcomes were expressed as averages of six values ± standard deviation across the research period. A One-way ANOVA test for intra-group comparison and Two-ways ANOVA test (GraphPad Prism-Version 7.0 for Windows) for paired/unpaired values was conducted, and a p-value < 0.05 was considered statistically significant. Each experiment was carried out three times independently. The pop-up function shows Kaplan–Meier curves for the relevant immune infiltrates and luminal A breast cancers. The penetration level is divided into two categories: low and high. The split infiltration percentage of patients is 50% and survival time between equal to two hundred months. The Cox model's hazard ratio and p value, as well as the log-rank p value for the KM curve, were calculated[50]. The p value of pathway and cell cycle enrichment analysis is computed from the fisher exact which is a proportion test that assumes a binomial distribution and independence for probability of any gene belonging to any test. The adjusted p value is computed using the Benjamini–Hochberg method for correction for multiple hypotheses testing [51].

Data availability

All data generated or analyzed during this study are included in this published article.

Received: 15 November 2024; Accepted: 17 March 2025
Published online: 22 March 2025

References

1. El Sohaimy, S. A., Masry, S. H. D. & Shehata, M. G. Physicochemical characteristics of honey from different origins. *Ann. Agric. Sci.* **60**, 279–287 (2015).
2. Taha, E. K. A., Al-Kahtani, S. & Taha, R. Comparison of the physicochemical characteristics of sidr (*Ziziphus* spp.) honey produced by *Apis florea* F. and *Apis mellifera* L. *J. Apic. Res.* **60**, 470–477 (2021).
3. Al-Ghamdi, G., Hussein, R. H. & Al-azragi, R. The antioxidant impact of Saudi sidr honey against acetyl salicylic acid- induced gastric ulcer. *Med. Sci.* **24**, 1923–1929 (2020).
4. Almalki, R. A., Alzahrani, D. A., Almalki, R. A. & Alzahrani, D. A. Morphological Investigation of Genus *Ziziphus* Mill. (Rhamnaceae) in Saudi Arabia. *Am. J. Plant Sci.* **9**, 2644–2658 (2018).
5. Al-Ghamdi, A. A. & Ansari, M. J. Biological and therapeutic roles of Saudi Arabian honey: A comparative review. *J. King Saud Univ. - Sci.* **33**, 101329 (2021).
6. Zerrouk, S., Seijo, M. C., Escuredo, O. & Rodríguez-Flores, M. S. Characterization of *Ziziphus lotus* (jujube) honey produced in Algeria. *J. Apic. Res.* **57**, 166–174 (2018).
7. Sampaio, L. A., Pina, L. T. S., Serafini, M. R., Tavares, D. D. S. & Guimarães, A. G. Antitumor effects of carvacrol and thymol: A systematic review. *Front. Pharmacol.* https://doi.org/10.3389/fphar.2021.702487 (2021).
8. Alkhalifah, T. S., Almuhaish, L. A. & Syed Mahin, P. M. Remineralization effect of zamzam water on initial artificial carious lesion of permanent teeth. *Cureus* https://doi.org/10.7759/cureus.32823 (2022).
9. Medina, C., Santos-Martinez, M. J., Radomski, A., Corrigan, O. I. & Radomski, M. W. Nanoparticles: pharmacological and toxicological significance. *Br. J. Pharmacol.* **150**, 552–558 (2007).
10. Burdușel, A. C. et al. Biomedical applications of silver nanoparticles: An up-to-date overview. *Nanomaterials* **8**, 681 (2018).
11. Bahari, N., Hashim, N., Md Akim, A. & Maringgal, B. Recent advances in honey-based nanoparticles for wound dressing: A review. *Nanomaterials* **12**, 2560 (2022).

12. Guruvu, N. R. et al. Synergistic antimicrobial activity of honey combined with silver nanoparticles topically applied in experimentally infected wounds in Wistar albino rats. *Natl. J. Physiol. Pharm. Pharmacol.* **12**, 1–1 (2021).
13. Alnegheimish, N. A. et al. Molecular subtypes of breast carcinoma in Saudi Arabia. A retrospective study. *Saudi Med. J.* **37**, 506–512 (2016).
14. AlRajhi, B. et al. Breast cancer awareness among women in Saudi Arabia: A systematic review. *Breast Cancer.* **15**, 913–924 (2023).
15. El-Arabey, A. A., Abdalla, M. & Abd-Allah, A. R. SnapShot: TP53 status and macrophages infiltration in TCGA-analyzed tumors. *Int. Immunopharmacol.* **86**, 106758 (2020).
16. Waheed, M. et al. Honey and cancer: A mechanistic review. *Clin. Nutr.* **38**, 2499–2503 (2019).
17. Ibrahim, E. H. et al. *Lepidium sativum* and its biogenic silver nanoparticles activate immune cells and induce apoptosis and cell cycle arrest in HT-29 colon cancer cells. *Biomater. Tissue Eng.* **11**, 195–209 (2021).
18. Verma, A. & Mehata, M. S. Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. *J. Radiat. Res. Appl. Sci.* **9**, 109–115 (2016).
19. Chirumamilla, P., Dharavath, S. B. & Taduri, S. Eco-friendly green synthesis of silver nanoparticles from leaf extract of Solanum khasianum: optical properties and biological applications. *Appl. Biochem. Biotechnol.* **195**, 353–368 (2023).
20. Almasaudi, S. The antibacterial activities of honey. *Saudi J. Biol. Sci.* **28**, 2188 (2021).
21. Baran, A., Kwiatkowska, A. & Potocki, L. Antibiotics and bacterial resistance-A short story of an endless arms race. *Int. J. Mol. Sci.* **24**, 5777 (2023).
22. Romário-Silva, D. et al. Antimicrobial activity of honey against oral microorganisms: Current reality, methodological challenges and solutions. *Microorganisms* **10**, 2325 (2022).
23. Hossain, M. L., Lim, L. Y., Hammer, K., Hettiarachchi, D. & Locher, C. A review of commonly used methodologies for assessing the antibacterial activity of honey and honey products. *Antibiot.* **11**, 975 (2022).
24. Czernel, G. et al. Biodirected synthesis of silver nanoparticles using aqueous honey solutions and evaluation of their antifungal activity against Pathogenic Candida Spp. *Int. J. Mol. Sci* **22**, 7715 (2021).
25. Hosny, A. M. S., Kashef, M. T., Rasmy, S. A., Aboul-Magd, D. S. & El-Bazza, Z. E. Antimicrobial activity of silver nanoparticles synthesized using honey and gamma radiation against silver-resistant bacteria from wounds and burns. *Adv. Nat. Sci. Nanosci. Nanotechnol.* **8**, 045009 (2017).
26. Hussein, S. Z., Mohd Yusoff, K., Makpol, S. & Mohd Yusof, Y. A. Gelam honey inhibits the production of proinflammatory, mediators NO, PGE 2, TNF-α, and IL-6 in carrageenan-induced acute paw edema in rats. *Evid. Based Complement. Altern. Med.* **2012**, 1–13 (2012).
27. Raju, G. S. R. et al. Nanoparticles mediated tumor microenvironment modulation: Current advances and applications. *J. Nanobiotechnol.* **20**, 274 (2022).
28. Sengul, A. B. & Asmatulu, E. Toxicity of metal and metal oxide nanoparticles: A review. *Environ. Chem. Lett.* **18**, 1659–1683 (2020).
29. Masad, R. J. et al. The immunomodulatory effects of honey and associated flavonoids in cancer. *Nutrients* **13**(4), 1269 (2021).
30. Quinn, K. M. Innovative approaches to immunotherapy in breast cancer. *J. Thorac. Dis.* **12**, 4536–4540 (2020).
31. Guo, L. et al. A high-risk luminal A dominant breast cancer subtype with increased mobility. *Breast Cancer Res. Treat.* **175**, 459–472 (2019).
32. Dasgupta, N. & Ramalingam, C. Silver nanoparticle antimicrobial activity explained by membrane rupture and reactive oxygen generation. *Environ. Chem. Lett.* **14**, 477–485 (2016).
33. Guo, Z., Zeng, G., Cui, K. & Chen, A. Toxicity of environmental nanosilver: mechanism and assessment. *Environ. Chem. Lett.* **17**, 319–333 (2018).
34. Lee, A. V., Oesterreich, S. & Davidson, N. E. MCF-7 cells–changing the course of breast cancer research and care for 45 years. *J. Natl. Cancer Inst.* **107**, djv073 (2015).
35. Hamadou, W. S. et al. Acacia honey-derived bioactive compounds exhibit induction of p53-dependent apoptosis in the MCF-7 human breast cancer cell line. *Pharmacogn. Mag.* **19**, 144–155. https://doi.org/10.1177/09731296221145076 (2023).
36. Zhang, H.-L. et al. Elucidating the molecular basis of ATP-induced cell death in breast cancer: Construction of a robust prognostic model. *World J. Clin. Oncol.* **15**, 208–242 (2024).
37. Aryappalli, P. et al. The IL-6/STAT3 signaling pathway is an early target of manuka honey-induced suppression of human breast cancer cells. *Front. Oncol.* https://doi.org/10.3389/fonc.2017.00167 (2017).
38. Portokalakis, I., Mohd Yusof, H. I., Ghanotakis, D. F., Singh Nigam, P. & Owusu-Apenten, R. K. Manuka honeyinduced cytotoxicity against MCF7 breast cancer cells is correlated to total phenol content and antioxidant power. *J. adv. biol. biotechnol.*, **8**(2), 1–10. https://doi.org/10.9734/JABB/2016/27899 (2016).
39. Ibrahim, E. H. et al. Origanum majorana harvested from Al-Soda, Saudi Arabia promotes mitotic arrest and apoptosis in colon cancer cells. *J. King Saud Univ. Sci.* **34**, 101878 (2022).
40. Gramah, H. A., Ahmad, Z. & Ibrahim, E. H. Facile synthesis of silver and gold nanoparticles using mangrove (*Avicennia marina*) leaves extract and its cytotoxicity and larvicidal activity—PubMed. *Pak. J. Pharm. Sci.* **37**, 297–305 (2024).
41. Ghramh, H. A., Al-Ghamdi, K. M., Mahyoub, J. A. & Ibrahim, E. H. Chrysanthemum extract and extract prepared silver nanoparticles as biocides to control *Aedes aegypti* (L.), the vector of dengue fever. *J. Asia. Pac. Entomol.* **21**, 205–210 (2018).
42. Arora, S. et al. Synergistic effect of plant extracts on endodontic pathogens isolated from teeth with root canal treatment failure: An in vitro study. *Antibiotics* **10**, 552. https://doi.org/10.3390/antibiotics10050552 (2021).
43. Ibrahim, E. H., Taha, R., Ghramh, H. A. & Kilany, M. Development of rift valley fever (RVF) vaccine by genetic joining of the RVF-glycoprotein Gn with the strong adjuvant subunit B of cholera toxin (CTB) and expression in bacterial system. *Saudi J. Biol. Sci.* **26**, 1676–1681 (2019).
44. Oves, M. et al. Antibacterial and cytotoxic efficacy of extracellular silver nanoparticles biofabricated from chromium reducing novel OS4 strain of *Stenotrophomonas maltophilia*. *PLoS One* **8**, e59140 (2013).
45. Masad, R. J. et al. The immunomodulatory effects of honey and associated flavonoids in cancer. *Nutrients* **13**, 1269 (2021).
46. Ghramh, H. A., Ibrahim, E. H. & Kilnay, M. Majra honey abrogated the normal and cancer cells proliferation inhibition by *Juniperus procera* extract and extract/honey generated AgNPs. *Anticancer. Agents Med. Chem.* **20**, 970–981 (2020).
47. Santos, A. et al. Comprehensive comparison of large-scale tissue expression datasets. *PeerJ* **3**, e1054 (2015).
48. Rouillard, A. D. et al. The harmonizome: A collection of processed datasets gathered to serve and mine knowledge about genes and proteins. *Database J. Biol. Databases Curation* **2016**, bae100 (2016).
49. Li, T. et al. TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res.* **77**, e108–e110 (2017).
50. El-Arabey, A. A. et al. Metformin as a promising target for DPP4 expression: Computational modeling and experimental validation. *Med. Oncol.* **40**, 277 (2023).
51. Li, W., Shih, A., Freudenberg-Hua, Y., Fury, W. & Yang Y. Beyond standard pipeline and $p < 0.05$ in pathway enrichment analyses. *Comput. Biol. Chem.* **92**, 107455. https://doi.org/10.1016/j.compbiolchem.2021.107455 (2021).

Acknowledgements

The authors extend their appreciation to University Higher Education Fund for funding this research work under Research Support Program for Central labs at King Khalid University through the project number CL/RP/6.

Author contributions

Authors Contribution Conceptualization, Essam H. Ibrahim and Hamed A. Ghramh, Methodology, Essam H. Ibrahim, Software, Amr A. El-Arabey; Formal analysis, Essam H. Ibrahim, and Amr A. El-Arabey; Resources, Essam H. Ibrahim; Data curation, Essam H. Ibrahim; Writing original draft, Essam H. Ibrahim, Amr A. El-Arabey and Khalid Ali Khan; Writing review & editing, Essam H. Ibrahim, Hamed A. Ghramh and Amr A. El-Arabey; Visualization, Essam H. Ibrahim; Supervision, Hamed A. Ghramh; Funding acquisition, Hamed A. Ghramh.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to A.A.E.-A.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.