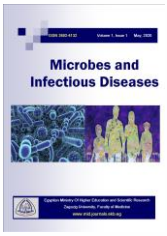
Microbes and Infectious Diseases 2025; 6(4): 6542-6557



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Isolation and molecular characterization of multidrug-resistant *Escherichia coli* with evaluation of Sidr honey, rich in antioxidants and anti-inflammatory, as an alternative therapeutic agent.

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ARTICLE INFO

Article history:

Received 15 December 2024

Received in revised form 7 January 2025

Accepted 11 January 2025

Keywords:

Sidr honey

phenolic compounds

flavonoids

resistant bacteria

semen

cytotoxicity

ABSTRACT

Background: Sidr honey is one of the best sources of bioactive substances with remarkable pharmacological qualities. Different botanical compounds and various elements make up honey's composition. Several variables affect how many of these components are present in honey, geographical setting, the flower's location, the season, and the procedures that were used. This study aimed to evaluate the chemical composition, antimicrobial activity, antioxidant properties, anti-inflammatory effects, and cytotoxicity of Egyptian Sidr honey, with a particular focus on its potential against multidrug-resistant bacterial strains. **Methods:** Egyptian Sidr honey was obtained from Saini and extracted using methanol. Analyzed using HPLC to detect the levels of phenolic compounds and flavonoids. On the other hand, **2**00 semen samples were collected from participants in El-Hussien Hospital in Egypt and screened for bacterial infections. The most resistant strain was identified using genetics tools. Sidr honey extract was examined for its antibacterial impact on multidrug-resistant bacterial stains. The antioxidant activity, cytotoxicity, and anti-inflammatory effects of Sidr honey were tested using *in vitro* validated methods. **Results:** Sidr honey contained twelve different phenolic compounds, with vanillic acid being the compound present in the highest concentration. Additionally, there were nine different flavonoids, with kaempferol being the common molecule found in both groups (phenolic compounds and flavonoids).While various bacteria could be seen in the semen samples, where 43% were Gram-positive microbes and only 57% had Gram-negative bacteria. *E. coli* was the most resistant bacterium, identified and deposited in the gene with the accession number PQ637169. Sidr honey extract showed a promising antibacterial action towards *E. coli* with an inhibition diameter of 2.6±0.5 mm, an antioxidant value with IC_{50} = 3.41 ±0.3 µg/ml, an anti-inflammatory role with IC_{50} = 3.41 ±0.3 µg/ml, and minimal toxicity towards Vero cells, where CC_{50} is 22.2±1.2 µg/ml. **Conclusion**: Egyptian Sidr honey had large variations of phenolic compounds and flavonoids and a promising potential versus multidrug-resistant *E. coil* isolated from Egyptian patients and it could be applied as an antioxidant and anti-inflammatory agent with high safety towards normal cells.

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DOI: 10.21608/MID.2025.344737.2398

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Introduction

Honey has long been recognized for its therapeutic qualities and has been utilized in traditional therapy. Infectious and inflammatory disorders have been treated with notable therapeutic outcomes. Honey compounds and their therapeutic use in cancer treatment have garnered more attention recently. Numerous findings demonstrated that different varieties of honey exhibited notable anticancer effects [1]. Time along the year, honeybee species, and geographical position of the equivalent floral source can all affect the honey composition [2, 3]. When it comes to honey, monofloral honey made from the sap of a single plant species is superior to polyfloral honey [4].

Sidr honey is derived from the nectar of *Ziziphus trees* (*Ziziphus* spp.) [5]. A country that produces a lot of good monofloral honey is Saudi Arabia. One example is ziziphus honey (*Ziziphus* spp.) (ZH), which is also referred to as Sidr honey [6]. Three primary plant species *Ziziphus nummularia*, *Z. mucronata*, and *Z. spina-christi* are the sources of ZH [7].

Various types of Sidr honey are harvested worldwide, yet little is known about their chemical and physical characteristics [8]. Due to its high cost and limited availability, Sidr honey is often subject to adulteration [9]. The color and properties of honey are significantly influenced by its geographic and floral origins [10-12]. Moreover, according to various national honey authentication standards and the Codex Alimentarius, the two primary criteria for honey authentication are its geographic and floral origins [13].

Infections caused by bacteria in the reproductive system have been connected to male impotence according to Gimenes et al. [14]. These infections may result in tissue inflammation and genital duct blockage. Additionally, bacteria may directly harm spermatozoa biology by decreasing their motility or viability [15, 16], although it is still unclear how much of an effect bacterial infections have on male fertility. While bacteriospermia was once thought to be negatively related to fertility, new research shows that bacteria are quite common in semen, even in fertile people with normal sperm characteristics [17]. It would be crucial to assess whether particular microbiological patterns are associated with the reproductive status of the seminal microbiome, which has received less attention than other body regions [18]. The study on Sidr honey's pharmacological properties and its potential application as an alternative therapeutic agent against multidrug-resistant *E. coli* (MDR *E. coli*) presents a novel and highly relevant approach in the context of rising antibiotic resistance. As the global challenge of MDR bacteria continues to escalate, exploring natural products like Sidr honey, with its antimicrobial, antioxidant, and anti-inflammatory properties, offers a promising avenue for the development of alternative therapies. This research not only highlights the therapeutic potential of Sidr honey but also underscores its role in the search for sustainable solutions to combat multidrug-resistant infections. The objective of the current study is to test the antibacterial impact of Sidr honey on isolated resistant bacteria from semen as well as *in vitro* assessment of the antioxidant, anti-inflammatory, and cytopathic impact of Sidr honey.

Materials and methods

Collection of honey and extraction

The Egyptian Sidr honey was bought from a honeybee colonies production unit in the Sinai area and kept in clean, dry, and dim lighting at 4°C. The monofloral origin was confirmed by examining the honey under a microscope to identify the most common pollen types [19]. Extraction was carried out using methanol, left at room temperature and then rotated for 200 rpm for 24 hours to obtain the extract [20].

Examination of various bioactive molecules using HPLC

HPLC (Agilent 2100), equipped with a UV/Vis detector and two LC pumps, was employed for the analysis. A C18 column (130 x 4.80 mm, particle size of 5 µm) was used, and chromatograms were collected and analyzed with the Agilent ChemStation. For phenolic acids separation, a mobile phase consisting of 0.2% methanol and phosphoric acid (50:50 v/v, isocratic mode) was used. The instrument's wavelength was set to 290 nm, with a liquid flow rate of 1.5 mL/min. For flavonoids, a binary mobile phase of methanol and water (50:50 v/v), calibrated to pH 2.7 with phosphoric acid, was used with an isocratic flow rate of 1.5 mL/min [21].

Semen specimens' collection

The urology branch at El-Hussien Hospital refers and/or checks on patients. Between January and June 2023, a total of 200 volunteers were recruited, with 100 participants in the healthy, fertile

group and 100 participants in the group of individuals with health conditions. For the seminal fluid examination bacteriological investigation, male infertiles served as controls, and semen was collected from them. The control group consisted of 100 randomly selected participants throughout the trial. Each specimen was subjected to a semen culture. The inclusion parameters were as follows: After a year of regular, unprotected intercourse, infertile guys (ages 20 to 39 years) from partners who failed to have babies. A sterile man with azoospermia. Some participants who have both oligozoospermia and asthenozoospermia. One hundred male participants who looked healthy and fit the same parameters as the subject made up the control group. Al-Azhar University's International Islamic Centre for Population Research and Development has granted ethical permission for the study.

Bacterial isolation and identification from seminal fluid

Seminal samples were processed for bacterial isolation and identification using the method described by Olana et al. [22]. The specimens were placed in nutrient broth, and colonies that grew were subcultured onto nutrient agar plates. For further identification, specialized media such as blood agar and MacConkey agar (Abcam, UK) were used. Isolated bacterial strains were incubated at 38°C for 24 hours and then at 38°C for an additional 24 hours under aerobic conditions. Gram staining and KOH tests were performed for morphological assessment, and biochemical tests (oxidase and catalase reactions) were conducted for bacterial confirmation [23, 24, 25, 26].

Screening for resistant bacterial isolates

The antibiotic susceptibility of the bacterial isolates was assessed using Mueller-Hinton agar (Abcam, UK) in accordance with the International Standard [27]. Antibiotic discs were placed on the surface of the agar, and the plates were incubated at 37°C for 18-24 hours [28, 29]. The antibiotics used and their concentrations were selected based on established standards for resistant bacterial screening: Erythromycin (15 µg), Clindamycin (2 µg), Oxacillin (1 µg), Cefoxitin (30 µg), Tetracycline (30 µg), Ciprofloxacin (10 µg), Aztreonam (10 µg), Bacitracin (10 µg), Norfloxacin (10 µg), and Amoxicillin (10 µg) were purchased from Sigma-Aldrich, USA. Additionally, Amoxicillin-clavulanic acid (30 µg) and Cefaclor (30 µg) were purchased from Merck, Germany; Amikin (10 µg) from Bristol-Myers Squibb, USA; and Azithromycin (15 µg) from Pfizer, USA.

Phylogenetic study and identification of 16S RNA for the most resistant strain

Bacterial isolates were grown for 24 hours in Luria-Bertani broth (LB) (Abcam, Uk). Following three rounds of centrifugation and recovery washing in 0.85% NaCl, the DNA fragments were harvested at 14,000 g for five minutes. The Gene JET Genomic DNA cleaning kit (Abcam, UK) was used to isolate genomic DNA according to the manufacturer's. Amplification was performed using the forward primer 8F (5′-CTG GCC TAA CAG ATG CAA GTC-3′) and the reverse primer (5′-GGC CGG GGC GTACAA GGC-3′) [66]. The amplified PCR product has undergone cleaning and sequencing. The Finch T.V. 1.5.0 program was used to alter the raw sequencing data. The BLAST program from the National Centre for Genetic Information (NCBI) was used to analyze the strain's 16S rRNA sequences (MD, USA). Aligning multiple sequences was done using ClustalW 2.2. The phylogenetic trees were constructed using MEGA X sign the neighbour-joining technique [30].

Evaluation of the antibacterial impact and minimal inhibitory concentration of Sidr honey

Using the agar diffusion procedure, 100 µl of extract filtrate was administered to fill the holes to assess the antibacterial activity of the aqueous extracts of Sidr honey towards the most resistant bacterial strain isolated from semen. The areas of inhibition were determined after the incubation phase. The effective dose was generated in a series of dilutions and evaluated for a variety of test organisms [31, 32].

Detection of the antioxidant impact of Sidr honey

The antioxidant test based on the electron transfer process was used to measure the sidr honey samples' DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) (Abcam, Uk) scavenging free radical capabilities. The specimens in various dilutions were left to incubate with DPPH for 20 minutes at ambient temperature. The spectroscopic method at 530 nm was used to measure the colour fluctuation of DPPH. Ascorbic acid served as our standard reference [33].

Determination of the anti-inflammatory role of Sidr honey

To evaluate the anti-inflammatory properties of Sidr honey extract, membrane stabilization testing was conducted. A series of specimen concentrations, ranging from 100 to 1000 µg/mL, were prepared. The samples that have been collected were infused with a hypotonic liquid. As negative and positive standards, indomethacin and purified water were both used. After adding 500 µL of the specimens to the fresh erythrocyte solution (2.8%) in 0.7 mL of saline, the mixture was incubated for two hours at 38°C. Following incubation, the mixture was centrifuged at 17,000 ×g for 25 minutes at 10°C. The absorbance of the specimen was then measured at 570 nm [34].

Assessment of the cytotoxic effect of Sidr honey

Vero (African green monkey cells) were used to assess the cytotoxic consequences of Sidr methanol extract. Following 24 hours of attachment until convergence, cells were given the extract at doses varying from 500 to 15.63 µg/mL and incubated for 24 hours at 38 °C. After adding the new medium, 100 µL of MTT solution (6 mg/mL) was introduced, and the mixture was incubated for 4 hours at 38°C. Absorbance was then measured at 580 nm using a microplate reader [35].

Statistical analysis

For data analysis, a Tukey's post hoc test was performed at $p < 0.05$ after a one-way ANOVA was used to assess for significance across the treatments. The examination of statistical data was carried out using GraphPad Prism 6.0.

Results

Chemical composition of Sidr honey

Characterization of phenolic compounds using HPLC identified 12 phenolic compounds at varying levels, including caffeic acid (4.02), chlorogenic acid (13.52), cinnamic acid (10.13), ellagic acid (8.52), ferulic acid (7.73), gallic acid (6.00), coumaric acid (5.21), sinapic acid (7.40), syringic acid (9.56), vanillic acid (10.80), hydroxycinnamic acid (12.20), and protocatechuic acid (5.11), as shown in Figure 1 and Table 1. Furthermore, HPLC analysis of flavonoids revealed nine distinct compounds with varying concentrations: 7-OH flavone (7.71), naringin (11.08), rutin (9.36), myricetin (9.00), quercetin (10.67), kamferol (24.61), luteolin (9.90), apegnin (8.8), and catechin (11.87), as depicted in Figure 2 and Table 2.

Bacterial isolation from semen

All **2**00 research individuals, 100 healthy control subjects and 100 diseased participants had their semen cultures performed. Only 70 of the 100 patients had positive semen cultivation. As shown in Figure 3A, of the 70 patients with 70 bacteriospermia, 43 percent had Gram-positive microbes and only 57 percent had Gram-negative bacteria. Bacteriospermia contained the most prevalent microbe, *E. coli*, which made up 65.0%. The rates of Gram-positive bacterial isolates for *S. aureus*, *B. subtilis*, and *E. faecalis* were 42%, 37%, and 21%, respectively. However, as shown in (Figure 3b), the proportions of the Gram-negative bacteria were *E. coli*, *K. pneumonia*, *P. mirabilis*, and *N. gonorrhoeae*, which were 65%, 21%, 7%, and 7.0%, sequentially. There was a significantly substantial distinction ($P < 0.05$) in the location of the isolated bacteria between infected and non-infected semen.

Antibacterial and screening for sensitivity

The antibiotic sensitivity evaluation was performed using the Kirby-Bauer disc diffusion method, which determined the minimum inhibitory concentration (MIC) for each bacterial isolate against the most commonly used antibiotics for the treatment of pyospermia. While some Gram-negative bacteria exhibited resistance to certain antibiotics, all Gram-positive bacteria were susceptible to the majority of the antibiotics. Additionally, as shown in Table 3 and Figure 4, *E. coli* was identified as the most prevalent and resilient bacterium among the collected specimens, highlighting its strong antibacterial resistance.

Identification of *E. coli* genetically

By applying 16S RNA, the most resilient bacterial isolate strain was identified as *Escherichia coli*. It was deposited in the gene bank with accession number PQ637169 (https://www.ncbi.nlm.nih.gov/nuccore/PQ637169) and, according to the phylogenetic tree (Figure 5), it shared 99.0% of its similarities with the isolates in the gene bank.

Antibacterial role Sidr honey versus the most resistant bacterial strain

The most resistant strains obtained from semen samples exhibited a notable response to the antibacterial effect of Sidr honey extract, with an inhibition zone of 2.6±0.5 mm. The MIC was determined to be 250±0.6 µg/ml, highlighting the

effectiveness of the honey against antibiotic-resistant bacteria, as shown in Figure 6.

Antioxidant of Sidr honey

Ascorbic acid, a well-known antioxidant, demonstrated a strong antioxidant activity with an IC_{50} value of 2.62±0.8 µg/ml. In comparison, Sidr honey extract exhibited slightly higher antioxidant activity with an IC_{50} value of 3.41 ±0.3 µg/ml. This result indicates the potent antioxidant potential of Sidr honey extract, which is comparable to that of ascorbic acid, as shown in Figure 7.

Anti-inflammatory of Sidr honey

As a standard anti-inflammatory agent, indomethacin demonstrated a potent anti-inflammatory effect with an IC_{50} value of 8.72±0.7 µg/ml. In contrast, Sidr honey extract showed a slightly higher IC_{50} value of 13.21±0.2 µg/ml, indicating its own strong anti-inflammatory activity. These findings suggest that while Sidr honey extract's anti-inflammatory effect is slightly less potent than that of indomethacin, it still exhibits significant potential, as depicted in Figure 8.

Cytotoxicity of Sidr honey

The cytotoxicity of Sidr honey extract was assessed using the MTT protocol to evaluate its effects on normal cells. The results demonstrated that Sidr honey extract exhibited a significant cytotoxic effect, with a CC_{50} value of 22.2±1.2 µg/ml. This indicates that at this concentration, 50% of the normal cells were inhibited, highlighting the extract's potential cytotoxic activity. The findings are illustrated in Figure 9.

IC_{50} (half maximal inhibitory concentration) and CC_{50} (half maximal cytotoxic concentration) are measures used to assess the effectiveness and safety of substances. IC_{50} measures the concentration required to inhibit a biological process by 50%, while CC_{50} measures the concentration that causes a 50% reduction in cell viability. A low IC_{50} value indicates higher effectiveness at lower concentrations, while a low CC_{50} value indicates lower toxicity, suggesting that Sidr honey has beneficial effects with minimal toxicity.

Table 1. Determination of various phenolic compounds in extract of Sidr honey

Retention Time	Molecule Name	Conc. (µg/ml)
3.0	**Caffeic acid**	**4.02**
5.0	**Chorogenic acid**	**13.52**
6.0	**Cinnamic acid**	**10.13**
8.0	**Ellagic acid**	**8.52**
9.0	**Ferullic acid**	**7.53**
11.0	**Gallic acid**	**6.00**
13.0	**Coumaric acid**	**5.21**
14.0	**Sinapic acid**	**7.40**
15.0	**Syringic acid**	**9.56**
16.2	**Vanilic acid**	**10.80**
17.5	**Hydeoxycinnamic acid**	**12.20**
20.7	**Protocatechulic acid**	**5.11**

Table 2. Detection of different flavonoids in extract of Sidr honey.

Retention Time	Molecule Name	Conc. (µg/ml)
3.0	**7-OH flavone**	7.71
4.2	**Naringin**	11.08
5.3	**Rutin**	9.36
6.0	**Myricetin**	9.00
8.0	**Quercetin**	10.67
11.0	**Kamferol**	24.61
13.0	**Luteolin**	9.90
14.0	**Apegnin**	5.8
15.0	**Catechin**	11.87

Table 3. Sensitivity pattern for various bacteria isolated from semen (Inhibition diameters were recorded by mm, as means ± SD, R: Resistant).

Isolates	E	DA	CEC	NOR	AM	AMC	OX	TE	FOX	TN	AN	ATM	AZM	B
G. positive bacteria														
S. aureaus	3.2±0.1	3.1±0.3	2.7±0.3	2.3±0.1	2.4±0.1	2.7±0.1	2.1±0.1	2.4±0.1	3.0±0.2	3±0.3	1.8±0.1	1.7±0.2	1.8±0.1	1.5±0.1
B. Subtilis	1.7±0.2	1.8±0.2	2.2±0.2	2.2±0.2	2.1±0.2	2.2±0.2	1.9±0.2	1.7±0.2	1.8±0.3	1.7±0.4	1.4±0.3	1.6±0.3	1.9±0.1	1.7±0.1
E. faecalis	1.8±0.1	2.2±0.1	2.1±0.1	R	3.1±0.1	2.1±0.3	2.0±0.1	2.1±0.2	1.7±0.2	1.8±0.1	1.7±0.1	1.6±0.1	1.8±0.1	1.8±0.3
G. negative bacteria														
E. coli	R	R	0.5±0.1	R	R	R	R	0.2±0.1	R	R	R	R	R	R
P. mirabilis	1.7±0.1	1.1±0.2	R	1.7±0.2	1.4±0.2	2.1±0.2	R	1.7±0.1	1.4±0.3	R	1.8±0.2	1.8±0.3	1.8±0.2	1.7±0.1
P. aeruginoa	1.3±0.1	R	1.6±0.2	1.7±0.2	1.7±0.4	2.2±0.3	1.3±0.1	1.5±0.2	1.4±0.2	1.6±0.2	1.8±0.1	1.7±0.1	1.9±0.1	1.4±0.2
N. gonorrhoeae	1.4±0.1	R	1.5±0.2	1.6±0.1	1.8±0.1	2.1±0.1	1.7±0.2	1.6±0.2	1.6±0.2	1.4±0.2	1.8±0.3	1.6±0.4	1.9±0.2	1.5±0.1

Abbreviations for antibiotics: E: Erythromycin, DA: clindamycin, CEC: Cefaclor, NOR: Norfloxacin, AM: Amoxicillin, AMC: Amoxicillin-clavulanic acid, OX: Oxacillin, TE: tetracycline, FOX: cefoxitin, TN: Ciprofloxacin, AN: Amikin, ATM: Aztreonam, AZM: Azithromycin, B: Bacitracin

Figure 1. HPLC chromatogram for various phenolic compounds in Sidr honey extract (The concentrations were measured by (µg/ml).

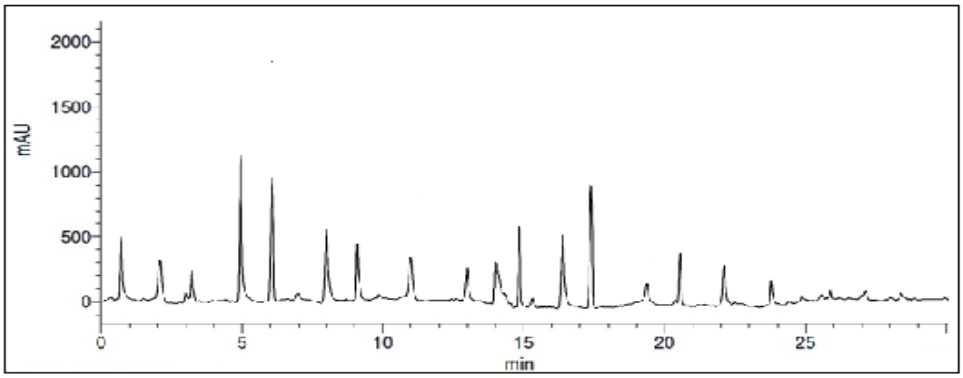


Figure 2. HPLC chromatogram for various flavonoids in Sidr honey extract (The levels were detected by (µg/ml).

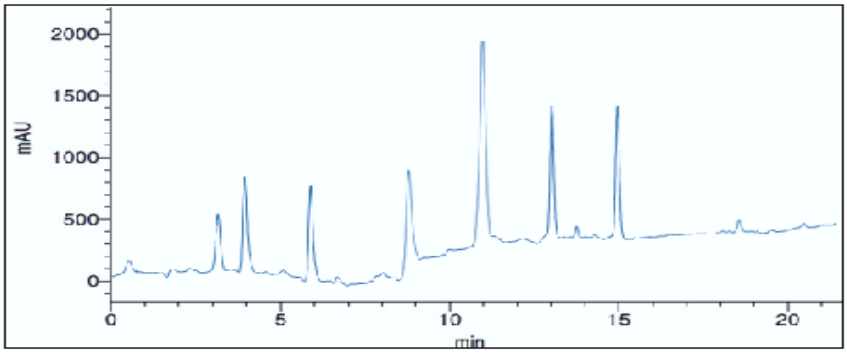


Figure 3. A: Various levels of Gram positive and Gram negative bacteria in semen samples; **B:** Distribution of different ratios of bacteria in two sub-groups of bacteria.

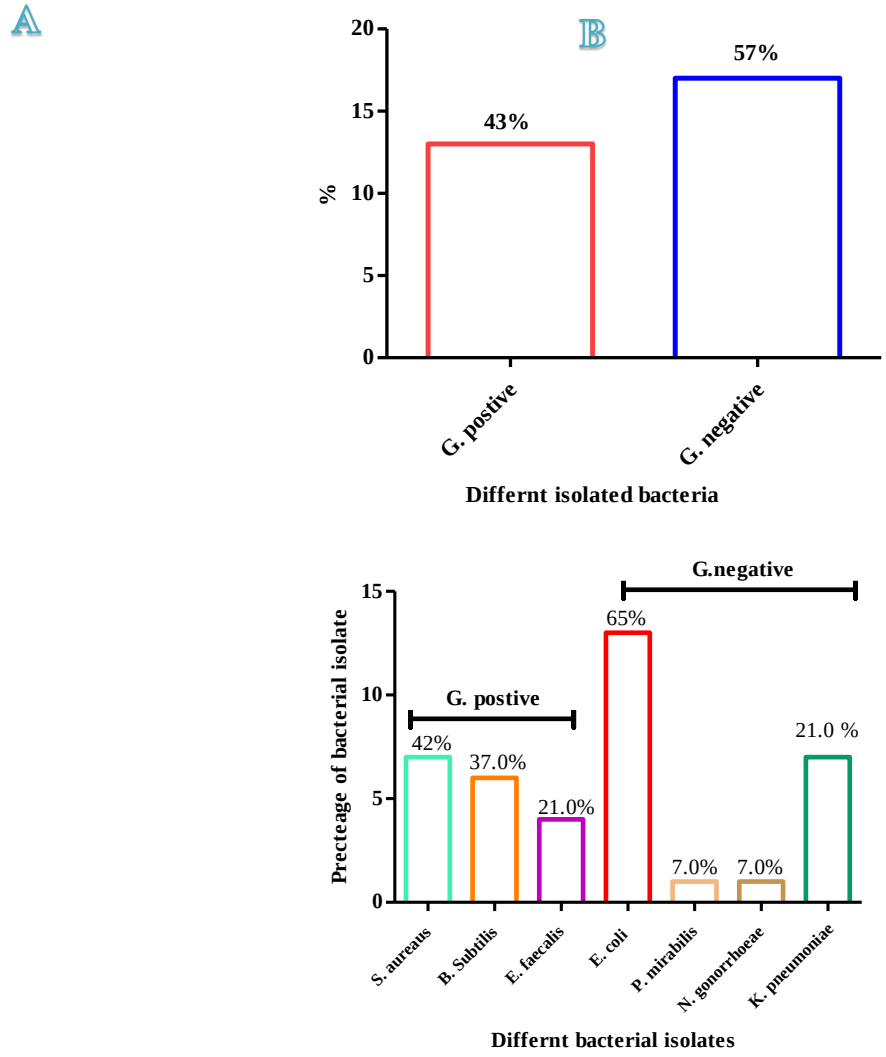


Figure 4. Testing the antibiotics impact towards bacteria isolated from infected semen to determine the most resistant strain in the study group.

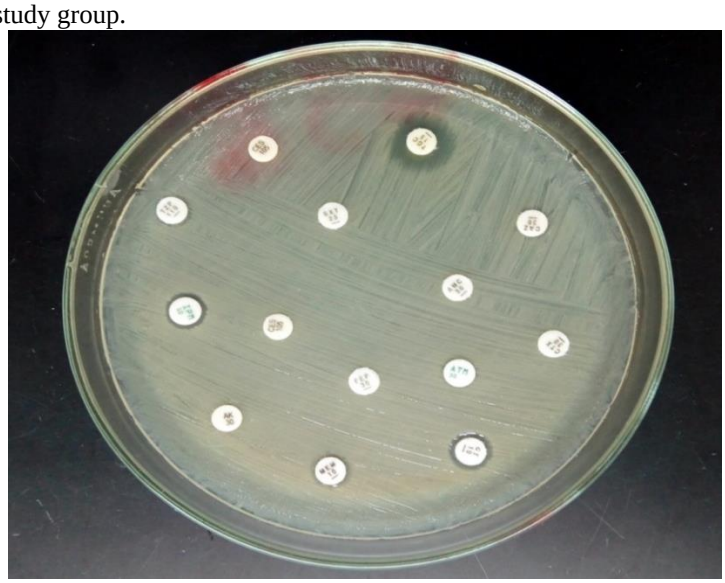


Figure 5. Phylogenetic tree for isolated *E. coli* as the most resilient bacterial strain from semen.

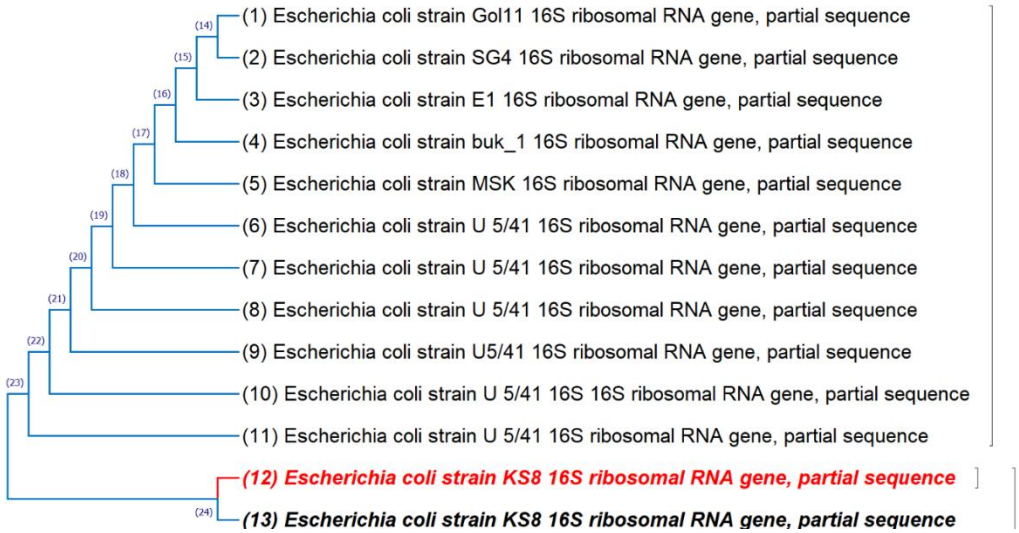


Figure 6. Antibacterial impact to Sidr honey towards resistant E. coli isolated from semen (Sh: Sidr honey extract, - ve ctrl: negative control which was methanol as solvent for extraction; G: Gentamicin as positive control.

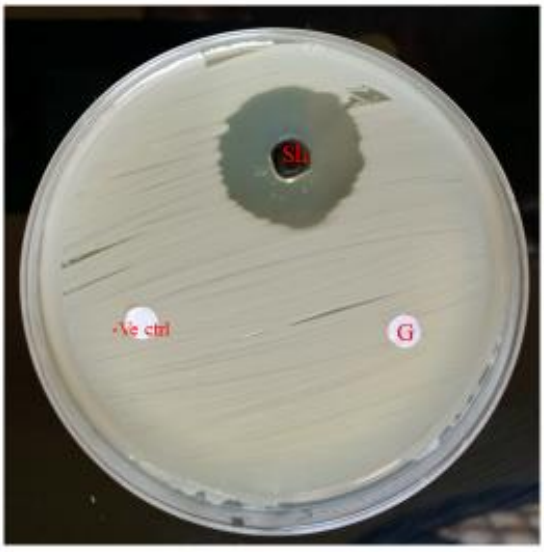


Figure 7. Using the DPPH method, the antioxidant effect of Sidr honey extract was determined (The outcomes recoded as means ± SD).

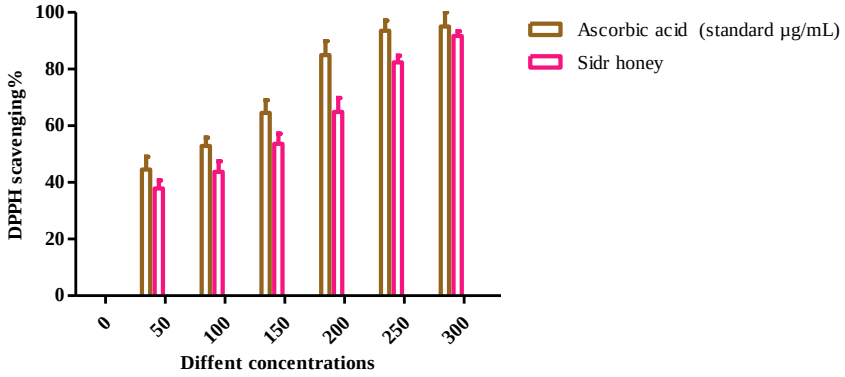


Figure 8. Hemolysis procedure for detecting anti-inflammatory properties of Sidr honey extract (The findings are shown as means ± SD).

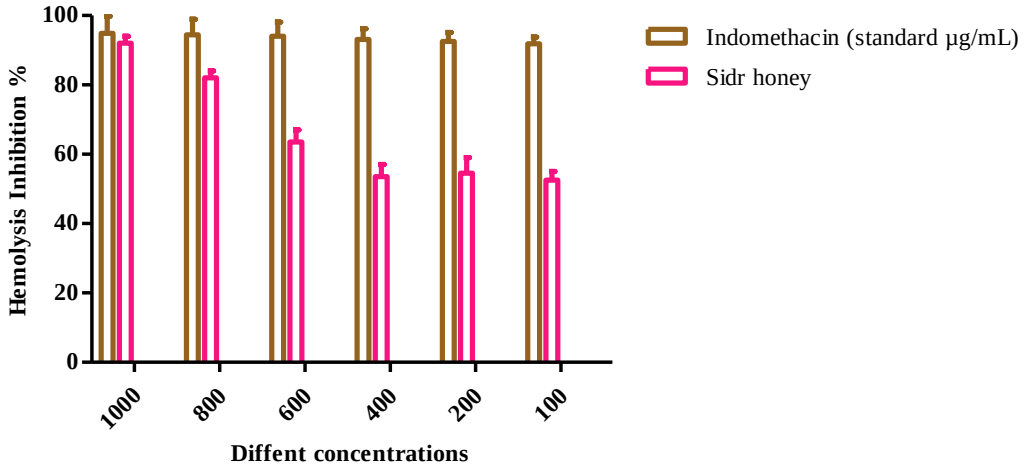
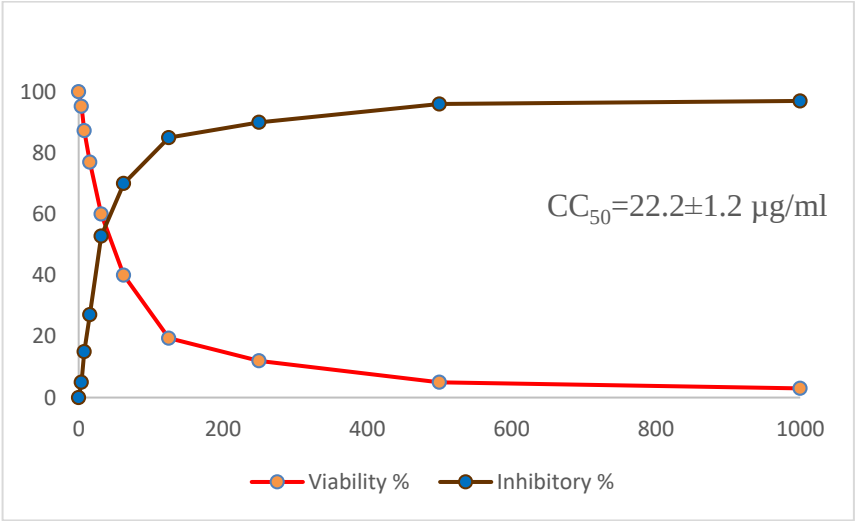


Figure 9. Evaluation of Sidr honey extract's cytotoxic influence on normal cells using the MTT test (The outcomes are shown as means ± SD).



Discussion

Honey may include intriguing bioactive chemicals with strong biological potential [36, 37]. Many parameters, including the source of honey, can affect this activity [1, 2]. Sugars, minerals, and vitamins are among the more than 200 components of honey that have been identified. Honey's high phenolic and flavonoid content is not the sole factor contributing to its biological activities; additional active families of chemicals should also be investigated [38, 39].

In the present investigation screening for various phenolic compounds and flavonoids was done in Egyptian Sidr honey extract using HPLC, and the outcome revealed the existence of twelve various phenolic compounds as well as nine various flavonoids with different levels where **Vanillic acid and Kaempferol were the most common molecules in the two groups**. Vanillic acid and kaempferol are significant due to their potent biological activities. Vanillic acid, a phenolic compound, is known for its antioxidant, anti-inflammatory, and antimicrobial properties [40] . It plays a crucial role in reducing oxidative stress and protecting cells from damage [41]. Kaempferol, a flavonoid, exhibits strong antioxidant, anti-inflammatory, and anticancer activities. It also contributes to improving immune function and reducing the risk of chronic diseases [42]. Both compounds enhance the therapeutic potential of Sidr honey in treating infections and inflammatory conditions. Honey's component phenolic and flavonoid chemicals are responsible for the majority of its biological actions. The bioavailability of different phytochemical components, as well as how

they are absorbed and metabolized, has been discovered to influence honey's impact on heart health [43]. Given that every variety of honey has unique health benefits, the potent cytotoxic, antioxidant, and anti-inflammatory effects observed in Sidr honey extract are not surprising. Sidr honey, being rich in bioactive compounds, has demonstrated a range of therapeutic properties, further supporting its potential for use in medicinal applications [44]. Twelve honeys gathered from different parts of Greece were examined for their phenolic acid profiles, and it was discovered that they were high in phenolic acids, specifically p-hydroxybenzoic acid [45]. In light of the detection and availability of one or more particular phenolic chemicals, some research has suggested chemical clues for identifying the honey's vegetative source [46]. According to Ferreres et al. [47], ellagic acid might be a good marker for Portuguese Erica species.

The bacteria that cause semen infections typically come from the patient's urinary tract or can be passed from one spouse to another through sexual activity [47]. *E. coli* is the most commonly isolated pathogen in male patients with semen infection or genital tract pathogens. This species' detrimental impact on the quality of sperm is partly caused by its impact on motility [48] and its compromised acrosome function, as Diemer et al. [49] showed at the ultrastructural level. Little research has been done on how Gram-negative uropathogenic bacteria affect the shape and function of sperm [50]. In the present investigation *E. coli* was reported as the most common resistant bacterial strain isolated from semen from male patients with fertility problems in El Hussien Hospital.

Egyptian Sidr honey has shown antibacterial efficacy versus the most resilient bacterial pathogen identified in semen in the current investigation. Honey's antibacterial qualities are also attributed to a variety of bioactive substances, such as phenolic acids, flavonoids, and enzymes [51]. According to investigations, honey can stop a variety of bacteria from growing. It has been demonstrated that honey works well against bacteria that are tolerant to antibiotics [52-54].

Honey's powerful antioxidant properties are one of its many advantages and the primary justification for its use as a food preservative [55] Flavonoids and phenolic acids are primarily responsible for honey's biological features, such as its antioxidant action [56]. By minimizing the harm that different oxidizing agents could do, the abundance of these chemicals in honey preserves human health [57, 58]. The present study illustrates that the Egyptian Sidr honey had a promising antioxidant action that might assist in its antibacterial role towards resistant bacterial strains.

The present report highlights the possible potential of applying Egyptian Sidr Honey as an anti-inflammatory agent with minimal toxicity. Honey, in general, has long been recognized for its therapeutic properties and has been utilized in traditional medicine across various cultures [59]. Sidr honey, in particular, stands out due to its rich bioactive composition, which includes phenolic compounds and flavonoids that contribute to its medicinal effects [60]. The findings of this study suggest that Sidr honey could serve as a valuable natural alternative for treating inflammation, with the added benefit of minimal cytotoxicity, making it suitable for further development in pharmaceutical applications. Microbial and inflammatory disorders have been treated with notable therapeutic results. Honey phytonutrients and their pharmacologic use in cancer treatment have garnered more attention recently. Numerous findings demonstrated that different varieties of honey exhibited notable anticancer effects [61]. Numerous studies indicate that honey may have anticancer properties through a variety of molecular pathways that have anti-inflammatory and immunomodulatory effects [62, 63]. The therapeutic benefits of Sidr Honey have been extensively studied [64]. Numerous additional investigations have also revealed significant antioxidant, anti-inflammatory, and anticancer activities, indicating that Sidr Honey is a promising area for further investigation into potential cancer treatment options [65].

Conclusion

Egyptian Sidr honey has a large variety of phenolic compounds and flavonoids with a considerable level that may help in its antibacterial impact towards resistant *E. coli* isolated from semen from Egyptian patients from El Hussien Hospital. Sidr Honey has promising in vitro antioxidant and anti-inflammatory potential with the least toxicity towards Vero cells to be used for larger application in the upcoming studies.

Funding

Non

Conflict of interest

Authors have no conflict of interest to declare

Data availability

Data available on request / reasonable request

Authors' contribution

Khalid M. Swidan: conception, design of the study and submitting the article to the journal, **Mahmoud K. A. Ismail:** drafting the article and data collection, and **Tarek Mohamed Abdelghany**: final approval.

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