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Research Article

# *In vitro* Assessment of Two commercial Honey Samples for Antibacterial and Antioxidant Activities

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**Abstract**

The current communication represents the antibacterial and antioxidant activities of two commercial honeys: Dabur Honey (DH) and Patanjali Honey (PH) against clinical isolates of *Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.* The antibacterial activities of PH and DH (both autoclaved and non-autoclaved), were determined alone and in combination with antibiotics, Gentamicin (GM) and Kanamycin (KM) against the test isolates. The autoclaved PH and DH (at concentrations $10\times10^3$ and $15\times10^3$µg/disc) had Zone of Inhibition Diameter (ZID) 9 – 17 mm and 9 – 13 mm, respectively, against the gram-negative bacteria *(P. vulgaris, E. coli, Ps. aeruginosa); S. aureus* was resistant to almost all concentrations of the honeys. The non-autoclaved honeys (at concentrations $10\times10^3$ and $15\times10^3$µg/disc) showed excellent activity against both gram-positive *(S. aureus)* and gram-negative bacteria tested (PH honey had ZID 10-27 mm, and DH honey had ZID10-30 mm). The $IC_{50}$ values of PH and DH, in 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) system, were $132.24\times10^3$ µg/ml and $66.73\times10^3$µg/ml, respectively; both the honeys contained steroids, quinones and terpenoides. In combination with KM and GM the autoclaved honey samples (PH and DH) had synergistic activity against *S. aureus* and *E. coli* ATCC 25922 standard strain, while GM-honey and KM-honey combinations had synergistic interaction against *Ps. aeruginosa* and *P. vulgaris*, respectively. Thus, PH and DH alone and in combination with GM/KM can be used against different bacterial strains causing infection to humans.

## Introduction

Honey has been in use for its healing, nutritional and therapeutic properties since ancient times, and currently it has been proved experimentally that honey possesses anti-bacterial, anti-inflammatory and anti-oxidant properties, which may be beneficial in combating multi-drug resistant bacteria as well as in preventing many chronic inflammatory processes [1]. Honey, which is a healthy food stuff and nutrition, serves as a good source of natural antioxidant, and thus it is free radical scavenger reducing the formation of free radicals, or neutralizing them that produce beneficial effects in human health [2]. The improved status of serum total anti-oxidation among young females with regular use of honey revealed it is one of the most acceptable form of food to keep balance between antioxidants and prooxidants minimizing the onset of many diseases [3]. Various studies explained the mechanism of action of different honeys against antibiotic resistant bacteria *in vitro* and their antibio film activity, and an important clinical advantage is that resistance to honey has not yet been detected in microorganisms causing human infection [4,5].

Mandal et al. [6] determined the antibacterial activity of honey against clinical isolates of *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella enterica* serovar Typhi. Das *et al.* [2,7] investigated the antioxidant properties of various unifloral honeys procured from West Bengal, India. Allen *et al.* [3,8] have revealed that the honey is effective against Methicillin-Resistant *S. Aureus* (MRSA), β-*haemolytic streptococci* and Vancomycin Resistant *Enterococci* (VRE). Cooper *et al.* [4,9] discovered the antibacterial activity of honey against strains of *S. aureus* from infected wounds. Visavadia *et al.* [5,10] conducted research on manuka *(L. scoparium)* honey, and showed its activity against several human pathogens, including *E. coli, Enterobacter aero genes, Salmonella typhimurium, S. aureus.* Hussein *et al.* [6,11] reported, Gelam honey has anti-oxidative and radical scavenging activities, which are mainly attributed to its phenolic content. Khalil *et al.* [7,12] discovered antioxidant property of Algerian honey, as indicated by their high phenolic, flavonoid, ascorbic acid and proline contents.

The importance of honey in medical science has already been described by Mandal *et al* [13]. Thus, honey, both natural and commercial, has been used traditionally over the years by the people in India as food, and as traditional medicine in the treatment of various health disorders, but only a few data based on the scientific studies are available to support the medicinal claims of commonly consumed honey in our part of the globe. Therefore, the current study has been undertaken to investigate the antibacterial and Antioxidative activities of two types of commonly consumed commercial honey samples: Dabur Honey (DH) and Patanjali Honey (PH) purchased from local market (Malda, India); to the best of our knowledge, this is the first study of its kind from our part of the globe.

## Materials and Methods

### Bacterial strains

The bacterial strains used in the study included *E. coli, Ps. aeruginosa, P. vulgaris,* and *S. aureus*; the *E. coli* ATCC 25922 strain was used as control. The identified bacterial isolates were kindly provided by Dr. N. K. Pal, Professor and Head, Department of Microbiology, Malda Medical College, Malda (India).




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**Table 1:** Anti bacterial activity of different concentrations of DH (autoclaved and non-autoclaved) against clinical isolates of bacteria and the standard strain.

| Bacterial strain | ZID (mm) at different concentrations of honey (×10³µg/disc) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Autoclaved | | | | Non-autoclaved | | | |
| | 2.5 | 5 | 10 | 15 | 2.5 | 5 | 10 | 15 |
| *E. coli* ATCC 25922 | 6 | 6 | 13 | 17 | 13 | 18 | 24 | 27 |
| *E. coli* (clinical) | 6 | 6 | 14 | 16 | 8 | 6 | 11 | 11 |
| *P. vulgaris* | 8 | 6 | 9 | 10 | 6 | 6 | 10 | 12 |
| *Ps. aeruginosa* | 6 | 6 | 10 | 12 | 6 | 12 | 12 | 13 |
| *S. aureus* | 6 | 6 | 9 | 6 | 6 | 6 | 21 | 24 |

DH: Dabur Honey; the values 2.5, 5, 10 and 15 indicate the concentrations of honey (×10³ µg/disc), and the values represented below each of the concentrations and against the bacterial isolates indicate the Zone of Inhibition Diameter (ZID; mm).

**Table 2:** Anti bacterial activity of different concentrations of PH (autoclaved and non-autoclaved) against clinical isolates of bacteria and the standard strain.

| Bacterial strain | ZID (mm) at different concentrations of honey (×10³µg/disc) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Autoclaved | | | | Non-autoclaved | | | |
| | 2.5 | 5 | 10 | 15 | 2.5 | 5 | 10 | 15 |
| *E. coli* ATCC 25922 | 8 | 7 | 9 | 10 | 9 | 11 | 14 | 20 |
| *E. coli* (clinical) | 8 | 10 | 11 | 13 | 9 | 17 | 22 | 21 |
| *P. vulgaris* | 9 | 10 | 13 | 12 | 6 | 10 | 10 | 20 |
| *Ps. aeruginosa* | 7 | 8 | 10 | 11 | 8 | 8 | 10 | 15 |
| *S. aureus* | 6 | 6 | 10 | 11 | 6 | 23 | 27 | 30 |

PH: Patanjali Honey; the indicative values for Zone of Inhibition Diameter (ZID; mm) and the honey concentrations are as mentioned in the Table 1.

### Honey samples and disc preparation

Two commercial honeys: Dabur Honey (DH) and Patanjali Honey (PH) were purchased from market (Malda, India), and were utilized in the study. One gram of honey diluted in 5ml of double distilled water (200µg/µl) was autoclaved at 121°C for 15 min. Similarly, non-autoclaved aqueous honey sample (200µg/µl) was also prepared for the study. The PH and DH were subjected to screening tests for bioactive compounds following standard protocol.

Both autoclaved and non-autoclaved honey samples (PH and DH) were utilized in disc preparation. The autoclaved blank paper discs (6 mm diameter; punched from what man No. 1 filter paper) were soaked either with diluted PH or DH, to prepare honey discs of different concentrations: 2.5×10³, 5×10³, 10×10³ and 15×10³µg/disc.

### Antibacterial activity

The antibacterial activity of PH and DH were determined for the *E. coli* ATCC 25922, *E. coli*, *Ps. aeruginosa, P. vulgaris,* and *S. aureus*; by disc diffusion method using sterile Nutrient Agar (NA) plates, each of which were inoculated with $10^8$ CFU from young broth culture of the test bacteria. The discs containing different concentrations of honey, as mentioned above, were placed aseptically on the inoculated NA plates, and incubated at 35$^0$C for 16-18 h. The sensitivity of the test bacterial isolates to PH and DH (autoclaved and non-autoclaved) were considered with Zone of Inhibition Diameter (ZID) ≥7 mm.

### Antibiotic susceptibility testing

Antibiotic sensitivity testing was performed by disc diffusion method following the NCCLS (National Committee for Clinical Laboratory Standards) guidelines for the bacterial isolates the *E. coli* ATCC 25922, *E. coli, Ps. aeruginosa, P. vulgaris,* and *S. aureus*:, tested against Gentamicin (GM) and Kanamycin (KM). The antibiotic discs, GM (10 µg/disc) and KM (10 µg/disc), were purchased from Hi-Media, India.

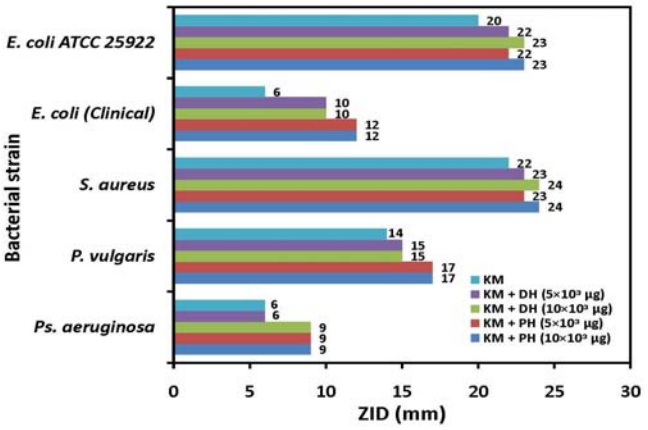


**Figure 1:** Combined antibacterial activity between KM (kanamicin) and honey samples against clinical bacterial isolates and the standard strain. PH: Patanjali Honey; and DH: Dabur Honey; ZID: Zone of Inhibition Diameter.

In order to determine the combined effect of honey and antibiotic (GM/KM) against the test isolates, PH and DH (both non-autoclaved), two different amount of each of the honey samples:1×10³ µg (5 µl) and 10×10³µg (50 µl), were dropped on GM and KM discs on the NA plates inoculated with $10^8$ CFU. The ZIDs were recorded after 16-18 h incubation at 35 $^0$C. The combined antibiotic (GM/KM)-honey (PH/DH) activity was considered synergistic when the ZID from the combined action for a given bacterial strain was increased compared to the ZIDs obtained from the single action of both antibiotics and honey samples.

### DPPH-free radical-scavenging assay for antioxidant activity

The free radical-scavenging activity for DH and PH was studied following the protocol of Habib *et al.* [14], through the evaluation of free radical-scavenging effect on 2, 2-Dipheny-1-Picrylhydrazyl (DPPH) radical. To 3.8 ml of methanolic DPPH solution (0.25 mM) aliquots (200 µl) of PH and DH (aqueous solution) at different concentration (25 × 10³, 50 × 10³, 75 × 10³, 100 × 10³, 125 × 10³ and 150 × 10³µg/ml) were mixed, and incubated in the dark for 30 min, following which the absorbance was measured colorimetric ally at 520 nm against methanol without DPPH as blank. The results were expressed as % inhibition of DPPH radical, which was calculated according to the equation: *% inhibition of DPPH = Absorption (control) — (Absorption honey)/Absorption (control) × 100;* where Absorption (control) is the absorbance of DPPH solution without the test sample (PH and DH). The $IC_{50}$values of PH and DH (honey concentration, mg/ml, which scavenges the DPPH radicals by 50 %) were calculated using linear regression of plots where the x-axisrepresented the concentration of honey and the y-axis represented the % inhibition (antioxidant activity).

## Results

The ZIDs obtained due to the action of DH (both non-autoclaved

and autoclaved), at different concentrations, against clinical isolates of *E. coli, P.vulgaris, Ps. aeruginosa* and *S. aureus* are presented in Table 1. The bacterial isolates were resistant to the autoclaved honey at concentrations $2.5\times10^3$ -$5\times10^3$ µg/disc, while the ZIDs ranged 9-16 mm for clinical isolates of *E. coli, P. vulgaris, Ps. aeruginosa* and *S. aureusat* concentrations $10\times10^3$ -$15\times10^3$ µg/disc; the *S. aureus* was resistant to the honey at concentration $15\times10^3$ µg/disc. The bacterial isolates of *P. vulgaris* and *S. aureus* were resistant to the non-autoclaved honey at concentrations $2.5\times10^3$-$5\times10^3$µg/disc; *Ps. aeruginosa* was resistant to the honey ($2.5\times10^3$µg/disc) and *E. coli* was resistant to the honey at concentration $5\times10^3$µg/disc; the ZIDs ranged 10-24 mm for the clinical isolates of *E. coli, P. vulgaris, Ps. aeruginosa* and *S. aureus* at concentrations $10\times10^3$-$15\times10^3$µg/disc.

The ZIDs obtained due to the action of PH (both non-autoclaved and autoclaved), at different concentrations, against the test isolates are presented in Table 2. The bacterial isolate of *S. aureus* was resistant to the autoclaved honey at concentrations $2.5\times10^3$-$5\times10^3$µg/disc; in case of *Ps. aeruginosa, E. coli* and *P. vulgaris* ZID ranged 7-10 mm at same concentrations, while the ZIDs ranged 9-13 mm for all the clinical isolates of *E. coli, P. vulgaris, Ps. aeruginosa* and *S. aureus* at concentrations $10\times10^3$-$15\times10^3$µg/disc. The bacterial isolates of *P. vulgaris* and *S.aureus* were resistant to the non-autoclaved honey at concentration $2.5\times10^3$µg/disc, while in case of *Ps. aeruginosa* and *E. coli,* ZIDs ranged 8-9 mm at the same concentrations. The ZIDs ranged 8-30 mm for clinical isolates of *E. coli, P.vulgaris, Ps.aeruginosa* and *S. aureus* in presence of $2.5\times10^3$-$15\times10^3$µg/disc of non-auto claved honey. The susceptibility patterns of *E. coli* (ATCC 25922 strain), which was used as control, against all honey types are represented in Table 1 and Table 2.

The ZIDs obtained in antibiotic action alone and in combination with two honey samples against *E. coli, P. vulgaris, Ps.aeruginosa* and *S.aureus* isolates are presented in Figure 1. The isolates of *E.coli* and *Ps.aeruginosa* showed resistance and *P. vulgaris* showed intermediately susceptibility to KM; *S.aureus* was sensitive to KM. The ZIDs obtained due to the action of GM alone and in combination with two honey samples against the test isolates are presented in Figure 2.

The scavenging activity (% inhibition) of the test honey samples (DH and PH) in DPPH system is represented in Figure 3. The lowest inhibition exerted by DH and PH honey samples were 17.24 % and 28 %, respectively, at concentration $25\times10^3$µg/ml, while the highest scavenging activities (55.5 % and 71.78 %, respectively) were due to $125\times10^3$µg/ml of honey (DH, PH). The highest concentration of the honey samples used was $150\times10^3$µg/ml, at which the scavenging activities were decreased to 42.47 % and 68.43 %, respectively. The $IC_{50}$ values calculated were $66.73\times10^3$µg/ml and $132.24\times10^3$µg/ml for DH and PH honeys, respectively.

## Discussion

In the current study, both the PH and DH (autoclaved and non-autoclaved) had excellent antibacterial activities particularly at concentrations $10\times10^3$ and $15\times10^3$ µg/disc (Table 1 and Table 2). Rakhi *et al.* [15], studied the antibacterial activity of five types of natural honeys (Baidhyanath honey; BH, Uttarakhand honey, DH, Wings honey and Alwar) at different concentrations (20 – 100 %; v/v)

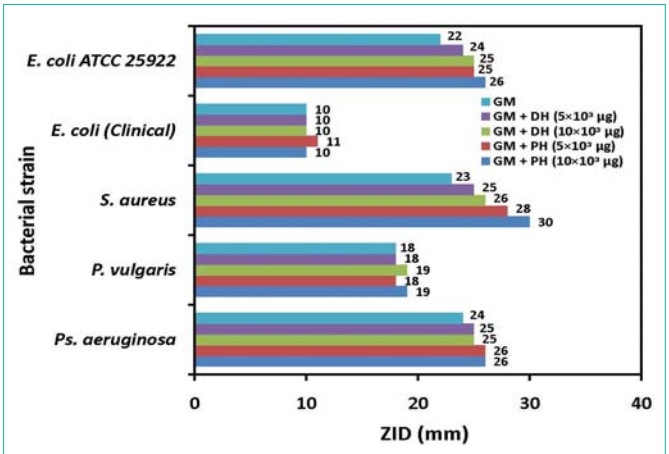


**Figure 2:** Combined antibacterial activity between GM (gentamicin) and honey samples against clinical bacterial isolates and the standard strain. PH: Patanjali Honey; and DH: Dabur Honey; ZID: Zone of Inhibition Diameter.

against *E. coli* and *S. aureus*, and found that BH was more effective among all the others, and ZID ranged 14-28 mm for *E. coli* and 20-36 mm for *S. aureus*. The honey samples such as DH, crude honey-1 and crude honey-2 exhibited inhibitory effect against *E. coli, Salmonella typhi, Ps.aeruginosa* and *P. vulgaris*; the highest antimicrobial activity was found by crude honey-2 (100 %) against *E. coli* and *S. typhi* (ZID; 50 mm each) followed by Dabur honey against *E. coli* (ZID; 48mm) and *S. typhi* (ZID; 46mm) [16]. The Sesame Honey (SH) and Eucalyptus Honey (EH) exhibited excellent activities against *Clostridium acetobutylicum* DSM1731 with ZID 18 mm and 25 mm, respectively, while SH was effective against *C. perfringens* KF383123 strain showing ZID 29 mm [17]. The Karnataka raw honey had ZID 15-17 mm, while the Kerala raw honey had ZID 14-15 mm against the test bacteria; the DH showed least sensitivity (ZID; 8-13 mm) to a number of clinical bacteria [18]. As has been reported by Sharma *et al.* [19], the ZID due to the action of Himachal Pradesh raw honey was 15-17 mm, while the Rajasthan raw honey showed antibacterial activity with ZID 14-16 mm; ZID was 8-14 mm for DH, against *Staphylococcus, Pseudomonas, Bacillus, Streptococcus, P. vulgaris* and *E.coli.* High sugar concentration (approximately 80 % w/v), low pH (3.2-4.5 for undiluted honey) and production of hydrogen peroxide, which upon dilution of honey is produced by glucose oxidase originating from the bees, are probably responsible for the antibacterial activity [19]. The

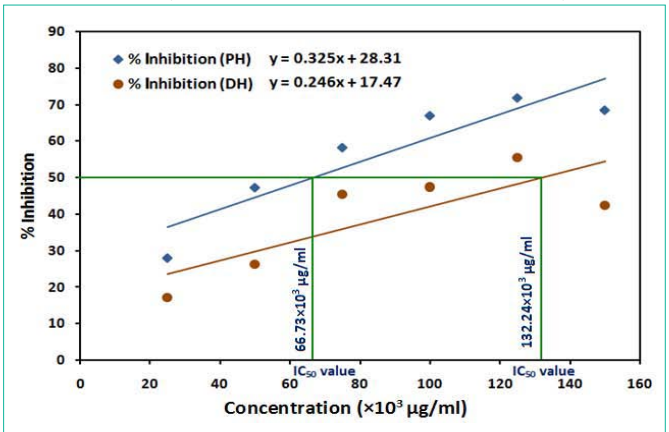


**Figure 3:** Antioxidant activity of PH: Patanjali Honey and DH: Dabur Honey in DPPH system.

earlier studies indicated too that the honey contains enzymes such as glucose oxidase, diastase, invertase, catalase and peroxidase [20] and these enzymes may play role in the antibacterial activity of honey. Al-Waili [21] reported that the bacterial conjunctivitis caused by a variety of human bacterial pathogens, such as *Proteus spp., S. aureus, E. coli, Ps. aeruginosa* and *Klebsiellaspp.*, was treated successfully with the topical application of honey. Ilechie *et al.* [22] documented the potency of Stingless Bee Honey (SBH), which was comparable with that of GM, and suggested that SBH might be a rational agent for the treatment of infective conjunctivitis, since the agent is less expensive and commonly available to the rural population.

The SH showed synergistic effect when combined with cefotaxime (CTX; 30 µg/disc) showing an increase in ZID against C. perfringens KF383123, from 29 mm for SH alone and 8 mm for CTX alone to 40 mm of the combined action; the SH had synergistic effect against *C. acetobutylicumin* combination with CTX, ciprofloxacin (CP; 5 µg/disc) and tobramycin (TOB ; 30 µg/disc), while the EH showed synergistic activity with CTX, CP, cephalexin (CN; 10 µg/disc), with TOB and Sulphamethoxazole (SMZ; 100 µg/disc) against *C. perfringens* [17]. MJawad [23] showed increase in the ZIDs of honey, compared to that of antibiotics ceftriaxone, CP and vancomycin, when used in combination with the antibiotics showing synergistic effect on methicillin resistant Staphylococci. Honey samples ($5\times10^3$ and $10\times10^3$µg/ml) used in combination with KM, in the present study, had increased ZIDs compared to the single action of KM for almost all the isolates, but decreased ZID was seen compared to the single action of the honey samples for *Ps. Aeruginosa* and *E. coli* clinical isolates; in case of *S.aureus* and *P. vulgaris* along with the *E. coli* ATCC 25922 standard strain increased ZIDs were found in combined action compared to the single actions of both antibiotic and honey indicating synergism between honey and KM. The GM in combination with both the honey samples (PH and DH), in the current study, had greater activity (in terms of ZIDs) for *Ps. aeruginosa, S. aureus* and *E. coli* (ATCC 25922), but the ZIDs were similar or increased very slightly (not greater than 1 mm) for *P. vulgaris* and *E. coli* clinical isolates. The PH and DH had increased ZIDs for *P. vulgaris, Ps. aeruginosa, S. aureus* and *E. coli* (ATCC 25922) in combination with GM, showing synergistic activity against the isolates in combination with GM.

The radical scavenging activity of honey varied from23.81% to 100% in the DPPH reaction, as per the report of Wilczyńska [24], who recorded that the dark honeys were highly active in DPPH system. Moniruzzaman *et al.* [25] showed strong correlation between the color intensities of the honey samples and their antioxidant parameters: phenolic acids, flavonoids, and reported the mean DPPH radical-scavenging activity of the Bangladeshi honey samples as 36.95 %; the highest DPPH radical-scavenging activity being 76.68 %.In the present study, in DPPH system, the honey samples had concentration dependant ($25\times10^3$ – $125\times10^3$µg/ml) antioxidant activities that ranged 17.24 - 55.5 % for DH, and 28 - 71.78 % for PH; at the highest concentration the capacities were reduced to 42.47 % and 68.43 %, respectively (Figure 3). Chinwe *et al.* [26] reported that the $IC_{50}$ value of the honey sample tested against DPPH was $12.74\times10^3$ µg/ml. The scavenging ability of multifloral honey samples expressed as $IC_{50}$, with respect to the DPPH, which ranged $3.17\times10^3$-$8.79\times10^3$µg/ml. Pontishoney from the northeast of Brazil had $IC_{50}$ values $4.2\times10^3$-$106.72\times10^3$µg/ml, and most of the values were above $20\times10^3$µg/ml [27]. In a study conducted by Ferreira *et al.* [28], the antioxidant values ranged $106.67\times10^3$-$168.94\times10^3$µg/ml, and according to the report of Beretta *et al.* [29], the values ranged $1.63\times10^3$-$47.62\times10^3$µg/ml. Das *et al.* [7] determined the antioxidant activities of different unifloral honeys, and reported the dark brown Hizal honey as the most potent DPPH radical scavenger ($IC_{50} = 23.92\times10^3$µg/ml). The honey samples used in the current study had $IC_{50}$ $66.73\times10^3$µg/ml and $132.24\times10^3$µg/ml, respectively for DH and PH honeys. The antioxidant properties of honey are due to its both enzymatic (catalase, glucose oxidase and peroxidase) as well as non-enzymatic substances (ascorbic acid, α- tocopherol, carotenoids, amino acids, proteins, flavonoids and phenolic acids) [30-33]. In the present study, the presence of steroids, terpinoids and quinones were determined qualitatively.

## Conclusion

Based upon the excellent antibacterial activity of the honey samples used, alone or in combination with the test antibiotics, in the current study, and the low $IC_{50}$ values ($66.73\times10^3$-$132.24\times10^3$ µg/ml) it can be concluded that the honey, both PH and DH, can be used alone or in combination with KM and GM in combating bacterial antibiotic resistances, as well as a good source of antioxidants; the possible benefits in clinical implications of bacterial infections, however, warrant further investigation [34].

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