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# SYNERGISTIC ANTIBACTERIAL EFFECT OF *APIS FLOREA BEE HONEY* AND CIPROFLOXACIN ON PATHOGENIC BACTERIA

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***Key words:*** *Antibacterial activity, Synergism,* Apis florea *bee honey, Ciprofloxacin, Disc diffusion method.*

**Abstract–**Honey is a natural gift to man from Mother Nature which is made available to us from the mysterious kingdom of the bees. The aim of the present study was to evaluate the synergistic antibacterial effect between the *Apis* honey and common antibiotic ciprofloxacin on multiple antibiotic resistant bacteria. Fifteen honey samples of *Apis florea* were harvested from various geographical areas of Coorg, Karnataka during 2020-2021. The honey samples and ciprofloxacin were subjected to Determination of antibacterial potency by Antibacterial disc diffusion assay separately against the test bacteria. Then followed Screening for synergistic activity and Data Analysis. The findings of the present study revealed that synergistic antibacterial sensitivity with inhibitory zones that were formed in the cultures against the selected bacterial strains showed significant variations. The highest antibacterial activity was recorded against *Staphylococcus aureus* (ATCC 6538) with 19.26 ± 0.23 mm. However, the least sensitivity range was recorded for *Erwinia nigrifluens* (ATCC 21922) with 8.97± 0.48 mm. *Bacillus cereus (*ATCC 31443) showed mm, ZI of 13.74±0.37, *Bacillus subtilis* (ATCC 32441) *with* 12.99±0.27, *Burkholderia glumae* (ATCC 25813) with 11.87± 0.58, *E.coli* (ATCC 25891) *with* 14.95± 0.41 mm, *Klebseilla sp.*(ATCC 31482) with 12.11± 0.59, *Pseudomonas aerugenosa* (ATCC 287858) *with* 17.53± 0.72 mm. The research findings of the present study on the synergistic antibacterial activity of ciprofloxacin and *Apis florea* honey on pathogenic bacteria showed potential usefulness of honey and antibiotics together in the clinical practice against the pathogenic effects of strains of bacteria.

## INTRODUCTION

Honey is a natural food of very high nutritive value which is obtained when nectar and sweet deposits from plants are gathered and stored in the honeycombs of honeybees. Honey has been used in medical practice since ancient times (Al-Jabri *et al.,* 2005; Richard, 2009).

In recent years, pathogenic microorganisms have developed multiple drug resistance due to the abundant and wide spread use of antimicrobial drugs that were commonly used in human medicine (Al-Waili *et al*., 2011; Noori *et al.,* 2013). The therapeutic use of honey has been rediscovered by medical provincial as it inhibits both Gram-positive and Gram-negative bacteria (Hegazi, 2011; Hegazi and Abd Allah, 2012). Honey exhibits several biological activities (Molan, 2002). It was used for the treatment of burns and wounds (Mullai and Menon, 2007), post-surgical wound infection (Namias, 2003), ulcers and bed sore (Brudzynski, 2006), bacterial gastro-enteritis in infants, liver diseases and as an antioxidant (Haffejee and Moosa, 1985; Hegazi and Abd El- Hady, 2009). The antibacterial activity of different honey samples was of specific interest many authors (Kwakman *et al.,* 2010; Halawani and M. Shohayeb, 2011).

The antibiotic and antiseptic effects of honey have been scientifically proven in several studies (Werner and Laccourreye, 2011). These effects are mainly due to the bio-chemical composition of honey that contains high sugar and low water concentrations with low pH. These properties generate the high osmolarity that produces the antimicrobial action (Namias, 2003). Honey also contains molecules inhibiting bacterial growth, such as hydrogen peroxide produced by glucose oxidase; and also the non-peroxide also known as phytochemicals (Montenegro and Mejías, 2013). The mixtures of honey and antibiotics have been reported for

synergistic effects against species of bacteria (Liu *et al.,* 2018; Laallam *et al.,* 2015).

Thus this study aims to investigate the synergistic antibacterial effects of mixtures of *Apis florea* bee honey and Ciprofloxacin against selected pathogenic bacteria.

## MATERIALS AND METHODS

### Study areas

The present study areas of Karnataka were of different biogeographical regions of Coorg (12.3375° N, 75. 8069° E) district. The honey samples collected were from regions of Abbe falls, Kushal Nagar and Somavarapet of Coorg district.

### Procurement of Apis florea bee honey samples

Fifteen honey samples of *Apis florea* species were harvested from various geographical areas of Coorg, Karnataka during 2020-2021. With the help of a local beekeeper, few bees were collected from the hive and identified for *Apis florea* species. Upon identification, the honey samples from the comb were collected under sterile conditions. Earlier studies of the honey from *Apis florea* bee were found to be effective against the bacteria (Bhushanam and Madhusudan, 2019). Each honey sample was first filtered with a sterile mesh to remove debris. All the samples were collected and transported in sterile sealed bottles or screwed cups with authentic labels. Four replications of bottles for each sample were kept under storage at 2-8 °C until tested as per the method proposed by Nzeako and Hamdi (2000).

### Determination of antibacterial potency of honey samples

### Collection of bacterial isolates

The test isolates were collected from American Type Collection Center (ATCC). These human pathogens are used for testing antibacterial activity (Bhushanam and Madhusudan, 2019). The clinical isolates were identified based on the standard microbiological technique (Harley *et al.,* 2010). The bacterial strains, *Bacillus cereus* (ATCC 31443), *Bacillus subtilis* (ATCC 32441), *Burkholderia glumae* (ATCC 25813), *Erwinia nigrifluens* (ATCC 21922), *Escherichia coli* (ATCC 25891), *Klebsiella sp.* (ATCC 31482), *Pseudomonas aeruginosa* (ATCC 287858) and *Staphylococcus aureus* (ATCC 6538) were used to determine the antibacterial activity of each sample of honey.

### Culturing of bacterial strains

The test isolates were maintained on Mueller-Hinton Agar by slant–streak technique and incubated at 37 °C for 24 h (Laallam *et al.,* 2015). The slants with strains were stored at 4 °C. Under aseptic conditions, pure colonies of bacterial isolates from slants were picked with an inoculating loop and suspended in 3-4 ml of Mueller-Hinton broth in sterile test tubes and incubated for 24 h at 36-37 °C (Andargarchew *et al.,* 2004). Multiple slants were stored for further use.

### Antibacterial disc diffusion assay

Bacterial inoculum suspensions containing $10^6$–$10^8$ CFU/ml were prepared in sterile saline (0.9 g/l) and spread on Mueller-Hinton (MH) agar plates. The antibacterial activities of honey were tested using the agar disc diffusion method against the pathogens. Using sterile forceps, Whatman's filter discs (Ø = 5 mm), impregnated with saturated honey dilutions of 75 %, 80%, 85%, 90% and 95% (v/v % of honey: water), were placed on the inoculated plates and incubated at 37 °C for 24 h. the clear zone of inhibition around the discs indicate the presence of antibacterial activity of honey (Harley *et al*., 2010). This zone of inhibition was measured in mm including the diameter of the disc.

Experiments were carried out in triplicates. The broad spectrum kanamycin was used as positive control (Hegazi *et al.,* 2014). The newer fluoroquinolone antibiotic ciprofloxacin was also tested by antibiotic disc diffusion method.

### Screening for synergistic activity

Screening for synergistic antibacterial activity was carried out by using ciprofloxacin antibiotic discs saturated with 50µl of test honey samples ranging from 75 to 95% dilutions. The same procedure as in antibacterial disc diffusion assay was applied. The experiment was carried out in triplicates for statistical relevance and Mean ± SE of results was calculated (Hegazi *et al.,* 2014).

### Data Analysis

Data were analysed by subjecting to F-test and analysis of variance to determine the significant levels at 5% ($p<0.05$).

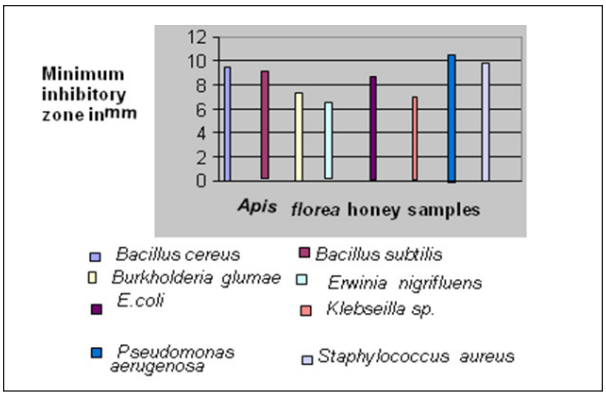
## RESULTS

### Antibacterial efficacy of honey

Most of the honey samples with various dilutions

have proved to possess, significant antibacterial potency against the selected bacterial isolates such as *Bacillus cereus* (ATCC 31443), *Bacillus subtilis* (ATCC 32441), *Burkholderia glumae* (ATCC 25813), *Erwinia nigrifluens* (ATCC 21922), *Escherichia coli* (ATCC 25891), *Klebsiella sp.* (ATCC 31482), *Pseudomonas aeruginosa* (ATCC 287858) and *Staphylococcus aureus* (ATCC 6538).

The honey samples collected from regions of Abbe falls, Kushal Nagar and Somavarapet of Coorg district were tested against the selected test isolates and exhibited significant inhibitory zones indicating pronounced antibacterial activity (Table 1). The Coorg honey of *Apis florea* species showed highest antibacterial activity against *Pseudomonas aeruginosa* (ATCC 287858) with 10.8 ± 0.97 mm and the lowest being 6.0 ± 0.18 mm (Fig.1). However, the least sensitivity range was also recorded for *Erwinia nigrifluens* (ATCC 21922) with 6.0 ± 0.32 mm (Fig.1).




(n=5, significant at p>0.05)

**Fig. 1.** Showing minimum inhibitory zones at 75 to 95 % honey (dilutions) samples against test pathogens

**Antibacterial efficacy of Ciprofloxacin**

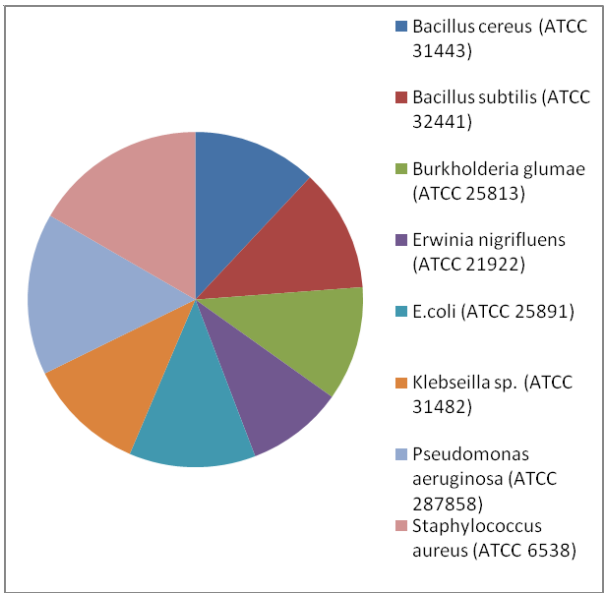
The antibacterial sensitivity with inhibitory zones that were formed in the cultures against the selected bacterial strains showed significant variations with Ciprofloxacin discs. The highest antibacterial activity was recorded against *Staphylococcus aureus* (ATCC 6538) with 14.23 ± 0.71 mm (Fig. 2). However, the least sensitivity range was recorded for *Erwinia nigrifluens* (ATCC 21922) with 7.99± 0.73 mm.

*Bacillus cereus* (ATCC 31443) *showed mm range of ZI of 10.23*±0.62, *Bacillus subtilis* (ATCC 32441) *with* 10.11±0.38 *Burkholderia glumae* (ATCC 25813) *with* 9.43± 0.62, *E.coli* (ATCC 25891) *with* 10.42± 0.52 mm,

**Table 1.** Antibacterial activity of diluted *Apis florea* honey from Coorg, Karnataka.

| Abbe falls, Coorg | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration of *Apis florea* honey (v/v%, Honey: water) | *Bacillus cereus* (ATCC 31443) | *Bacillus subtilis* (ATCC 32441) | *Burkholderia glumae* (ATCC 25813) | *Erwinia nigrifluens* (ATCC 21922) | *E.coli* (ATCC 25891) | *Klebseilla sp.*(ATCC 31482) 287858) | *Pseudomonas aerugenosa* (ATCC 6538) | *Staphylococcus aureus* (ATCC |
| 75 | 6.93±0.03 | 6.91±0.53 | 6.52±0.73 | 6.34±0.13 | 6.93±0.02 | 6.13±0.05 | | 7.34±0.16 |
| 7.86±0.14 | | | | | | | | |
| 80 | 9.29±0.17 | 8.31±0.65 | 7.78±0.58 | 6.36±0.52 | 9.85±0.29 | 8.36±0.48 | 9.93±0.21 | 10.51±0.29 |
| 85 | 9.66±0.82 | 9.52±0.89 | 8.36±0.71 | 7.17±0.27 | 10.79±0.03 | 8.75±0.03 | 11.88±0.37 | 12.65±0.04 |
| 90 | 11.82±0.71 | 10.37±0.67 | 10.93±0.36 | 7.49±0.02 | 11.27±0.07 | 9.29±0.14 | 13.73±0.02 | 14.91±0.07 |
| 95 | 12.14±0.95 | 11.58±0.16 | **11.87±0.58** | 8.64±0.65 | 12.35±0.81 | 10.53±0.52 | 15.08±0.62 | 16.96±0.16 |
| 100 | 12.38±0.47 | 12.76±0.63 | 11.03±0.29 | 8.82±0.16 | **14.95±0.41** | 12.02±0.74 | **17.53±0.72** | **19.26±0.23** |
| Kushalnagar, Coorg | | | | | | | | |
| 75 | 6.87±0.52 | 6.52±0.28 | 6.49±0.28 | 6.16±0.72 | 6.72±0.54 | 6.09±0.53 | 6.93±0.04 | 7.24±0.62 |
| 80 | 7.84±0.49 | 7.40±0.09 | 6.97±0.03 | 6.22±0.06 | 7.97±0.33 | 7.43±0.92 | 8.25±0.07 | 9.66±0.51 |
| 85 | 9.46±0.73 | 9.22±0.66 | 7.85±0.21 | 6.88±0.71 | 9.51±0.92 | 8.55±0.59 | 9.93±0.22 | 10.98±0.82 |
| 90 | 11.35±0.21 | 11.03±0.43 | 9.67±0.50 | 6.95±0.38 | 12.13±0.62 | 9.96±0.52 | 12.08±0.93 | 13.65±0.74 |
| 95 | 11.62±0.03 | 12.92±0.56 | 10.52±0.38 | 7.71±0.0.72 | 12.26±0.05 | 10.98±0.69 | 14.52±0.57 | 16.37±0.05 |
| 100 | **13.74±0.37** | **12.99±0.27** | 10.66±0.63 | **8.97±0.48** | 13.64±0.02 | 11.51±0.64 | 16.55±0.36 | 18.71±0.03 |
| Somvarpet, Coorg | | | | | | | | |
| 75 | 6.54±0.69 | 6.37±0.22 | 6.06±0.53 | 6.11±0.58 | 6.67±0.53 | 6.33±0.47 | 6.82±0.93 | 7.15±0.83 |
| 80 | 7.11±0.32 | 7.06±0.35 | 6.08±0.37 | 6.19±0.04 | 7.93±0.57 | 6.84±0.22 | 8.17±0.53 | 8.86±0.94 |
| 85 | 8.63±0.59 | 7.69±0.83 | 6.36±0.92 | 6.73±0.55 | 9.08±0.12 | 7.31±0.93 | 9.64±0.85 | 9.91±0.32 |
| 90 | 10.41±0.06 | 10.05±0.47 | 7.74±0.61 | 6.82±0.91 | 11.45±0.61 | 9.92±0.28 | 11.59±0.94 | 12.64±0.48 |
| 95 | 10.62±0.45 | 10.39±0.53 | 8.54±0.32 | 7.50±0.43 | 11.93±0.52 | 10.31±0.73 | 13.22±0.06 | 15.16±0.05 |
| 100 | 10.65±0.22 | 10.49±0.29 | 9.31±0.05 | 8.26±0.11 | 12.61±0.30 | **12.11±0.59** | 14.69±0.55 | 16.65±0.47 |

(n=5, significant at p>0.05)



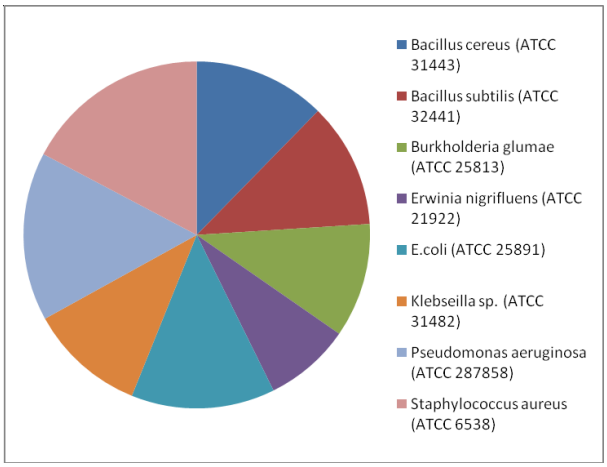

(n=5, significant at p>0.05)

**Fig. 2.** Showing minimum inhibitory zones of Ciprofloxacin discs against bacterial strains.

*Klebseilla sp.* (ATCC 31482) with 9.69± 0.71 *Pseudomonas aeruginosa* (ATCC 287858) with 13.33± 0.94 mm.

**Synergistic Antibacterial efficacy of Ciprofloxacin and *Apis florea* honey**

The synergistic antibacterial sensitivity with inhibitory zones that were formed in the cultures against the selected bacterial strains showed significant variations. The highest antibacterial activity was recorded against *Staphylococcus aureus*




(n=5, significant at p>0.05)

**Fig. 3.** Showing minimum inhibitory zones of Ciprofloxacin discs treated with *Apis florea* honey samples against bacterial strains.

(ATCC 6538) with 19.26 ± 0.23 mm. However, the least sensitivity range was recorded for *Erwinia nigrifluens* (ATCC 21922) was 8.97± 0.48 mm (Fig. 3).

*Bacillus cereus* (ATCC 31443) showed a zone of inhibition of 13.74 ± 0.37 mm, *Bacillus subtilis* (ATCC 32441) *with* 12.99±0.27, *Burkholderia glumae* (ATCC 25813) *with* 11.87± 0.58, *E.coli* (ATCC 25891) *with* 14.95± 0.41 mm, *Klebseilla sp.*(ATCC 31482) with 12.11± 0.59 *Pseudomonas aeruginosa* (ATCC 287858) *with* 17.53± 0.72 mm.

## DISCUSSION

In the present investigations the combination of ciprofloxacin honey showed different levels of growth inhibition on all the bacteria tested. The results of synergistic antibacterial assay revealed that *Staphylococcus aureus*, *Bacillus cereus, Bacillus subtilis, E.coli* and *Pseudomonas aerugenosa* were most susceptible against the antibiotic disc treated *with Apis florea* honey.

Similar findings were recorded by Hegazi *et al.*(2014), Mukherjee *et al*. (2011), Laallam *et al.* (2015) and Ejim *et al.* (2011). Many natural compounds have previously been shown to have potential to inhibit antibiotic resistance in bacteria (Gibbons, 2008). Three antibiotics, from an initial selection of fifteen antibiotics proved to be synergistic in combination with Manuka honey against MRSA and three were additive against *P. aeruginosa*. One combination (Manuka honey and tetracycline) exhibited enhanced activity against tested bacteria (Al- Jabri *et al.,* 2005). It is likely that the botanical origin of honey influences its biological activity because different antibacterial components have been identified in different honey samples (Kwakman *et al.,* 2011).

## CONCLUSION

The research findings of the present study on the synergistic antibacterial activity of ciprofloxacin and *Apis florea* honey on pathogenic bacteria showed good and acceptable results. The findings indicate the potential usefulness of honey and antibiotics together in the clinical practice against the pathogenic effects of strains of bacteria.

**Conflict of Interests**

The authors have not declared any conflict of interests.

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