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Pharmacological Assessment of Cutaneous Wound Healing by Natural *Apis* Honey on Animal Models

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Abstract: Honey is a nutritional food with health-promoting properties. Antiseptics are topical agents that act to prevent growth of microorganisms. A range of *Apis florea* bee honey from Karnataka was used to investigate the prevention of infection and promote healing of wounds in rat models as honey is a tissue-regenerative agent. It contributes to all stages of wound healing, and thus has been used in direct topical application and also in dressings. Most honey samples with various dilutions have proved to possess, significant antibacterial potency against selected bacterial isolates by disc diffusion assay. The Coorg honey of *Apis florea* species showed highest antibacterial activity against *Staphylococcus aureus* (ATCC 6538) with 19.26±0.23 mm and the least susceptibility was recorded for bacterial strain *Erwinia nigrifluens* (ATCC 21922) with 8.97±0.48 mm. The Kolar honey of *Apis florea* species showed highest antibacterial activity against *Bacillus cereus (*ATCC 31443) with 8.79±0.16 mm and least susceptibility was recorded for *Staphylococcus aureus* (ATCC 6538) with 7.01±0.55 mm. The Bengaluru honey of *Apis florea* species showed highest antibacterial activity against *Staphylococcus aureus* (ATCC 6538) with 9.95±0.23 mm and least susceptibility was recorded for *E.coli* (ATCC 25891) with 6.27±0.22 mm. The present study also checked the wound healing ability of honey when applied topically in several rat models. The control rats were healed by 20 ± 1.07 days. The Kanamycin treated rats were healed on 10±1.45 days. 13 ±1.07days were recorded to heal the wounds by *Apis florea* honey samples of Abbe Falls, Coorg regions. 18 ±1.54 days and 17 ±1.03 days were recorded against Champion Reef regions of Kolar district and Varthur regions of Bengaluru district.

Key words: *Apis florea* honey, Antibacterial potency, excision wounds, wound healing.

Introduction

Natural honey is composed of 82% of water, carbohydrates, proteins, phytochemicals, antioxidants, and minerals. the amount of compounds with medical activities vary among the various types of honey. The sugars in honey include, fructose (38.2%), glucose (31.2%), sucrose (0.7–1%), disaccharides and higher saccharides (9%)[46]. Flavonoids, organic acids, phenolic acid, vitamins, and enzymes present in the honey may improve wound healing. The

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deposition of fibroblasts and collagen formation may also be promoted by the amount of amino acids found in honey[17].

A wound is a disturbance in the normal structure and function of the epidermis. The epidermis is considered as the first line of defense and protection against trauma. Wound healing is a complex process with many interdependent immunological and physiological mediators to restore the cellular integrity of the damaged tissue[34]. With the emergence of drug-resistant bacteria, many antimicrobial agents have become ineffective in wound treatment. Thus, the use of natural honey as a wound treatment agent is used as alternative medication[23, 30, 10] . The use of honey has gained clinical popularity for possible use in wound treatment and in regenerative medicine[24, 31, 29]. Topical honey treatment has shown to possess antimicrobial properties, promote autolytic debridement, stimulate growth of wound tissue in dormant wounds, stimulate anti-inflammatory activity that swiftly reduces pain, edema and exudates production[43].

Natural honey is a viscous fluid; its jelly consistency creates a surface layer over the wound that inhibits the entrance of bacteria and protects the wound from dehydration[35]. Its high sugar content creates a higher osmotic gradient that pulls fluid up through the subdermal tissue and offers an additional glucose source for flourishing cellular components in the wounded area[44]. The low pH of honey increases tissue oxygenation, while free radicals, which lead to tissue damage, are removed by flavonoids and aromatic acids[42]. According to the international guidelines on the proper use of antimicrobials in medicine, honey and other alternative therapeutics were used for the treatment of skin lesions on animal models[41]. Honey exerts bacteriostatic and bactericidal actions[45, 5, 20]. This present work looks into the antibacterial activity and wound healing efficacy of variuos *Apis florea* honey samples from Karnataka.

Materials And Methods

Study areas

The present study areas of Karnataka, India were of different biogeographical regions of Coorg district (12° 19’45 North latitude to 75º 53’44 East longitude), Bangalore district (12^0 58’ to 13^0 65’ North latitude to 77^0 35’ to 77^0 40’ East longitude with an elevation of 928m) and Kolar district (12^0 46’ to 13^0 58’ North latitude and 77^0 21’ to 78^0 35’ East longitude).

Procurement of Apis bee honey samples

One hundred and twenty five honey samples of *Apis florea* were harvested from various geographical areas of Karnataka during 2019 to 2022. With the help of a local beekeeper, few bees were collected from the hive and identified for *Apis* species. Upon identification, the honey samples from the comb were collected under sterile conditions. 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 to 8 ^{0}C until tested as per the method proposed by Nzeako and Hamdi[40] as well as Bhushanam and Madhusudan[14].

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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. The clinical isolates were identified based on the standard microbiological technique. 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[14].

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[26]. The slants with strains were stored at 4 ^{0}C. Under aseptic conditions, pure colonies of bacterial isolates from slants were picked with an inoculating loop and suspended in 3 to 4 ml of Mueller-Hinton broth (Hi- Media) in sterile test tubes and incubated for 24 h at 36 to 37°C[8]. Multiple slants were stored for further use.

Antibacterial disc diffusion assay

Bacterial inoculums suspension containing 10 ^{6} to 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 of Kirby- Bauer method against the pathogens. Using sterile forceps, whatman filter discs (Ø = 6 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 indicates the presence of antibacterial activity of honey[21]. 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[22].

Pharmacological wound healing potency of Albino rats

Pharmacological effects of various *Apis florea* honey was evaluated on infected excision wounds of Albino rats. Twenty five male Albino rats weighing 250 to350 g each were used in the present study. The rats were kept in the animal unit at one week prior to initiation of the study. The rats were given commercial pellet and water throughout the study to ensure stabilization of their good health. Rats were anesthetized with an injection of Ketamine (50 mg/kg) and Xylazine (5mg/kg). Under anesthesia, the back of both sides of the body were shaved. Following this procedure, rats were returned to their cages for 24 h to allow any edema caused by the shaving procedure to recede. The wound site was prepared following the excision wound model[1]. Initially, the rats were anesthetized as described above and a circle of diameter of 15mm was marked one each right side of the thigh of animals skin surface, and the skin was gently dissected out. The area was measured immediately by tracing out the wound area using a sterile transparent tracing paper and the area was recorded. Treatment was initiated only after 2 days of excision as the wound was exposed for the bacterial infection. After 2 days of excision, the wound was swabbed with potent concentration of honey. Simultaneously, the wound area of each animal was measured while the animals were under anesthesia on the days of post surgery. Each application was

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evaluated in 5 rats per group and results shown were a mean of 5 determinations [1, 28]. A group Albino rats with excision but without treatment were used as control.

Measurement of wound contraction

The excision wound margin was traced after wound creation by using transparent paper and the respective area was measured using a graph paper. Wound contraction was measured at every 2 days' interval, until complete wound healing, and expressed in percentage of the healed wound area[42]. The evaluated surface area was then used to calculate the percentage of wound contraction, taking the initial size of wound 15mm as 100%, by using the following formula:

$$\% \text{ Wound Contraction} = \frac{\text{Initial wound size} - \text{Specific } n^{th} \text{ day wound size}}{\text{Initial wound size}} \times 100$$

The data obtained from period of excision wound healing was subjected to analysis of Mean ± Standard Deviation.

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 *Staphylococcus aureus* (ATCC 6538) with 19.26±0.23 mm and the lowest being 7.15±0.83 mm. However, the least sensitivity range was recorded for bacterial strain *Erwinia nigrifluens* (ATCC 21922) with 8.97±0.48 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)	*Pseudomonas aerugenosa* (ATCC 287858)	*Staphylococcus aureus* (ATCC 6538)
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±0.72	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)

The honey samples collected from regions of Champion reefs, Oorgaum and Coromandel of Kolar district were tested against the selected test isolates and exhibited significant inhibitory zones indicating pronounced antibacterial activity (Table 2).

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The Kolar honey of *Apis florea* species showed highest antibacterial activity against *Bacillus cereus (*ATCC 31443) with 8.79±0.16 mm and the lowest being 6.13±0.04 mm. However, the least sensitivity range was recorded for bacterial strain *Staphylococcus aureus* (ATCC 6538) with 7.01±0.55 mm.

Table 2. Antibacterial activity of diluted *Apis florea* honey from Kolar, Karnataka.

Champion Reefs, Kolar								
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)	*Klebsilla sp.*(ATCC 31482)	*Pseudomonas aerugenosa* (ATCC 287858)	*Staphylococcus aureus* (ATCC 6538)
75	6.13±0.04	6.11±0.27	6.13±0.02	6.18±0.06	6.17±0.13	6.09±0.22	6.08±0.07	6.07±0.22
80	7.42±0.05	6.48±0.18	6.27±0.17	6.26±0.04	6.19±0.11	6.17±0.11	6.11±0.05	6.17±0.53
85	7.45±0.13	7.15±0.34	6.22±0.27	6.53±0.08	6.22±0.17	6.24±0.90	6.24±0.83	6.28±0.94
90	7.59±0.33	7.68±0.21	6.43±0.92	6.94±0.07	7.58±0.12	6.37±0.11	6.92±0.66	6.29±0.22
95	8.26±0.22	7.92±0.29	6.67±0.94	6.98±0.12	7.69±0.22	6.39±0.24	7.80±0.76	6.34±0.28
100	8.79±0.16	8.16±0.37	6.91±0.55	7.16±0.64	8.64±0.54	7.10±0.33	8.60±0.45	6.47±0.48
Oorgaum, Kolar								
75	6.29±0.13	6.14±0.18	6.11±0.25	6.08±0.01	6.11±0.02	6.12±0.24	6.09±0.33	6.07±0.11
80	6.57±0.27	6.79±0.23	6.27±0.34	6.10±0.04	6.14±0.04	6.22±0.37	6.11±0.53	6.18±0.27
85	6.91±0.51	6.8±0.27	6.59±0.43	6.15±0.01	6.16±0.22	6.31±0.28	6.24±0.94	6.27±0.31
90	7.11±0.91	6.9±0.72	6.81±0.27	6.19±0.31	6.91±0.24	7.52±0.33	6.32±0.66	6.39±0.11
95	7.59±0.14	7.18±0.44	6.93±0.54	6.34±0.17	7.13±0.22	7.94±0.23	6.45±0.84	6.44±0.83
100	8.22±0.59	8.19±0.61	7.10±0.12	6.58±0.16	7.54±0.09	8.66±0.41	7.63±0.91	6.92±0.17

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Coromandel, Kolar								
75	6.16±0.22	6.19±0.45	6.19±0.22	6.14±0.33	6.12±0.26	6.08±0.25	6.07±0.01	6.04±0.23
80	6.28±0.18	6.46±0.26	6.28±0.44	6.17±0.19	6.28±0.35	6.09±0.11	6.11±0.64	6.08±0.83
85	6.39±0.26	6.82±0.14	6.39±0.17	6.22±0.16	6.34±0.42	6.14±0.22	6.16±0.22	6.19±0.11
90	6.54±0.17	6.92±0.23	6.53±0.72	6.28±0.27	6.86±0.24	6.38±0.14	6.92±0.37	6.28±0.23
95	6.83±0.11	7.10±0.22	6.97±0.12	6.29±0.19	6.95±0.17	6.81±0.55	7.10±0.01	6.29±0.01
100	7.10±0.15	7.20±0.64	7.32±0.22	7.10±0.28	7.10±0.48	6.97±0.23	7.20±0.61	7.01±0.55

(n=5, significant at p>0.05)

The Bengaluru honey of *Apis florea* species showed highest antibacterial activity against *Staphylococcus aureus* (ATCC 6538) with 9.95±0.23 mm and the lowest being 6.11±0.17 mm (Table.3). However, the least sensitivity range was recorded for bacterial strain *E.coli* (ATCC 25891) with 6.27±0.22 mm.

Table 3. Antibacterial activity of diluted *Apis florea* honey from Bengaluru, Karnataka.

Varthur, Bengaluru								
Concentration of *Apis florea* honey (v/v%, Honey: water)	*Bacillus cereus (*ATCC 31443)	*Bacillus subtilis* (ATCC 32441)	*Burkholderia glumae* (ATCC 25813)	*Erwinia nigriflu ens* (ATCC 21922)	*E.coli* (ATCC 25891)	*Klebsei lla sp.*(ATCC 31482)	*Pseudom onas aerugeno sa* (ATCC 287858)	*Staphyloc occus aureus* (ATCC 6538)
75	6.04±0.05	6.03±0.11	6.08±0.26	6.07±0.04	6.07±0.34	6.09±0.01	7.13±0.15	7.52±0.11
80	6.08±0.33	6.04±0.08	6.09±0.53	6.08±0.03	6.09±0.24	6.14±0.25	7.16±0.06	7.61±0.09
85	6.09±0.22	6.08±0.28	6.09±0.22	6.08±0.11	6.10±0.27	6.18±0.37	7.19±0.22	8.62±0.43
90	6.11±0.58	6.09±0.45	6.10±0.11	6.10±0.07	6.10±0.03	6.19±0.38	7.22±0.61	9.14±0.87
95	6.24±0.67	6.19±0.36	6.11±0.46	6.11±0.04	6.13±0.11	6.22±0.99	7.23±0.53	9.84±0.57
100	6.84±0.83	6.21±0.33	6.21±0.76	6.14±0.22	6.18±0.22	7.12±0.26	7.91±0.11	9.95±0.23
Jakkur, Bengaluru								
75	6.05±0	6.09±0		6.08±0	6.11±0	6.02±0	6.09±0.0	6.95±0.37

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	.27	.11	6.07±0.04	.12	.66	.11	1	
80	6.08±0.62	6.11±0.23	6.08±0.01	6.09±0.11	6.15±0.33	6.07±0.37	6.11±0.28	7.16±0.38
85	6.16±0.63	6.23±0.17	6.17±0.06	6.17±0.31	6.16±0.24	6.09±0.34	6.19±0.19	7.63±0.76
90	6.19±0.62	6.47±0.94	6.18±0.67	6.19±0.67	6.18±0.32	6.17±0.66	6.26±0.47	7.93±0.04
95	6.24±0.73	6.58±0.33	6.28±0.33	6.28±0.64	6.19±0.17	6.23±0.22	6.38±0.92	8.16±0.56
100	7.12±0.92	6.95±0.36	6.43±0.11	6.37±0.92	6.27±0.22	6.26±0.11	7.18±0.22	8.26±0.57
Kengeri, Bengaluru								
75	6.08±0.72	6.07±0.11	6.04±0.11	6.08±0.22	6.07±0.85	6.08±0.11	6.91±0.04	6.11±0.17
80	6.09±0.17	6.08±0.23	6.05±0.23	6.09±0.11	6.08±0.22	6.09±0.14	7.64±0.34	6.24±0.33
85	6.18±0.38	6.09±0.37	6.11±0.24	6.11±0.17	6.09±0.13	6.18±0.27	7.74±0.88	6.58±0.19
90	6.21±0.73	6.19±0.53	6.23±0.79	6.13±0.14	6.15±0.34	6.19±0.53	8.13±0.43	6.96±0.44
95	6.28±0.22	6.19±0.73	6.34±0.81	6.18±0.52	6.19±0.37	6.28±0.34	9.17±0.09	7.23±0.38
100	6.37±0.59	6.59±0.18	9.59±0.14	6.27±0.29	6.23±0.11	6.37±0.91	9.21±0.77	7.51±0.83

(n=5, significant at p>0.05)

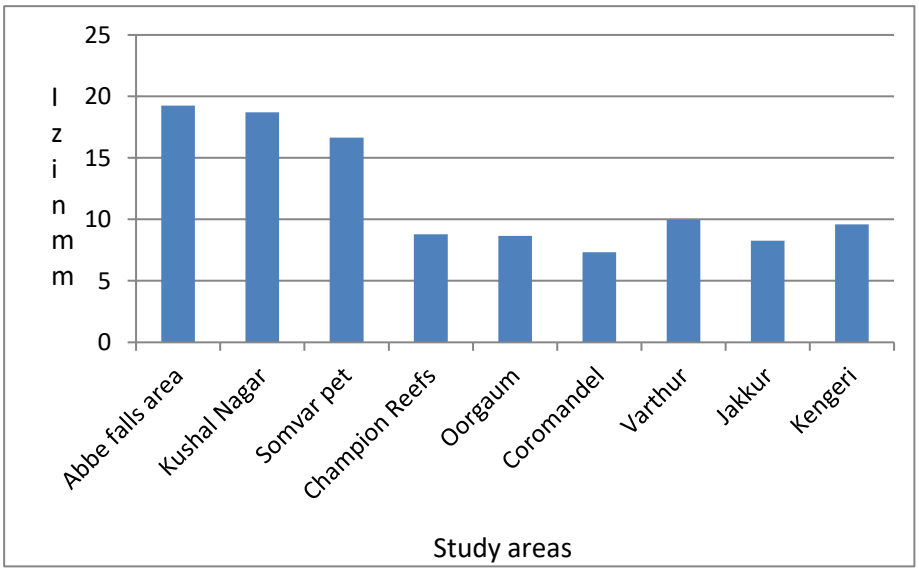

Fig.1. Showing minimum inhibitory zones (IZ) in mm of *Apis florea* honey samples

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Wound healing potency of Apis honey samples on experimental rats

The natural honey samples of the present study areas that retained antibacterial potency against control isolates were used in the wound healing of experimental Albino rats. The wound healing experiments on the Albino rats showed significant variations.

Fig. 2. Wound healing in Albino rat model

Table.4. Showing Mean number of days (Mean ± Standard Deviation) for the healing of wounds on experimental animal models in vitro.

Type of Wound	Mean No. of Days				
	Control	Treatment With Kanamycin	Treatment with Apis florea honey Coorg		
			Abbe Falls	*Kushal Nagar*	*Somvarpet*
Excision	20 ± 1.07	10±1.45	13 ±1.07	14 ±1.38	15 ±1.52
	Percentage of wound Healing on 10th day				
	50	100	76.92	71.43	66.67
	Control	Treatment With Kanamycin	Treatment with Apis florea honey Kolar		
			Champion Reefs	Oorgaum	Coromandel
	20 ± 1.07	10±1.45	18 ±1.54	19 ±1.01	19 ±1.82
	Percentage of wound Healing on 10th day				
	50	100	55.56	52.63	52.59
	Control	Treatment With Kanamycin	Treatment with Apis florea honey Bengaluru		
			Varthur	Jakkur	Kengeri
	20 ± 1.07	10±1.45	17 ±1.03	18 ±1.62	17 ±1.08
	Percentage of wound Healing on 10th day				
	50	100	58.82	55.58	58.81

Significant at $p < 0.005$ levels

In the present investigations, the excision wounds were assessed by gross inspection of epithelialisation and wound healing. The research findings of the present study reiterates that honey can aid wound healing when applied topically on rat models. The control rats were

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healed by 20 ± 1.07 days (Table. 4). The Kanamycin treated rats were healed on 10±1.45 days. The high potency of *Apis florea* honey from Coorg district (Abbe Falls) healed the test wounds in 13 ±1.07days. Similarly, 14 ±1.38 days and15 ±1.52 days were recorded to heal excision wounds by *Apis florea* honey of Kushalnagar and Somvarpet regions. 18 ±1.54 days were recorded for Champion Reefs regions of Kolar district. Similarly, 19 ±1.01 and 19 ±1.82 days were recorded to heal excision wounds by *Apis florea* honey of Oorgaum and Coromandel regions of Kolar district. 17 ±1.03 days were required to heal the excision wounds by *Apis florea* honey samples of Varthur regions of Bengaluru district. Similarly, 18 ±1.62 and 17 ±1.08 days were recorded for *Apis florea* honey of Jakkur and Kengeri regions of Bengaluru district (Table 4).

Discussion

Antibacterial potency is the effect influenced by the agent preferably the chemicals that inhibit or slow down the growth of bacteria in the given media. Honey has been demonstrated in many studies to have antibacterial effects, attributed to its high osmolarity (Sugar content), low pH, high hydrogen peroxide, high moisture content, high ash content and other uncharacterized compounds. Low pH alone is inhibitory to many pathogenic bacteria[6]. All the factors such as low pH, high sugar content and peroxide content are combattingly responsible for antibacterial activity of medicinally important and potent honey[41]. A study of 345 samples of New Zealand honeys found antibacterial activity of diluted honeys[37]. The author had also suggested the influence of phytochemical origin and geographical origin of honey in the antibacterial activity. In the present study, most honey samples of Coorg exhibited potent antibacterial activity.

The Coorg honey of *Apis florea* species showed highest antibacterial activity against *Staphylococcus aureus* (ATCC 6538) with 19.26±0.23 mm and the lowest being 7.15±0.83 mm. However, the least sensitivity range was recorded for bacterial strain *Erwinia nigrifluens* (ATCC 21922) with 8.97±0.48 mm.

Earlier studies by Albaridi[2], Anand *et al.*[7], M Bhushanam & S Madhusudan[13,14] and Matzen *et al.*[32] mentioned the use of diluted honey in controlling the bacterial growth and the dilutions could be confirmed through in vivo and clinical studies. The present findings are in accordance with previous studies reported that different honey types possess different efficacies and mechanisms against the same bacteria1[2, 16, 3, 4, 15]. Nzeako and Hamdi[40] reported antibacterial activity of *Pseudomonas, Acinobacter* and *Staphylococcus* was noticed at 40 per cent dilutions of Saudi Arabian honey. Andargarchew *et al.*[8] reported antibacterial activity against *E.coli, S.aureus, P. aeruginosa, S.shiga, S.typhi, P. vulgaris, K.aerogenes* and *P.mirabilis* at various dilutions of *A.melliferan* honey. French *et al.*[18] reported antibacterial activity of honey against coagulase negative *Staphylococci*. Noori *et al.*[38] reported against *Streptococcus, E.coli* and *Staphylococuss aureus*. Mitra *et al.*[33] reported antibacterial activity of honey against *S.aureus, E.coli, P.aeruginosa* and *Klebsiella*.

The usage of honey in the clinical fields is presumed to be one of the ancient methods but renewed the development in the recent days[36]. The cutaneous wounds are injury made to the integument that causes cellular death, capillary damage in varying degrees and coagulation of

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proteins. The loss of protective function of the skin as a barrier to micro organisms results in infection. The bacteria contaminate the wound surface and begin to multiply and proliferate in the wound area. Wound healing days on human population were recorded as 20.23 ± 4.45 in treated group while 28.38 ± 7.06 days in the control group[27].

Honey with high osmorlarity, low pH and high peroxide content favors the outflow of fluid from wound tissue, aiding cleansing, reducing edema and decreasing pain. The pure honey and diluted honey, when applied to wounds, permits movement of water through osmosis, thus contributing to the cleaning of wounds[19, 12]. Also, the movement of fluid from underlying tissue and capillaries in response to this osmotic pull will lead to improvements in the increased levels of dissolved oxygen and nutrients. Thus, the nutrient content of honey stimulates the cell growth and provides energy for the dividing cells on the surface of wounds[35, 36, 17, 21]. Honey along with wound healing prevents scar formation that makes a difference in effects for the outcomes wound infection, scar quality, pain and patient satisfaction as the evidence is low to very low-certainty[25, 26].

In the present investigations the cutaneous wounds were assessed by gross inspection of epithelialisation and wound healing. The high potency of *Apis florea* honey from Coorg district showed 13 ± 1.07 to 15 ± 1.52 days for healing of wounds upon the treatment than the control animals (20 ± 1.07 days). The high potency of *Apis florea* honey from Bengaluru district showed 17 ± 1.03 to 18 ± 1.62 days for healing of wounds upon the treatment than the control animals (20 ± 1.07 days). The high potency of *Apis florea* honey from Kolar district showed 18 ± 1.54 to 19 ± 1.82 days for healing of wounds upon the treatment than the control animals (20 ± 1.07 days). Similar findings were reported by Georgina[19], Molan[36], Adikwu and Alozie[1], Bangroo *et al.*[9] and Bhavin e*t al*[11] reported wound healing in human patients using honey. Manuka honey dressing has long been available as a non-antibiotic treatment in the management of chronic wound infections. Vandamme *et al.*[45] reported systemic wound healing using honey on human patients. Molan[37] studied wound healing in mice, rats and buffalo calves. According to Nur- Azida *et al.*[39] in the in-vitro antibacterial study, Aquacel-Ag and Aquacel-Manuka honey dressings gave better zone of inhibition for Gram positive bacteria compared to Aquacel-Tualang honey dressing.

Conclusions

The research findings of the present study on the antibacterial activity of *Apis florea* honey from Karnataka on pathogenic bacteria showed good and acceptable results. Variations in the antibacterial activity could be attributed by the *Apis* honey quality, floral varieties, diversity of geographical regions. Hence identification of appropriate honey type to control the specific bacterial growth is required. Further deciphering of phytochemicals in the effective honey variety is important in order to use the honey against specific pathogens. The excision wounds were healed rapidly by the potent *Apis florea* honey from Coorg district.

Conflict of Interests

The authors have not declared any conflict of interests.

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