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Traditional and Molecular Identification of Bacterial Contamination from Honey (Processed and Unprocessed) and Their Control by Phytotherapy

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NMA designed the study and wrote the protocol. Authors HS and BM carried out practical work. Authors SA performed the statistical analysis. Authors NMA and SA wrote the first draft of the manuscript. Author SA managed the analyses of the study. Authors RGM, IS, AN and SN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Honey used as a potential source of medicine traditionally. Today honey is contaminated due to improper handling which leads to cause various infections. So, the aim of current study was to isolate and identify the bacterial contamination from both commercial (processed) and natural (unprocessed) honey of Pakistan. These bacteria were treated with the medicinal plants and antibiotics to cure the infectious diseases. In the present study, bacterial population was investigated from five commercial and five natural honey products. Ten pathogenic bacteria were isolated from all honey products. Only one was isolated from processed honey, while the remaining

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nine were isolated from unprocessed honey. *Bacillus cereus, Bacillus anthracis, Clostridium botulinum, Clostridium septicum, Bacillus weihenstephanensis, Clostridium acetobutylicum Proteus mirabilis, Myroides odoratimimus* were identified through ribotyping and traditional microbial techniques. They are recognized as a common soil and water dwelling bacteria. Antibacterial effect of medicinal plants and standard antibiotics was evaluated against these pathogens through agar disc diffusion method. Among the tested medicinal plants, *Bacillus cereus* and *Bacillus anthracis* were strongly inhibited by methanolic extract of *N. sativa* and *Bacillus weihenstephanensis* was inhibited by *Citrus sinensis*. On the other hand, *Aloe vera* and *Cinnamon umverum* had no effect on all the tested pathogens. All tested bacterial pathogens were strongly inhibited by medicinal plants as compared to tested antibiotics (Cefixime and ampicillin). It was concluded that the high number of pathogenic bacteria in unprocessed honey indicated secondary contaminations of honey by poor handling. Phytotherapy indicated that medicinal plants could be used as a potential source of therapeutic agents to prevent the transfer of infectious diseases.

Keywords: Bacterial contamination; antibacterial activity; plant extracts; antibiotic susceptibility; ribotyping.

1. INTRODUCTION

Honey is a prehistoric therapy for the treatment of various infectious and noninfectious diseases. Honey has been stated as the valuable source of energy having both antimicrobial and antioxidant characteristics. Its composition contains aromatic substances, carbohydrates, amino acids, organic acids, minerals, pollen grains, pigments, and waxes [1]. Sugar and water are a most important component of honey. Antibacterial activity of various types of honey was already reported in worldwide [2-4]. Many microbes are associated with foods [5]. These microbes are soil associated, also found in air, plants as well as plant products. Microorganisms like yeast, bacteria, and molds are found in honey during harvesting, storing and maturation. The primary microbial resources are a digestive system of the honey bee, air, dust, pollens, and flowers [6] while the secondary resources are equipment's, insects, animals, water and human handling [7]. Other factors (high humidity, high temperature for storage) can also affect the quality of honey [7]. Martins et al. [8] illustrated that honey associated microbes can be involved in many activities like enzyme production, antibiotics, toxins, amino acids, increase vitamins, and promote metabolic rates.

According to Migdal et al. [9] Penicillium, Mucor, *Acosphaera apis, Acosphaera major,* strains of *Saccharomyces, Schizosaccharomyces* and *Torula* are predominate among yeasts. *Bacillus* and *Clostridium* genus, are regularly found in honey. Sulfite-reducing *Clostridium* is an indicator organism, whose presence in honey provides evidence of contamination or pollution [10]. *Bacillus cereus* and *Clostridium botulinum* are regularly found in honey and cause illness in humans [11]. Pucciarelli et al. [12] revealed the presence of *Escherichia coli*, *Staphylococcus*, *Enterococcus* and *Salmonella* in yateí honey. Although most of these bacteria are in indolent forms and they can barely survive in honey due to its many properties. Still, there is a need for caution, especially when giving honey to children under the age of one and in its use in wound management. As when honey is diluted in milk, water or any other medium there is a chance that these bacteria can become active and cause illness. The raw honey may taste better, but when it comes to bacterial contamination it is not safe.

Various remedies are available to prevent the infectious diseases. Synthetic drugs and natural resources are available to deceased the growth of infectious agents. Medicinal plants are one of the natural and traditional source to cure the infectious diseases. Various researchers reported the medicinal importance of herbs that could be used as antibacterial, antifungal, and antiviral agents [13-17]. They reported the antibacterial activity of *Morus nigra*, *Cedrus deodara*, *Zanthoxylum armatum*, *Momordica charantia Citrus sinensis*, *Elaeagnus umbellate*. Antibacterial activity is responsible due to the presence of secondary metabolites such as phenols, flavonoids, tannins, saponins, glycosides, antioxidants and others.

There is a lack of information on Pakistani honey. There are reports on the healing effect on burns and wounds and some chemical and physical properties, but there is a paucity of data on the pathogenic bacterial contamination of Pakistani honey. The goal of this study was to assess the

bacterial contamination in both unprocessed and processed honey available to consumers in Pakistan, with emphasis on pathogenic bacteria. Morphological, biochemical tests and molecular characterization have been done to identify bacterial contamination. The effect of various medicinal plants and antibiotics have been used in current research to control these bacterial agents.

2. MATERIALS AND METHODS

2.1 Sample Collection and Isolation of Bacteria

Total ten honey samples were taken (five each of processed and unprocessed honey). Unprocessed honey samples (citrus, sunflower, alfalfa, eucalyptus and lavender) were collected from different areas of Punjab and Khyber Pakhtunkhwa, Pakistan. Processed honey samples *viz*., Langnese, Lifestyle, Marhaba, Young's and Salman's were purchased from the local market Lahore, Pakistan. Processed honey samples are purified honey while unprocessed honey samples are not purified and directly collected from the local areas. The honey samples were then stored in sterilized bottles in the laboratory to avoid more contamination. Honey has high thickness and viscosity which poses difficulties to spread it over nutrient agar plates, so it was diluted with distilled autoclaved water (dH_2O). Approximately one ml of honey was diluted in 10 ml of water (1:10 ml). Diluted honey was streaked on nutrient agar plates with the help of sterilized loop, placed in 37℃ incubator (MMM group Medcenter Enrich tungen GmbH) for 24-48 hrs. Pure colonies were also obtained by spread plate method [18].

2.2 Morphological, Biochemical and Physiological Characterization of Bacterial Isolates

Selected pathogenic strains were initially characterized by observing cell morphology and Gram's, endospore, acid fast and capsule staining protocols [19,20]. A thorough biochemical investigation was carried out *via* various biochemical tests including the IMViC procedures ("I" is for indole test; "M" is for methyl red test; "V" is for Voges-Proskauer test, and "C" is for citrate test) and by using differential and selective media. Optimum conditions for bacterial strains were determined on the basis of the growth curve for different time intervals at 590 nm, different temperatures (17℃, 27℃, 37℃, 47℃ and 57℃) and pH ranges (4, 5, 6, 7, 8, 9 and 10).

2.3 Extract Preparation of Medicinal Plant

Various parts of medicinal plants were collected for extract preparation i.e. black seeds/kalonji (*Nigella sativa*), the bark of cinnamon (*Cinnamon umverum*), leaves of green tea (*Camellia sinensis*) and peppermint (*Mentha piperita*) and true aloe (*Aloe vera*). Methanolic extracts of plants were prepared by dissolving 60 g of the plant in 360 ml methanol then placed in a shaker (Irmeco GmbH, Germany) for 24 hrs. The extracts were filtered and concentrated on rotary vacuum evaporator (R/201B/II) for 3 to 4 hrs. The concentrated extracts were finally soaked in methanol in the ratio 1:6. Filter paper discs of 5 mm were prepared with the help of a paper puncture and sterilized.

2.4 Antibacterial Assay

Plant extract susceptibility was also checked by agar disc diffusion method [22,22]. Whatman No. 1 filter paper disc (5 mm diameter) was impregnated with crude (10 µl) plant extracts was placed on Nutrient agar (NA) which was previously swabbed with bacterial pathogens. The sterile disc impregnated with only methanol used as a negative control. All the plates were incubated at 37ºC for 24 hrs. After 24 hrs the zone of inhibition appearing around the discs were measured and recorded in millimeter (mm) [23]. The diameter of the clear zones (if greater than 5 mm) around each disc was measured with the help of scale [24]. Clear zones around the discs showed the sensitivity of bacterial isolates. Each experiment was conducted thrice, and the mean of the results was calculated for both the test and control.

2.5 Antibiogram Analysis

The antibiotic susceptibility of the bacterial isolates was checked against ampicillin (AMP, 10 mcg), cefixime (CFM, 5 mcg), erythromycin (E, 15 mcg), ofloxacin (OF, 5 mcg) and ticarcillin (TI, 75 mcg) through agar disc diffusion method [25]. Antibiotics used as a positive control. The plates were then incubated overnight at 37℃ for 24 hrs and the zone of inhibition appearing around the discs were measured and recorded in millimeter (mm) [23]. The diameter of the clear zones (if greater than 5 mm) around each disc was measured with the help of scale [24].

2.6 Genomic DNA Isolation

DNA was isolated by phenol-chloroform method with slight modifications [26]. Agarose gel electrophoresis (1%) was done to assure that the samples contain isolated genomic DNA. After running gel, DNA bands in the gel were visualized using short wave ultraviolet light provided by a transilluminator and photographed was taken through Stratagene Eagle Eye still video system.

2.7 Ribotyping

Ribotyping is aimed at molecular characterization of pathogenic isolates, so their 16SrDNA was partially amplified through polymerase chain reaction. Amplification was done using Universal primers 16S-27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 16S-1522R (5′- AAGGAGGTGATCCAGCCGCA-3′) (Penicon). Polymerase chain reaction (PCR) was performed in a thermocycler for 35 reaction cycles. The total reaction mixture (50 µl) was taken. Initial denaturation was done at 94ºC for 5 min, annealing was done at 52℃ and elongation at 72℃ for 30 sec, 40 sec, and 30 sec, respectively. Final extension was given at 72℃ for 10 min.

2.8 Amplified DNA Extraction and Gel Electrophoresis

To confirm the amplification, PCR products were loaded on 1% agarose gel. The gel was run following the same procedure for half hrs at 80 volts and the bands of amplified DNA were visualized under the UV light by using the trans-illuminator. After amplification anticipated band were eluted/cut and kept in Eppendorf. Then using GF-1 DNA recovery kit by Vivantis, the gene was cleaned.

2.9 Sequencing

The amplified sequence of 16S rDNA gene was sent to Molecular Biological Products, Korea for sequence analysis. The nucleotide sequences were Basic Local Alignment Search Tool (BLAST) searched for blastn and or/ blastx algorithms in NCBI. The phylogenetic tree was constructed using Treedyn software.

2.10 Statistical Data

Each experiment was repeated in triplicate and Standard Deviation from absolute data was calculated (http://easycalculation.com/statistics/standard - deviation.php).

3. RESULTS

3.1 Morphological and Biochemical Identification

Ten bacterial pathogens were selected which showed hemolysis on blood agar. Out of ten pathogens, nine of these were isolated from unprocessed honey samples and only 1 from processed samples. Gram's staining indicated that seven isolates were identified as Gram's positive bacteria and three as Gram's negative. *Bacillus cereus, Bacillus anthracis, Clostridium botulinum, Clostridium septicum, Bacillus weihenstephanensis, Clostridium acetobutylicum* are Gram-positive rod bacteria while *Proteus mirabilis, Pseudomonas aeruginosa, Myroides odoratimimus* are Gram's negative rods. *Staphylococcus aureus* is a Gram's negative cocci bacteria. These bacterial strains were also identified using biochemical tests, differential and selective media (Table 1).

3.2 Molecular Identification

The genomic DNA of all biochemically analyzed bacterial isolates were isolated (Fig1.A) and 1.5 kbp fragment of 16SrDNA was amplified (Fig1.B). After amplification, gene fragment was eluted from 1% agarose gel (Fig1.C) and sequenced. The homology of these amplified products was analyzed through National Center for Biotechnology Information (NCBI) nucleotide data blast system (Table 2). It was recorded that these bacteria showed 99% to 100% homology with the following bacteria *viz., Bacillus cereus* (H1), *Clostridium botulinum* (H2), *Proteus mirabilis* (H3), *Clostridium septicum* (H4), *Bacillus anthracis* (H5), *Staphylococcus aureus* (H6), *Pseudomonas aeruginosa* (H7), *Myroides odoratimimus* (H8), *Bacillus weihenstephanensis* (H9), *Clostridium acetobutylicum* (H10). The accession numbers and homology percentage (%) are shown in Table 2.

3.3 Optimized Temperature and pH

The optimum temperature and pH for all bacterial pathogens were also recorded. The optimum temperature and pH for *B. cereus*, *C. botulinum, P. mirabilis*, and *S. aureus* was recorded as 37℃ and pH 5 while 27℃ and pH 7 was shown by *C. septicum* and *B. weihenstephanensis* (Fig 2.A and Fig 2.B).

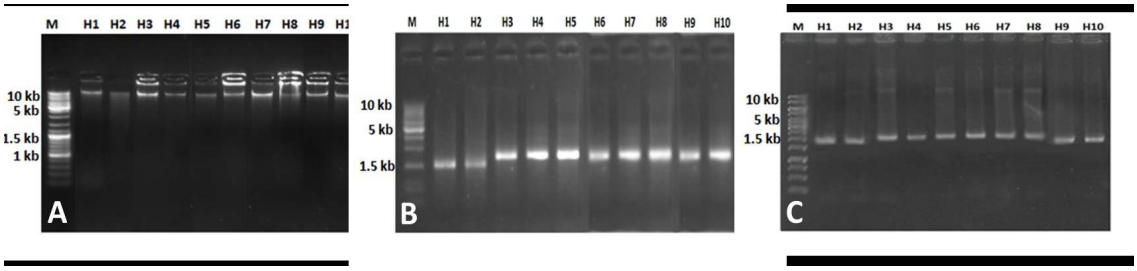

Fig. 1. Genomic DNA extraction, DNA elution and ribotyping. A, 1% agarose gel of genomic DNA. B, 1% agarose gel of PCR products. C, 1% agarose gel of gene clean products

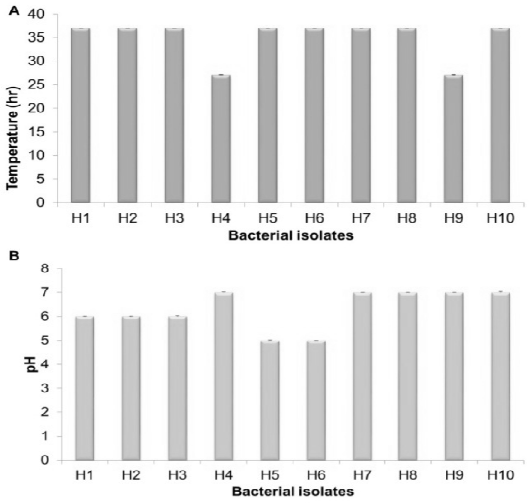

Fig. 2. Effect of physical parameters on the bacterial isolates. (A) Effect of temperature; (B) Effect of pH

Bacillus cereus (H1), Clostridium botulinum (H2), Proteus mirabilis (H3), Clostridium septicum (H4), Bacillus anthracis (H5), Staphylococcus aureus (H6), Pseudomonas aeruginosa (H7), Myroides odoratimimus (H8), Bacillus weihenstephanensis (H9), Clostridium acetobutylicum (H10)

3.4 Inhibitory Effect of Medicinal Plants

Nigella sativa indicated the strongest inhibition of *Bacillus cereus* (H1) and *Bacillus anthracis* (H5) with 10.0±0.0 mm and 10.0±0.0 mm zone of inhibition whereas moderate inhibition of *Pseudomonas aeruginosa* (H7) and *Clostridium acetobutylicum* (H10) was recorded (6.0±0.05 mm and 5.3±0.02). Similarly, *Camellia sinensis* showed the maximum inhibition of *Bacillus weihenstephanensis* (9.5±0.02 mm) while moderate inhibition was observed in the case of other tested microbes. *Aloe vera* and *Cinnamon umverum* either had no effect or low effect on all tested bacterial pathogens (Table 3). On the other hand, the moderate antibacterial activity of *Mentha piperita* was recorded against *Bacillus cereus* (H1), *Clostridium botulinum* (H2), *Pseudomonas aeruginosa* (H7), *Myroides odoratimimus* (H8), *Bacillus weihenstephanensis* (H9), and *Clostridium acetobutylicum* (H10). It was revealed that all plants had a bacterioCIDAL effect except *Aloe vera* and *Cinnamon umverum*.

3.5 Inhibitory Effect of Antibiotics

Antibiogram assay revealed that Ofloxacin was strongly inhibited the all tested pathogens (Table 4). Ampicillin and Ticarcillin showed the moderate inhibition of *Myroides odoratimimus* and *Clostridium septicum* (4.4±0.02 mm and 4.1±0.06 mm). Similarly, Erythromycin indicated the moderate inhibition of *Bacillus cereus* and *Clostridium botulinum* (6.5±0.02 mm and 5.4±0.04 mm).

4. DISCUSSION

Honey is the highly viscous and sweet substance that is produced by honey bees from the nectars of flowering plants. It is produced globally, in virtually all countries of the world and is considered among very major energy foods. It possesses the image of a naturally healthy product. But, nowadays honey is manufactured in an environment that is polluted through diverse sources of contamination. These can be either environmental or apicultural ones [5]. The occurrence of microbes in honey may affect its worth and safety [6]. In Pakistan, honey has not only be used as a food and but also as a medicine. In particular, children and old people are thought to need honey and its derivatives more. The antibacterial activity of honey products also recommended by Awan et al. [27]. There is a common notion in our country that unprocessed honey is tastier and healthy than the processed one. The drive of this study was to investigate and compare pathogenic bacterial contamination in both processed and unprocessed honey.

Table 1. Morphological and biochemical characteristics of isolated bacteria

		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
Staining techniques	Gram's staining	+	+	-	+	+	+	-	-	+	+
	Endospore staining	+	+	-	+	+	-	-	-	+	+
	Acid fast staining	-	-	-	-	-	-	+	-	-	-
	Capsule staining	-	+	-	-	+	-	+	-	-	-
Biochemical tests	Motility	+	+	+	+	-	-	+	-	+	+
	Indole	-	-	-	-	-	+	-	-	-	-
	Citrate	+	-	+	-	+	+	+	-	+	+
	MR	-	-	-	-	+	-	-	-	-	-
	VP	+	-	-	-	-	+	-	-	+	-
	Urease	-	+	+	+	-	+	+	+	-	+
	Catalase	+	+	+	-	+	+	+	-	+	+
	Oxidase	+	-	-	-	+	-	+	+	+	-
	Nitrate	+	-	+	-	+	+	-	-	+	-
	TSI	K/A	A/A,H_2S	G	A/A,H_2S	K/A	A/A	K/K	K/A	K/A	A/A, H_2S
	H_2S	-	+	+	+	-	-	-	-	-	-
	Glucose	+	+	-	+	+	+	-	+	+	+
	Lactose	-	+	-	+	-	+	-	-	-	-
	Sucrose	+	+	-	+	+	+	-	+	+	+
	Litmus	Ac-R	A	AlkP	A-R	AcP	A	AlkP-R	-	Ac	A
	Gelatin	+	+	-	-	+	-	+	-	+	+
Differential and selective media	MacConkey	-	-	+	-	-	-	+	+	-	-
	Eosin-methylene blue	-	-	+	-	-	-	+	+	-	-
	Mannitol salt	-	+	-	+	-	+	-	-	-	-
	Phenyl ethyl alcohol	+	+	-	+	+	+	-	-	+	+
	Casein	+	+	-	+	+	-	-	-	+	+
	Starch	+	-	-	-	+	-	-	-	+	-
	Blood	β	β	α	α	β	β	α	α	β	α

Bacillus cereus (H1), Clostridium botulinum (H2), Proteus mirabilis (H3), Clostridium septicum (H4), Bacillus anthracis (H5), Staphylococcus aureus (H6), Pseudomonas aeruginosa (H7), Myroides odoratimimus (H8), Bacillus weihenstephanensis (H9), Clostridium acetobutylicum (H10), Presence (+), Absence (-), (K/A), (A/A), (K/K), (Ac-R), (A), (A-R), (AcP), (AlkP-R), (AlkP)

Table 2. Molecular characterization of isolated bacteria

Strain	Query length	Query cover	E value	Identity	Species	Accession no.
H1	998	100%	0.0	100%	*Bacillus cereus*	KM975629
H2	910	100%	0.0	100%	*Clostridium botulinum*	KM975630
H3	854	100%	0.0	100%	*Proteus mirabilis*	KM975631
H4	1013	100%	0.0	100%	*Clostridium septicum*	KM975632
H5	854	100%	0.0	100%	*Bacillus anthracis*	KM975633
H6	925	100%	0.0	99%	*Staphylococcus aureus*	KM975634
H7	1104	100%	0.0	99%	*Pseudomonas aeruginosa*	KM975635
H8	1000	100%	0.0	100%	*Myroides odoratimimus*	KM975636
H9	997	100%	0.0	100%	*Bacillus weihenstephanensis*	KM975637
H10	882	100%	0.0	100%	*Clostridium acetobutylicum*	KM975638

Table 3. Zone of inhibition recorded against bacterial contamination

Medicinal plants	Zone of inhibition (mm) M±SD									
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
Aloe vera	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	2.8 ±0 .06	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.5 ±0.05
Nigella sativa	10.0 ±0.0	3.0 ±0.0	3.0 ±0.0	2.0 ±0.0	10.0 ±0.0	2.5 ±0.02	6.0 ±0.05	2.8 ±0.04	2.5 ±0.14	5.3 ±0.02
Cinnamom umverum	4.0±0.0	3.5±0.02	1.3±0.07	1.0 ±0.0	3.5±0.02	1.0 ±0. 0	5.0 ±0.0	3.8 ±0.02	0.0 ±0.0	3.25 ±0.02
Camellia sinensis	5.3 ±0.04	5.5±0.02	7.8 ±0.04	4.3±0.02	8.3±0.04	5.8 ±0.04	6.3±0.04	6.5 ±0.05	9.5±0.02	5.5 ±0.09
Mentha piperita	4.5 ±0.05	4.5±0.02	3.5 ±0.02	1.0 ±0.0	2.0 ±0.0	0.0 ±0.0	4.8±0.06	5.0±0.04	4.3±0.02	7.8 ±0.04

Growth inhibition was recorded as (0) for no sensitivity, (>1-4) for low, (>4-9) for moderate and (>9-18) for high sensitivity. Bacillus cereus (H1), Clostridium botulinum (H2), Proteus mirabilis (H3), Clostridium septicum (H4), Bacillus anthracis (H5), Staphylococcus aureus (H6), Pseudomonas aeruginosa (H7), Myroides odoratimimus (H8), Bacillus weihenstephanensis (H9), Clostridium acetobutylicum (H10), Mean ± Standard Deviation (M±SD)

Table 4. Antibiotics susceptibility against bacterial contamination

Antibiotic	Zone of inhibition (mm) M±SD									
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
Ampicillin	2.0±0.08	1.6±0.03	1.1±0.01	2.5±0.05	3.9 ±0.06	0.9±0.03	1.9±0.04	4.4±0.02	2.1±0.02	1.9±0.02
Cefixime	0.0±0.0	0.0±0.0	1.1±0.01	0.0±0.0	0.0±0.0	0.5±0.0	0.0±0.0	2.0±0.02	0.0±0.0	0.0±0.0
Erythromycin	6.5±0.02	5.4±0.04	3.6±0.06	7.0±0.08	0 .0±0.0	0.0±0.0	0.6±0.03	7.4±0.03	7.1±0.01	1.6±0.05
Ofloxacin	11.1±0.01	12.5±0.02	11.0±0.0	13.0±0.04	1 3.6±0.02	10.5±0.05	13.0±0.04	16.5±0.09	11.3±0.02	10 .6±0.03
Ticarcillin	2.25±0.02	3.75±0.06	1.3±0.01	4.1±0.06	3.5±0.07	2.0±0.04	2.1±0.03	2.0±0.12	0.0±0.0	2.9±0.0 3

Growth inhibition was recorded as (0) for no sensitivity, (>1-4) for low, (>4-9) for moderate and (>9-18) for high sensitivity. Bacillus cereus (H1), Clostridium botulinum (H2), Proteus mirabilis (H3), Clostridium septicum (H4), Bacillus anthracis (H5), Staphylococcus aureus (H6), Pseudomonas aeruginosa (H7), Myroides odoratimimus (H8), Bacillus weihenstephanensis (H9), Clostridium acetobutylicum (H10), Mean ± Standard Deviation (M±SD)

Current research work revealed that unprocessed honey that is raw and untreated harbored more bacteria than the packaged ones. None of the bacteria isolated were honey's natural inhabitant but were contaminations which entered honey during different stages of its production and packaging. These included the bacteria that commonly contaminate soil and water [28]. In current research *Bacillus* and *Clostridium* species were identified. Our findings are consistent with the results of Van der Vorst et al. [11]. Buba et al. [29] also revealed the presence of *Bacillus*, *Kliebsiella* and *Staphylococcus aureus* in a variety of honey samples. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Proteus vulgaris* cause several human infections [19]. The presence of gram negative bacteria indicated recent contamination by a secondary source [6]. They were aerobes, facultative anaerobes, and anaerobes. Most of them produced endospores while only one was found to have a capsule. The presence of bacteria in honey is not surprising. However, the number of pathogenic bacteria in honey is alarming. The production of honey, as well as the storing process, account for the presence of bacteria in honey. The results concluded that processed honey is less contaminated than the one which is not processed i.e. raw. The companies that are marketing honey sterilize them after collection and keep them in sterile packaging with minimum human handling and proper temperature control. Although it can be debated that whether raw honey tastes better or the processed one, but when it comes to bacterial contamination there is no doubt that processed is more safe and clean.

In the present study, the bacterial contamination had been tried to control using medicinal plants and antibiotics. The antibacterial activity of medicinal plants/herbs and antibiotics against bacterial pathogens were investigated through agar disc diffusion method. Findings from the current study revealed that methanolic extract of medicinal plants has potential inhibitory effects on all tested bacteria except *Aloe vera* and *Cinnamon umverum*. Thiruppathi et al. [30] studied the antibacterial activity of extracts of *A. vera* gel against *B. subtilis, S. aureus, E. coli, K. pneumoniae*, and *P. aeruginosa*, respectively. Pawar et al. [31] also indicated the antibacterial activity of leaf extracts of *A. vera* against *S. aureus*. Similarly, leaf extracts of *Mentha piperita* possess potent antimicrobial activity. Similar to our result, the biological activity of *Mentha piperita* against the pathogenic bacteria were reported by [32]. Suresh et al. [33] demonstrated that extracts of cinnamon had active effect against many pathogenic bacteria. It was suggesting that all medicinal plants contain active phytochemical constituents, responsible for eliminating the bacterial pathogens [34,35].

The antibacterial effect of these plants depends on the presence of bioactive phytochemical constituents [36]. On the other hand, some plants had no antibacterial activity, it means that the extracts may be active against other bacterial species which were not tested [37]. The bioactive constituents have the ability to damage the cell membrane, enter to the bacterial cell wall, causes structural changes in the permeability membrane and lead to cause death [38,39]. On the other hand, these constituents involved in the inhibition of DNA, RNA, and protein synthesis [40].

5. CONCLUSION

The findings revealed that the medicinal plants are most effective against bacterial pathogens. The therapeutic value of honey could be enhanced when using along with the medicinal plants, which may reduce the growth of infectious agents. Medicinal plants may be more potential due to the presence of phytochemical constituents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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