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Evaluation of Antimicrobial Efficacy and Physico Chemical Properties of Raw and Processed Honey

Amaravathi D

Research Scholar, Department of Zoology, Bangalore University, Bengaluru, India
E mail id: *amaravathi.db11[at]gmail.com*

Abstract: ***Honey is used as a traditional medicine from ancient period. It is noticed in many literatures. It is used as the purpose of wound healing, gastrointestinal and cardiovascular problems and for the treatment of many infectious diseases, and also had a high health promoting properties it as confirmed many recent research investigations. Hence here examining a Phytochemical composition includes that Moisture content, pH, total solids, density of honey, colour, Alkaloids, Flavonoids, tarpinoids, phenols, saponins, steroids and tannins in both Raw and Processed honey. The antibacterial activity in Raw and Processed Honey Shows strong anti-bacterial activity, Gram positive and Gram negative bacteria were more susceptibility in Raw honey when compared to Processed honey. This reveals that honey apart from their role as food activities and supplements and also to be utilized as effective antibacterial agents for the treatment of infections.***

Keywords: Anti-microbial activity, Honey, Bacterial Strains, Anti-oxidants, Phytochemicals

1. Introduction

Honey is produced by bees from plant nectars. It is one of the most valued and appreciated natural products known to mankind from ancient times it has been practically used as human domestic needs for food and sweet products since many years ago (Sanaa, 2007). It is a sweet natural product that is produced by honey bee from floral nectar, transform through the hypo pharyngeal gland that secretes enzyme and store in honey comb to mature (jasna et al., 2001). which is consumed for its high nutritional value. Concerning its nutrient value, represents an interesting source of natural macro and micro nutrients, consisting of a saturated solutions of sugars, of which fructose and glucose are main contributors but also of a wide range of minor constituents, especially phenolic compounds, with antioxidants, bacteriostatic, anti-inflammatory and anti-microbial properties as well as wound and sunburn healing effects.

There has been increasing interest in traditional medicine through the world for the last two three decades (Tanaka et al., 2009). There is a strong realisation that traditional medicine was incorrectly challenged with enmity in the past by modern medicine and WHO urged developing countries to investigate and make use of the indigenous source of natural substances in order to meet the goal of primary health care (world health organisation, 2002). This method of treatment is affordable, readily available, and accessible and most importantly acceptable to people. By adopting and applying on large scale and interesting it with modern medicine, the dream of health for all can be converted into reality (Elujoba et al., 2005).

Honey is well known for its antibacterial activity, which was first reported in (Dustmann in, 1979). Since ancient times, it has been used for treatment and prevention of wound infections. In ancient communities it was regarded as an important medical treatment for all kinds of health problems (Zaghloul et al., 2001). Miraculous healing properties of honey are mentioned in almost all the Holy scripture including the Holy Quran, the Holy Bible and the Holy Torah (Namias, 2003). The ancient Egyptians, Assyrians, Chinese, Greeks and Romans used honey for almost every wound and diseases of gastrointestinal tract (Molan, 2001). The Greek Physician, Hippocrates (460-377 BC), Who is consider to be the father of modern medicine prescribed honey for different clinical conditions including wounds and gastritis. Honey has been extensively subjected to laboratory and clinical investigation for the last two to three decades (Cooper et al., 2008). Ayurveda, an ancient Indian system of health care treats as food for heath while recommending it as an ancient medicine for some condition's using it eternally as well as orally (Molan, et al., 1992).

The bacteriological effect depends on the structure of the honey which is linked to its origins. At a macro level low water activity inhibits growth. Enzymes such as glucose oxidase produce hydrogen peroxide (White et al., 1963). Which has an Antimicrobial effect but production is limited by other enzymes activity. Peroxides are however destroyed by heat treatment and to maintain potential activity honey must be processed and stored with a care. Other substances present are also believed to contribute to the antibacterial activity. Manuka honey is for example believed to be high aromatic acids and phenolic compounds and flavonoids, which in addition to their antioxidants properties show antimicrobial activity (Russel et al., 1998; Allon et al., 1991, Weston et al., 1999 and Cushine, Lamb., 2005). Similar results have been reported for some other geographic honeys. Recent data suggests that peptides present may also contribute to antibacterial properties particularly in treatment of antibiotic resistant bacteria strains (Kwakman et al., 2011).

Honey has been shown to have anti-inflammatory effects in humans consuming around 70g/d (Al- waili, Boni, 2003). And also had evidence in mice models that honey has a positive effect in models of IBD and colitis (Bisel. Et al., 2002). Comparison to the effects of honey in burns and wound healing (Postmes, 2010). There is though conflicting data concerning dental caries with some reports suggesting a protective effect (Decaix, 1976) Whilst others liken the

effects to sucrose (Bowen, Lawrence, 2005). Honey inhibits growth of *Helicobacter pylori*, the bacteria associated with development of gastric ulcers. Recent work has highlighted the potential antibiotic effect of honey with a number of intestinal pathogens (Lin, 2010). Other researcher has shown positive vascular effects in diabetic patients postulating that this could be used in the management of the condition (Bahrami et al., 2009; Deibert et al., 2010; Erejuwa., 2012). There are numerous reports of the anti-microbial activity of honey against a wide range of bacterial and fungal species (Chute, 2010; Kwakman et al., 2010). The antimicrobial activity could be attributed to osmotic effect of honey, the low pH of honey being between 3.2 and 4.5 (Cooper et al., 2002). Subrahmanyam (1998) compared between honey and silver sulphadiazine on treatment of patients with burns and found less inflammation, lower infection rates and faster healing in patients treated with honey.

2. Materials and Methods

2.1 Collection of Samples

Two raw honey samples were collected from Malur (RHM) and Nelamangala (RHN) directly from beekeeper's respectively. Other two processed honey samples Lion honey (PH1) and Prakruthi honey (PH2) were collected from local shops. The raw honey samples were filtered in advanced for the removal of body parts, since, they were directly extracted from the honey comb. All samples were kept in air tight containers at room temperature (28-30^{0}C) before use.

2.2 Evaluation of Physico – Chemical Properties of Honey

2.2.1 Determination of Colours

Optical density measurements were used for colour classification (Gidamis et al., 2012). Honey samples were heated in a water bath at 50^{0}C to dissolve any fine crystals and filtered to remove any coarse particles which may affect the measurements. Colorimeter was used to measured absorbance at OD 560nm and blank with deionised water. Absorbance values obtain was compared with slandered USDA, colour classification for honey.

2.2.2 Determination of honey density

By mass to volume ratio relationship can procure a density of honey. By the use of a dropper, exactly 1ml of sample was introduced in a 5ml measuring cylinder. The mass was then measured using an electronic balance. The density of different sample was calculated from the following equation

$$\text{Density} = \frac{(\text{Mass of honey} + \text{Cylinder}) - \text{Mass of cylinder}}{\text{Volume of honey}}$$

2.2.3 Determination of moisture content

Moisture content of each honey sample was determined by measuring 5g of sample, placed in pre-weighed beaker. The sample was dried to constant weight in oven at 105^{0}C for 4hrs under vacuum.

$$\text{Moisture} = \frac{M_1 - M_2}{M_1 - M_0}$$

Where,

M_0- weight of empty beaker

M_1- weight of fresh sample + beaker

M_2- weight of dried sample + beaker

2.2.4 Determination of total solids

A total solids present in honey samples was determined using the relationships:

Total solid % = 100 – moisture content.

2.2.5 Determination of pH

The pH values of honey samples are determined by using a pH meter. 10g of honey was dissolved in 75ml cooled, boiled distilled water using a glass rod. Duplicate measurements on two separate test portion from the sample were used. The mean value was calculated (Helrich et al., 1990).

2.2.6 Determination of protein by Lowry's method

1ml of sample was taken and 0.1ml of 2N sodium hydroxide was added. Solution was hydrolysed at 1000C for 10min in boiling water bath. The hydrolysate cooled to room temperature and 3ml of freshly prepared complex forming agent was added. The solution mixed well and 0.1ml folin reagent was added. The absorbance read at 660nm after 30-60min incubation at room temperature.

2.2.7 Phytochemical analysis of raw and processed honey

The samples were screened for the following compounds: alkaloids, flavonoids, tarpinoids, phenols, saponins, steroids and tannins using slandered laboratory techniques (Harbonme 1992; Sofowara, 1993).

2.3 Evaluation of anti-microbial activity of raw and processed honey

A total six bacterial stains both Gam positive and Gram negative bacteria were collected and stored at 4^{0}C for further use. And The Tetracycline Antibiotic was used in 5mg/ml concentration against every bacterial strain. Extraction of both raw and processed honey was performed by using organic solvents, such as methanol, ethanol and ethyl acetate. All the extracts were collected in sterilised glass tube and used within 24hrs for the evaluation of bacteriostatic bactericidal activity. Antibacterial activities of all the extracts were evaluated by zone of inhibition using the agar well diffusion assay (Perez et al., 1990; Goyal et al., 2009; Kaushik et al., 2009).

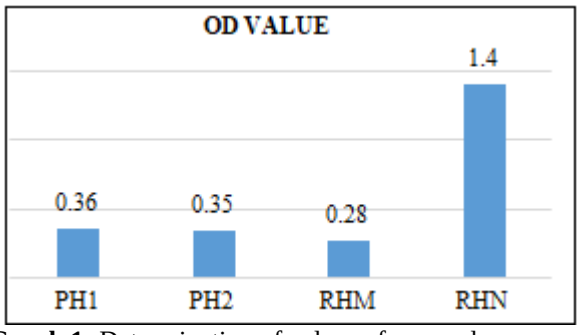
3. Results and Discussion

3.1.1 Honey colour analysis

Colour grading has been used by the honey industry for many years. Different shades of honey colours varies from deep amber to dark molasses. A diversity of honey colours was recorded in the samples studied, which ranged from white coloured to light amber colour honeys. The ranking of colour intensity of the honey samples revealed an array of colours of Raw and Processed honey. On the average, colours of raw honeys were darker than those of processed honey samples (Table 1 and Graph1).

Table 1: Determination of colour of raw and processed honey

Honey Type	OD Value	Colour
PH 1	0.36	White
PH2	0.35	White
RHM	0.28	Extra white
RHN	1.40	Light amber



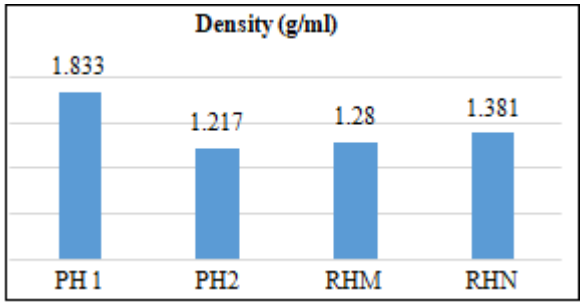
Graph 1: Determination of colour of raw and processed honey

3.1.2 Density of honey samples

Density is an important parameter of practical importance. The density of the samples varied from 1.833 to 1.212 as shown in (Table 2 and Graph 2). Highest density 4.833 was recorded in processed Honey (PH1) and 1.381 was recorded in Raw honey(RHN). Lowest density of 1.217 was recorded in Processed honey (PH2) and 1.28 in Raw honey (RHM).

Table 2: Density of raw and processed honey

Honey type	Density (g/ml)
PH 1	1.833
PH2	1.217
RHM	1.28
RHN	1.381



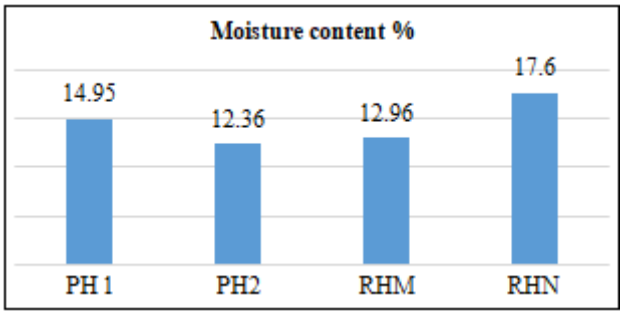
Graph 2: Density of raw and processed honey

3.1.3 Moisture content

Moisture content is an important parameter of honey quality and defines the amount of water in honey. The moisture content of the samples varied from 12.36% to 17.06% as shown in (Table 3 and Graph 3). Highest moisture content of 14.95% was recorded in processed honey (PH1) and 17.6% was recorded in Raw honey (RHN). Lowest moisture content of 12.36% was recorded on Processed honey (PH2) and 12.96% in Raw Honey (RHM) respectively.

Table 3: Moisture content in raw and processed honey

Honey type	Moisture content %
PH 1	14.95
PH2	12.36
RHM	12.96
RHN	17.6



Graph 3: Moisture content in raw and processed honey

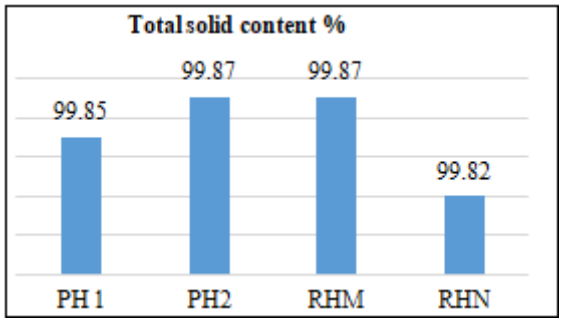
3.1.4 Total solids

The results of the total soluble solids are presented in (Table 4 and Graph 4). The total soluble solids of the honey ranged from 99.82% to 99.87%. The processed honey had the maximum total solid content of (99.876%), Whereas that from the processed honey had the lowest percentage of (99.85%). Raw honey had the maximum total solid content of (99.876%), whereas that from the processed honey had the lowest percentage of (99.82%).

Total solid is measured of dissolved solids in the honey samples. For all the honey samples, total soluble solids were generally more than80% and can be considered of high grade and highly stable upon storage. According to the grading system of the United States Department of Agriculture (USDA), honey with total soluble solids greater or equal to 81.4% is considered of higher grade(Aand B), while that falling between 80% and 81.3% is considered to be of lower grade C. Thus the honey investigated in this study can be stable with regard to fermentation upon storage and thus of high grade. Maximum number of sugars are the total solids in honey. These account for about 80% or more of solids by weight.

Table 4: Total solid content in raw and processed honey

Honey type	Total solid content %
PH 1	99.85
PH2	99.87
RHM	99.87
RHN	99.82



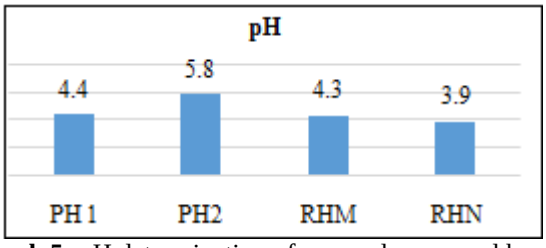
Graph 4: Total solid content in raw and processed honey

3.1.5 Determination of pH

The pH values of the honey samples from different and reveals that all the samples were in the acidic range of pH (Table 5 and Graph 5). The importance of acidic pH range in foods cannot be over emphasized. It prevents the honey samples from constant contamination by various species of micro- organisms and thus helps to ensure longer shelf life.

Table 5: pH determination of raw and processed honey

Honey type	pH
PH 1	4.4
PH2	5.8
RHM	4.3
RHN	3.9



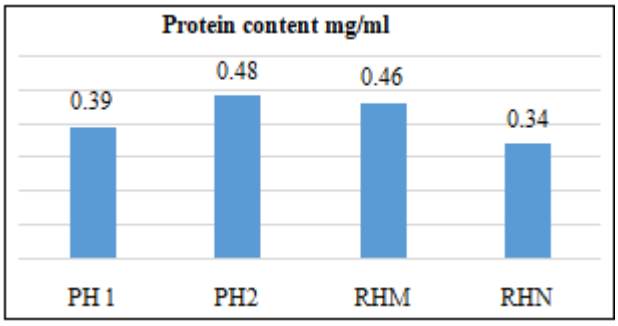
Graph 5: pH determination of raw and processed honey

3.1.6 Determination of protein by Lowry's method

The protein contents of Raw and Processed honey samples are different. The results of protein content are presented in (Table 6 and Graph 6). The protein content ranges from 0.48mg/ml to 0.39mg/ml. The processed honey had the maximum protein content 0.48mg/ml and the lower protein content 0.39mg/ml was recorded. Honey from the raw honey had the maximum protein content 0.46mg/ml, whereas that from the raw honey had the lower protein content 0.34mg/ml.

Table 6: Protein content of raw and processed honey

Honey type	Protein content mg/ml
PH 1	0.39
PH2	0.48
RHM	0.46
RHN	0.34



Graph 6: Protein content of raw and processed honey

3.2 Phytochemical analysis of raw and processed honey

The study revealed the presence of the following Phytochemicals in raw and processed honey (Table 7). Phytochemical screening revealed the presence of alkaloids, flavonoids, terpinoids, and phenolic in the crude extracts of the raw honey samples; whereas saponins, steroids and tannins were absent in processed honey; whereas phenolic and steroids are absent. These classes of compounds are known to possesstherapeutic properties against several pathogens and are therefore supporting its traditional use in curing diseases.Flavonoids helps in healing of wounds and treatment of skin diseases due to their ability to neutralize the activity of wounds, and inflammation. Plants containing alkaloids are used in the treatment of malaria, cold and cough (Thomson 1987). Treatment of heart diseases could be because of flavonoids, saponins and glycosides which stimulates heart, especially saponins that remain within gastrointestinal tract. Some interact directly with dietary with cholesterol producing an insoluble complex which prevents the cholesterol from being absorbed. Dietary saponins reduce plasma cholesterol level in primate thus having the potential to lower the risk of coronary heart diseases in humans.

Table 7: Phytochemical analysis of raw and processed honey

S. no	Parameters	PH1	PH2	RHM	RHN
1	Alkaloids	+	+	+	+
2	Flavonoids	+	+	+	+
3	Tarpinoids	+	+	+	+
4	Phenolics	-	-	+	+
5	Saponins	+	+	-	-
6	Steroids	+	+	-	-
7	Tannins	-	-	-	-

Where,
+= Present
-= Absent

3.3 Antibacterial Susceptibility testing

The belief that honey is a nutrient a drug and an ailment has been carried into our days, and thus, an alternative medicine branch called Apitherapy, has been developed in recent years, honey and other bee products offering treatments against many diseases including bacterial infections. At present a number of honey are sold with standardized levels of anti-bacterial activity. Tables 8, 9, 10, 11, 12, and 13 shows the results of in vitro susceptibility of the extracts of raw and processed honey having varying degree of antibacterial activity against Gram- positive and Gram-negative bacteria using methanol, ethanol, and ethyl acetate.

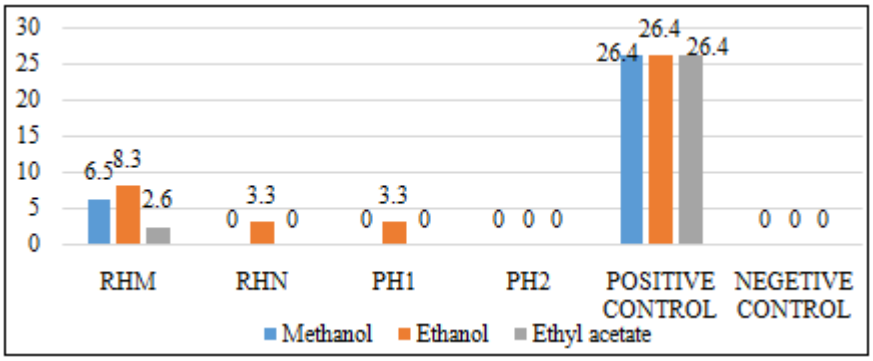
The test organisms show the highest activity in methanol by comparing ethanol and ethyl acetate. This may be due to better solubility and polarity of the active components in methanolcompared to ethanol and ethyl acetate. If such component is present in these raw and processed honey extracts, they could be used for the management of ailments caused by these pathogenic bacteria and give impressive results which could only be determined in vivo.

3.3.1 In case of gram positive bacteria *Streptococcus,* the maximum inhibition as produced by extracts was observed against positive control (26.4mm zone size)< RHM (8.3mm zone size) <RHN (3.3mm zone size) and PH1 (3.3mm zone size). However, PH2 and negative control was found to be inactive as n zone of inhibition observed against *Streptococcus*. The susceptibility of bacteria is present in a (Table 8 and graph 7).

Table 8: Antibacterial activity of extracts against *Streptococcus* (Gram positive)

Extracts	RHM	RHN	PH1	PH2	Positive control	Negative control
Methanol	6.5	NZ	NZ	NZ	26.4	NZ
Ethanol	8.3	3.3	3.3	NZ	26.4	NZ
Ethyl acetate	2.6	NZ	NZ	NZ	26.4	NZ

NZ= No Zone

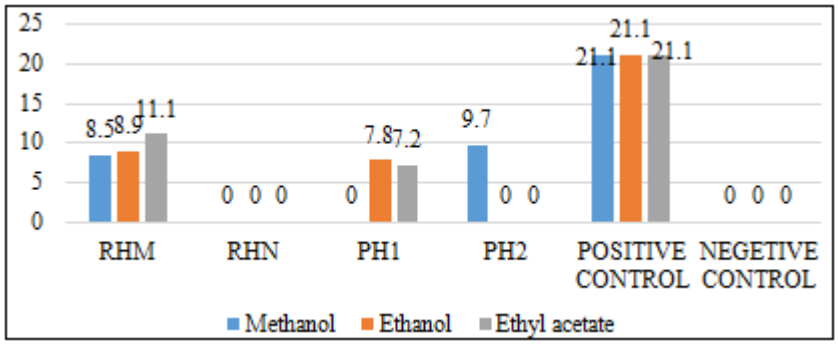


Graph 7: Antibacterial activity of extracts against *Streptococcus* (Gram positive)

3.3.2 In case of Gram positive bacteria *Listeria*, the maximum inhibition as produced by extracts was observed against positive control (21.1mm zone size)<RHM (11.1mm zone size) <PH2 (9.7mm zone size) and PH1 (7.8mm zone size). However, RHN and negative control was found to be inactive as no zone of inhibition observed against *Listeria*. The susceptibility of bacteria is presented in (Table 9 and Graph 8).

Table 9: Antibacterial activity of extracts against *Listeria* (Gram positive)

Extracts	RHM	RHN	PH1	PH2	Positive control	Negative control
Methanol	8.5	NZ	NZ	9.7	21.1	NZ
Ethanol	8.9	NZ	7.8	NZ	21.1	NZ
Ethyl acetate	11.1	NZ	7.2	NZ	21.1	NZ

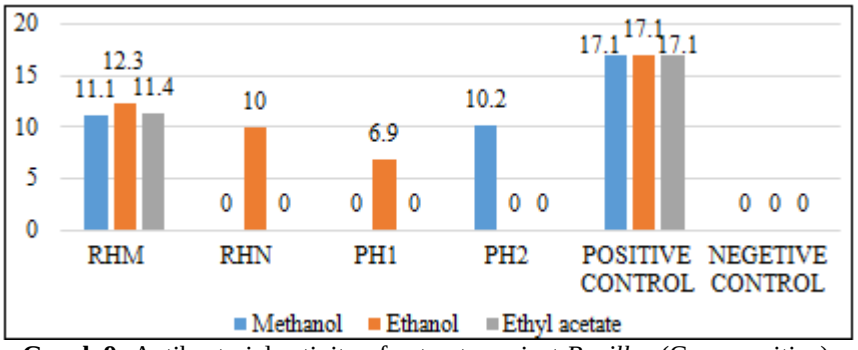


Graph 8: Antibacterial activity of extracts against *Listeria* (Gram positive)

3.3.3 In case of Gram positive bacteria *Bacillus*, the maximum inhibition as produced by extracts was observed against positive control (17.1mm zone size)<RHM (12.3mm zone size) <PH2 (10.2mm zone size) < RHN (10.0mm zone size) <PH1 (6.9mm zone size). However negative control was found to be inactive as no zone of inhibition observed against *Bacillus*. The susceptibility of bacteria is presented in (Table 10 and Graph 9).

Table 10: Antibacterial activity of extracts against *Bacillus* (Gram positive)

Extracts	RHM	RHN	PH1	PH2	Positive control	Negative control
Methanol	11.1	NZ	NZ	10.2	17.1	NZ
Ethanol	12.3	10.0	6.9	NZ	17.1	NZ
Ethyl acetate	11.4	NZ	NZ	NZ	17.1	NZ

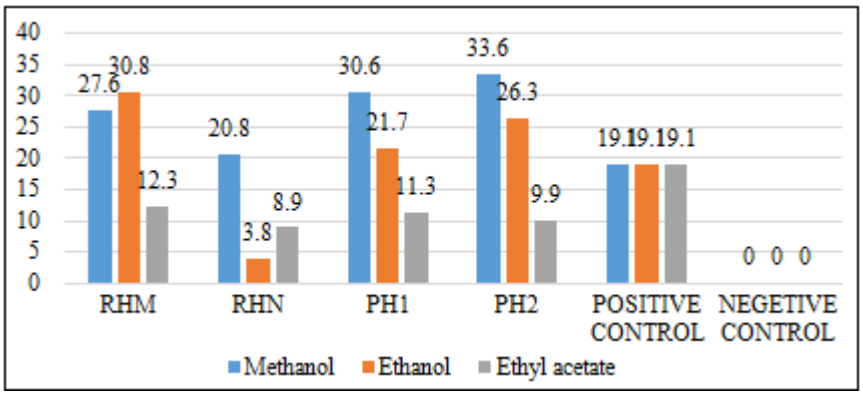


Graph 9: Antibacterial activity of extracts against *Bacillus* (Gram positive)

3.3.4 In case of Gramnegative bacteria *Pseudomonas* The maximum inhibition as produced by extracts was observed against PH1 (3.6mm zone size) <PH2(30.6 mm zone size) <RHM (27.6mm zone size)<RHN (20.8mm zone size) < positive control (19.1mm zone size). However negative control was found to be inactive as no zone of inhibition observed against *Pseudomonas*. The susceptibility of bacteria is presented in(Table11 and Graph 10).

Table 11: Anti-bacterial activity of extracts against *Pseudomonas* (Gram negative)

Extracts	RHM	RHN	PH1	PH2	Positive control	Negative control
Methanol	27.6	20.8	30.6	33.6	19.1	NZ
Ethanol	30.8	3.8	21.7	26.3	19.1	NZ
Ethyl acetate	12.3	8.9	11.3	9.9	19.1	NZ

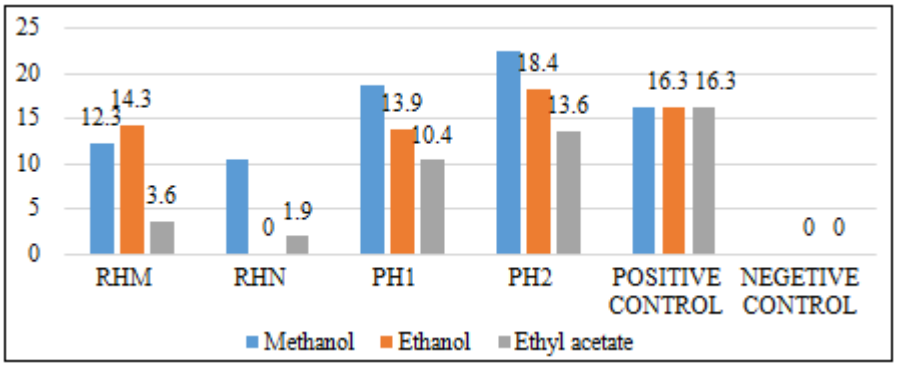


Graph 10: Anti-bacterial activity of extracts against *Pseudomonas* (Gram negative)

3.3.5 In case of gram negative bacteria *Escherichia coli,* the maximum inhibition as produced by extracts was observed against PH2 (22.6mm zone size) <PH1(18.7 mm zone size) < positive control (16.3mm zone size) <RHM (14.3mm zone size) <RHN (12.6mm zone size). However negative control was found to be inactive as no zone of inhibition observed against *Escherichia coli.* The susceptibility of bacteria is presented in (Table12 and Graph 11).

Table 12: Anti-bacterial activity of extracts against *Escherichia coli (*Gram negative)

Extracts	RHM	RHN	PH1	PH2	Positive control	Negative control
Methanol	12.3	10.6	18.7	22.6	16.3	NZ
Ethanol	14.3	NZ	13.9	18.4	16.3	NZ
Ethyl acetate	3.6	1.9	10.4	13.6	16.3	NZ

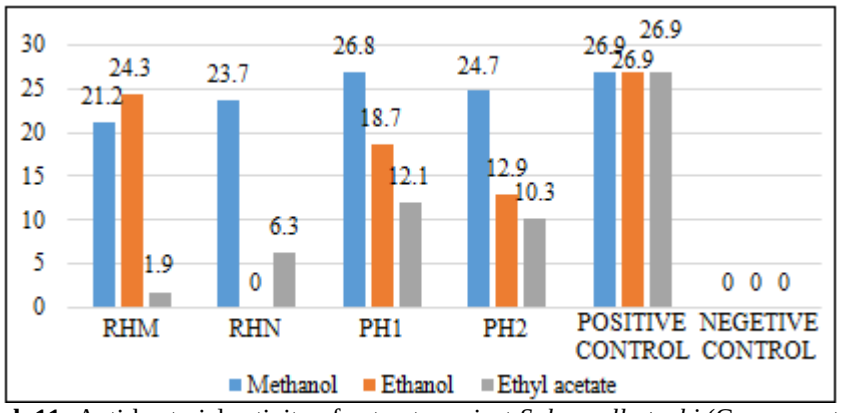


Graph 11: Anti-bacterial activity of extracts against *Escherichia coli* (Gram negative)

3.3.6 In case of gram negative bacteria *Salmonella typhi* the maximum inhibin as produced by extracts was observed against positive control (26.9mm zone size)<PH1 (26.8mm zone size) <PH2(24.7 mm zone size) <RHM (24.3mm zone size) <RHN (23.7mm zone size). However negative control was found to be inactive as no zone of inhibition observed against *Salmonella typhi.* The susceptibility of bacteria is presented in (Table13 and Graph 12).

Table 12: Anti-bacterial activity of extracts against *Salmonella typhi (*Gram negative)

Extracts	RHM	RHN	PH1	PH2	Positive control	Negative control
Methanol	21.2	23.7	26.8	24.7	26.9	NZ
Ethanol	24.3	NZ	18.7	12.9	26.9	NZ
Ethyl acetate	1.9	6.3	12.1	10.3	26.9	NZ



Graph 11: Anti-bacterial activity of extracts against *Salmonella typhi* (Gram negative)

It is clear from the above results that Gram positive bacteria and Gram negative bacteria were more susceptibility to raw honey. When compared to processed honey. Both raw honey and processed honey possess low pH, hence it is acidic in nature which is an favourable condition for bacterial growth. The bacteria were susceptible to honey due to the presence of phytochemical components. Hence honey can be used as an antibiotic in place of chemical standard antibiotic.

4. Conclusion

The unpredictable antibacterial activity of non-standardised honey may hamper its introduction as an antimicrobial agent due to variation in the in vitro antimicrobial activity of various honey at present a number of honeys are sold with standardized levels of antimicrobial activity. The medical grade of honey (Revamil, Medihoney), which has the potential to be a tropical antibacterial prophylaxis because of its broad spectrum bactericidal activity, or to be a treatment for topical infections caused by antibiotic resistant as well as antibiotic sensitive bacteria, should be considered for therapeutic use. Moreover, mountain, Manuka, capillano and eco honey have exhibited inhibitory activity against H. pylori isolates at concentration10%(v/v) _ (Ndip et al., 2007), demonstrating that locally produced honey possess excellent antibacterial activity comparable to the commercial honey therefore it is necessary to study other locally produced but yet untested honeys for their antimicrobial activities.

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