Anti-microbial, anti-oxidant and wound healing properties of different types of honey

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Abstract : Honey is a complex mixture of carbohydrates, proteins, vitamins , water , minerals and anti-oxidants. The increasing demand for honey by each passing day is due to its medicinal properties. As a result of which different honey samples were collected from different geographical areas and floral origins to check the anti-oxidant, anti-microbial and wound healing properties of the honey samples. Different methods were used to determine the anti-microbial activity of the samples like agar dilution method and standard well diffusion method. Khadikraft honey, Heather honey , Lavender honey and many other honey samples has shown great anti-microbial activity. Anti-oxidant property was also determined for different honey samples collected from Malaysia, Bangladesh and India .The anti-oxidant property of honey is due to the presence of compounds like phenols, flavonoids, flavonols as well as its radical scavenging ability. As a result the total phenolic content, total flavonoid content and the DPPH radical scavenging activity of the honey samples were determined to check the anti-oxidant potential. Many honey samples has shown great anti-oxidant potential namely Sourwood honey, Longan, honey, BDH-8 and Coriander honey and many samples were not as efficient as these samples . One of the most important biological property of honey is its wound healing property. To test this property 6 honey formulations were prepared, the effectiveness of Tualang honey was compared with hydrofibre and hydrofibre silver and also the effectiveness of Teucrium polium honey was analysed. Albino rats and Sprague Dawley rats were taken and were wounded at different areas .They were then treated with these honey formulations and honey samples to check their wound healing property. Effectiveness of the samples were tested by measuring the reduction in wound area and the best results were shown by honey 76% chitosan formula, Tualang honey and Teucrium polium honey.

Index terms : Anti- oxidant , Bacteria, Honey, Phenolics, Physicochemical, Resistance, Teucrium polium, Wound excision, Wound incision.

1.INTRODUCTION

Honey is a complex mixture of sugar and water. Besides this honey also contains several vitamins and a lot of minerals . Honey contains several vitamins which involves ascorbic acid , pantothenic acid, niacin and riboflavin while minerals found are copper, calcium, iron, magnesium, manganese, phosphorus , potassium and zinc. Other components of honey are amino acids, proteins, phenol anti-oxidants, flavonoids, organic acids , nitric oxide metabolites , etc. Honey also contains various enzymes like glucose oxidase, diastase, invertase , phosphatase, catalase and peroxidase. Evidence has shown that a variety of honey contains kynurenic acid which may contribute to the anti-microbial property of honey.

Honey contains many compounds which are responsible for its biological properties. One of the properties is anti-microbial activity which in most honeys is due to the enzymatic production of hydrogen peroxide. But there are honey samples known as non-peroxide honey (Manuka honey) which shows great anti-bacterial activity even when hydrogen peroxide production is blocked. The anti-microbial activity of honey also depends on the pH of honey and its sugar content. Honey has a pH ranging from 3.2 to 4.5 which is an acidic pH and low enough to inhibit the growth of microorganisms. Honey also has high sugar content and low moisture content , along with the acidic property of its gluconic acid and the antiseptic property of its hydrogen peroxide which are responsible for its anti-microbial activity. In vitro studies has shown that compounds like hydrogen peroxide , Methylglyoxal and an antimicrobial peptide Bee Defensin 1 are responsible for the anti-microbial activity of honey [10].

Another important property of honey is its anti-oxidant potential. The anti-oxidant potential of honey depends on, the presence of polyphenolic compounds like phenol, flavonoid , flavonols, catechin , cinnamic acid derivatives as well as on its radical scavenging activity. The anti-oxidant potential is also strongly correlated with the colour of the honey sample. Darker the colour more is the presence of phenolic compounds and thus higher is the anti-oxidant potential. Therefore anti-oxidant potential of different honey samples can be determined by measuring the total phenolic content, total flavonoid content as well as their free radical scavenging activity[4].

From ancient times honey has been preferred for wound healing purpose because of its medicinal properties. Because of its viscous nature when applied on wounds , provide a protective barrier to prevent the growth of microorganism[10]. Many wounds are caused by microorganisms which are resistant to antibiotics. Honey has been reported to contain special anti-bacterial and antibiotic properties , due to which they are effective in wound healing. More recently, honey has been reported to have inhibitory effect on 60 bacterial species including aerobes , anaerobes, Gram positive as well as Gram negative bacteria [8]. Honey also has the ability to stimulate human monocytes to produce cytokines . A lot of marketed dressings lose their moisturizing effect and adheres to the surface of the wound thereby damaging the newly formed epithelium but in case of honey , it maintains the integrity of the wound because it is non-adherent in nature [7].

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2. MATERIALS AND METHODS :

2.1 Chemical composition

Honey normally contains anti-oxidant , anti- inflammatory and anti- microbial properties that varies depending on their origin and geographical locations. The presence of phenols and flavonoids determines their anti-oxidant property. Some very important phenolics and flavonoids which are responsible for anti-oxidant property includes Caffeic acid , Isofeluric acid , p- Coumaric acid, Gallic acid, Syringin acid, Quercetin, Luteolin, Chrysin , Galangin. Other very important constituents of honey includes 1,2-dicarbonyl compounds like Glyoxal, 3-deoxyglucosulose and Methylglyoxal which are formed during caramelization reactions as degradation products from reducing carbohydrates , have been identified as very important contributers to the non- peroxide antibacterial activity (José M. Alvarez-Suarez 1,2,*).

Recent studies have shown that honey reduced the activities of cyclooxygenase 1 and cyclooxygenase 2, which were responsible for inflammation , thus showing anti-inflammatory effect. Also in inflammatory model of colitis , honey was as effective as prednisolone treatment. Corticosteroids and other medicines may have many serious side effects, but honey shows anti-inflammatory activity without major side effects. Recently , Gelam honey has shown a decrease in inflammatory mediators such as COX – 2 and TNF – alpha , thereby showing anti-inflammatory property (Natalia G Vallianou1*,2014).

3. ANTI – MICROBIAL ACTIVITY :

3.1 Types of honey sample

Honeys used in this study were Manuka honey (Australia),Heather honey (United Kingdom), locally marketed Indian honey and honey that was procured from local beekeepers in a South Indian village [1].

In this study , 28 honey samples along with one commercial sugar solution was taken from different types of sources including commercial sources of medical grade honey , supermarkets and were stored until use. Also the Gamma irradiated and non – irradiated preparation of the same medical grade honey were available and were tested separately [2].

To analyse the difference in anti-microbial activity , 13 different samples were taken , namely, Artificial honey, Bulgarian lavender, L.x allardii, L. angustifolia , Lavender 1, Lavender 2, Lavender 3 , Manuka , Medihoney , Paterson"s curse , Red stringy bark 1 , Red stringy bark 2 , Rewa Rewa and Rosemary [3].

3.2 Types of microorganisms used :

These honeys were tested for anti-microbial activity against 152 strains of *Pseudomonas aeruginosa* that were isolated from different sources like Chronic suppurative otitis media (56), burn wound infection (17), Diabetic foot ulcers (25), blood (14), environmental isolates (40), Control used was *P. aeruginosa* ATCC [1].

10 wound bacterial isolates were recovered from wounds of horses, , healthy skin samples from a group of horses at livery, equine wounds presented to external veterinary practitioners including Methicillin resistant Staphylococcus aureus and *P. aeruginosa* against which the anti-microbial activities of the honey samples were tested [2].

Bacterial isolates like *E. coli* and *P. aeruginosa* collected from the culture collection of University of New South Wales were taken to check the anti-microbial activity of the honey samples [3].

3.3 Methodology :

The test was done using Agar dilution method to check the anti- microbial activity of these honeys against *P. aeruginosa*. Dilutions of honey were prepared using sterile Mueller Hinton Agar to get concentrations ranging from 1% to 25 % (v/v). Agar plate without honey was used as control. MIC (minimum inhibitory concentration) of chloroxylenol was tested and was used as control . MIC of all the 4 honey samples were tested to check the minimum concentration of honey which can inhibit the growth of different strains of *P. aeruginosa* [1].

These 29 honey products were tested for any kind of microbial contamination by culturing them aerobically on 5% sheep blood agar and MacConkey agar , overnight at 37 degree Celcius. Any contaminated honey products were eliminated from the second part of study. 10 bacterial isolates were collected from different sources and for each isolate Antibiogram test was done using disc diffusion method on Diagnostic Sensitivity Test Agar . Isolates were stored at – 80 degree Celcius using a commercial microbead preservation system.

To perform this test at first honey agar solution was prepared by dissolving honey in sterile distilled water using aseptic measures and then mixing it with an equal volume of double strength nutrient agar. A final volume of 20 ml was used for

each petri dish. Different concentration of honey used were 16%, 14%, 12%, 10%, 8%, 6%, 4% and 2% (v/v).

The 10 bacterial isolates stored in microbeads, were now recovered and streaked onto nutrient agar plate and incubated at 37 degree celcius . For each isolate, 4 to 5 colonies were taken and transferred into a sterile glass capped tube containing sterile distilled water and mixed well. The turbidity was then adjusted and confirmed using colorimeter. Then the suspension of each isolate was prepared and the honey agar plates were inoculated with the bacterial isolates and incubated aerobically overnight , at 37 degree Celcius. After 16-24 hours of culturing , plates were observed for the presence or absence of colonies for each isolate for each honey concentration [2].

Artificial honey is usually made by using sucrose, maltose, fructose, glucose and sterile deionized water. All the honey samples were stored in the dark ,at room temperature until use. Dilutions of each honey sample was prepared by diluting honey in sterile water to make concentrations of 10% , 5%, 2.5% and 1% (w/v). All these honey samples at 4 different concentrations were tested for anti-microbial activity against *E.coli* and *P.aeruginosa* and zones of inhibition were recorded. This test was performed using standard well diffusion method.

The bacterial isolates *E.coli* and *P.aeruginosa* were cultured in nutrient broth. Each bacterial culture was then mixed with cooled nutrient agar and 15 ml of it was used for each petri dish and was allowed to cool. 5 wells were made on each agar plate ,using flamed cork borer. One well was filled with sterile water which was the control and the other 4 were filled with 4 different concentrations for each honey sample and the plate was incubated at 37 degree Celcius , for 24 hours . After 24 hours , the plates were observed for zones of inhibition and the diameter for each concentration was measured [3].

4. ANTI- OXIDANT PROPERTY

Anti-oxidant property in a particular sample is measured by determining the phenolic content, flavonoid content and DPPH free radical scavenging activity of the sample because these components are responsible for the anti-oxidant activity.

4.1 The anti-oxidant property was tested for 4 different Malaysian honey samples (Gelam, Longan, Rubber tree and Sourwood). Gelam honey was sourced from Federal Agriculture Marketing Authority (FAMA) Malaysia, while the other samples Longan, Rubber tree and Sourwood were sourced from beekeepers from Perak , Malaysia. Standard used was Manuka honey.

1. Total phenolics :

Total phenolic content of the honey samples were tested using modified spectrophotometric Folin-Ciocalteu method. During this method each honey sample was mixed with distilled water and the volume was made upto 10 ml .

This honey extract made was then mixed with Folin's reagent . After 3 minutes 10% Sodium Carbonate was added to the reaction mixture and the volume was adjusted using distilled water . The reaction mixture was then kept in dark for 90 minutes and then the absorbance was read at 725 nm using T60 UV/VIS spectrophotometer. Gallic acid was used in calculating the standard curve. Result was reported as mean or standard deviation .

2. Total flavonoids :

The total flavonoid content in all the honey samples were tested using colorimetric assay .

Each honey sample was mixed with distilled water and the volume was adjusted to 10 ml.

The honey extract made was again mixed with distilled water . Then 5% w/v Sodium nitrite was added to the reaction mixture . After 5 minutes , 10% w/v aluminium chloride was added and then after 6 minutes 1M sodium hydroxide was added to the reaction mixture and the volume was adjusted using distilled water . The mixture was then then shaken properly and the absorbance was measured at 510 nm. Standard solution of Catechin was used to make the calibration curve and the results were expressed as mg Catechin equivalents per kg of honey.

3. DPPH free radical scavenging activity :

This test was done to check the DPPH free radical scavenging activity of the honey samples .

During the method each honey sample was mixed with distilled water and the volume was adjusted to 10 ml.

The honey extract made was then mixed with methanolic solution containing DPPH radicals. The mixture was vigorously shaken and incubated for 15 minutes in the dark until the absorbance remained unchanged . The reduction in DPPH free radical was determined by measuring the absorbance at 517 nm. Reference used was Butylated hydroxytoluene (BHT). Radical scavenging activity (RSA) can be calculated using the formula –

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%RSA = ([A DPPH – A S] / A DPPH) X 100, where

A S – absorbance of the solution when the sample extract has been added at a particular concentration.

A DPPH – absorbance of DPPH solution [4].

4.2 The anti-oxidant property was tested for 5 different monofloral and 3 different multi floral honey samples collected from different parts of Bangladesh.

1. Total phenolics :

The total phenolic content of all the honey samples were tested using modified spectrophotometric Folin-Ciocalteu method.

Each honey sample was mixed with Folin's reagent and then after 3 minutes , 10 % of Sodium carbonate solution was added to the reaction mixture and the volume was adjusted using distilled water . The mixture was then kept in the dark for 90 minutes and then the absorbance was measured at 725nm using a T60 UV/VIS spectrophotometer. The standard curve was calculated using Gallic acid and the results were reported as mean + or – standard deviations and were expressed as mg of Gallic acid equivalents per kg of honey.

2. Total flavonoids :

The total flavonoid content in all the honey samples were tested using Colorimetric assay developed by Zhishen.

During this process each sample was mixed with distilled water. Then the reaction mixture was added with 5% w/v sodium nitrite . Then after 5 minutes 10% w/v aluminium chloride was added , followed by the addition of 1M sodium hydroxide after 6 minutes and the volume was adjusted using distilled water. The mixture was then properly shaken and the absorbance was measured at 510 nm . Calibration curve was made using a standard solution of Catechin and the results were expressed as mg Catechin equivalent per kg honey.

3. DPPH free radical scavenging activity :

All the honey samples were tested for their DPPH free radical scavenging activity . The method used for this test was proposed by Ferreira et al. During the process honey solution was mixed with methanolic solution containing DPPH radicals . The mixture was then shaken vigorously and left in dark for 15 minutes . Reduction in DPPH free radicals in the mixture was determined by measuring the absorbance at 517 nm. Butylated hydroxytoluene was used as reference and Radical Scavenging Activity was measured using the formula ,

%RSA = ([A DPPH – A S]/ A DPPH) X 100 , where

A S - absorbance of the solution when the sample solution has been added at a particular level

A DPPH – absorbance of DPPH solution [5].

4.3 Four different Indian honey samples were taken to check the anti-oxidant property namely, cotton honey , coriander honey Dalbergia honey and Murraya honey .

1. Total phenolics :

The total phenolic content of all the 4 honey samples were tested using Folin-Ciocalteu method . Each sample of honey was dissolved in distilled water and mixed well. The mixture was then filtered using a Whatman no. 1 filter paper. Then 0.2 N Folin's reagent was added to the mixture for 5 minutes , followed by the addition of sodium carbonate solution. All the sample mixtures were then incubated at room temperature in dark for 2 hours and then the absorbance was measured at 760 nm against a methanol blank. The calibration curve was made using Gallic acid and the results were expressed as mg of Gallic equivalents (GAE) per 100 g of honey.

2. Total flavonoids :

The total flavonoid content of all the honey samples were tested using a method described by Kim et al. (2003) and modified by Blara et al. (2005). Calibration was done by using different concentrations of Quercetin. Honey solution was mixed with 5% sodium nitrite , followed by the addition of 10% aluminium chloride after 5 minutes. The honey samples were then mixed well and neutralised using 1M sodium hydroxide solution after 6 minutes . The absorbance was then measured at 510 nm and the results were expressed in mg Quercetin equivalents (QE) per 100 kg of honey, average of 3 replications.

3 .DPPH free radical scavenging activity :

Radical scavenging activity of all the honey samples were determined using method described by Meda et al. (2005) based on DPPH inhibition. Honey samples at 3 different concentrations were mixed with methanolic soliution containing DPPH radicals . Blank sample used was Methanol . The mixtures were then kept at room temperature for 15 minutes and the absorbance was measured at 517 nm . Positive controls used were Quercetin and ascorbic acid .

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The Radical Scavenging Activity was calculated using the formula ,

%Inhibition = [(Blank absorbance –Sample absorbance)]/ Blank absorbance x 100. For each honey sample mean of 3 IC50 (concentration causing 50% inhibition) values were determined [6].

4. WOUND HEALING PROPERTY

4.1 The research was done to compare the wound healing property of honey 75%- chitosan formula, pure honey, silver sulphadiazine and normal saline. Honey based hydrogel formulations were prepared using 3 different concentrations of honey with gelling agents, chitosan and carbopol 934.

Materials used for the research were honey, chitosan (low molecular weight), glacial acetic acid, triethanolamine (TEA) and methyl paraben.

Three different concentrations of honey (25%, 50%, 75%) were tested, each concentration formulated twice , once with carbopol 934 and other with chitosan. Cold mechanical method was used to prepare 6 honey hydrogel formulae. The two different hydrogels (chitosan and carbopol 934) were prepared by dissolving the correct amount of polymer in sterile water with constant stirring using magnetic stirrer for 1 hour. To maintain the pH of hydrogel , TEA was used to neutralize the carbopol hydrogel and methyl paraben was used as preservative and then the hydrogel was left for 24 hrs for complete swelling and polymer equilibration to occur. Different concentrations of honey were then added to the mixture and stirred well and the final weight was made upto 100g using aqueous solution. The final formulations were kept in proper containers and stored in refrigerator.

IN VIVO BURN HEALING EVALUATION:

For the experiment , 10 Albino mice were taken , which were of 30-35 kgs in weight and 8 weeks old in age. The animals were kept in polyethylene cages, at the temperature of 21 to 25 degree Celcius . The 10 albino mice were divided into 2 equal groups of males and females which were anaesthetized using anaesthetic ether . After the mice were anaesthetized , they were shaved on the back using electric clipper and then using a shaving cream. The shaved region was then disinfected using 70% ethanol. Burn wounds were then made using a cylindrical metallic rod (10mm diameter) . The metallic rod was heated over an open flame for 30 seconds and then was pressed over the shaved area on the skin surface of the mouse for 20 seconds. 4 burn wounds were made on the back of each mouse. All the 4 wounds on the back of each mouse were treated with F (Honey 75% - chitosan formula), H (pure honey – 100%), P (positive control, standard wound healing cream, silver sulphadiazine), N (negative control, normal saline).

Using a sterile cotton swab all the honey formulations were applied on all the 4 wounds on the back of each mouse once daily in a rotational manner (FHPN, HPNF, PNFH, …….) for 9 days.

MEASUREMENT OF WOUND AREA :

The change in burn edge diameter was measured using a digital caliper everyday before the application of treatment . Decrease in burn edge diameter from the original burn edge diameter was measured to determine the effect of honey in wound healing [7].

4.2 To determine the wound healing property of honey and to compare it with hydrofibre and hydrofibre silver , 36 female Sprague Dawley rats were taken and then divided into 3 groups, each group having 12 rats. All the rats were injected with anaesthesia and then 3 full thickness burn wounds were made on the dorsum of each rat. Then all the burn wounds on each rat were inoculated with either *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* or *Acinetobacter baumannii*. Then all the 3 wounds on each rat were treated with Tualang honey, hydrofibre and hydrofibre silver respectively. Using sterile cotton swabs samples were collected every 3 days (3, 6, 9, 12, 15, 18, 21) for quantitative and semi-quantitative microbiological assays.

ANAESTHESIA AND SURGICAL PROTOCOL :

The rats taken were placed in ventral position and their abdomen was immobilized for surgery. For the process to occur the dorsum of each rat was shaved and immediately before the surgery each rat was anaesthetized using an intramuscular injection that was a mixture of Ketamine (35.0 mg/kg) and Xylazine (5.0 mg/kg) in gluteal area. The shaved area was then cleaned using iodine , alcohol and hibiscrub.

3 full thickness wounds were made on the dorsum of each rat using a metallic rod that was heated over an open flame at 100 degree Celcius for 30seconds. Each wound was 10 mm by 10 mm and 20 mm apart. All the burn wounds on each rat were inoculated with one of the three burn wound contaminants. For group A all the wounds were inoculated with *Pseudomonas aeruginosa* (Group-A) , for group B all the wounds were inoculated with *Klebsiella pneumonia* (

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Group-B) and for group C they were inoculated with *Acinetobacter baumannii* (Group-C).

Then on each rat the first wound was treated with undiluted Tualang honey,the second wound was treated with hydrofibre and the third wound was treated with hydrofibre silver. All the dressings were then coated with plain gauze and then covered with crepe bandage. Reduction in wound size was measured to check the wound healing activity of honey [8].

4.3 To determine the wound healing property of honey 36 Sprague Dawley rats were taken and were divided into 4 equal groups, each containing 9 rats. On each rat 2 full thickness wounds were made over the dorsal thoraic region . Wounds were then treated twice a day with Teucrium polium honey , until the healing was achieved . Out of the 4 groups , 2 were incision wound groups and 2 were excision wound groups along with the respective control groups. All the rats were anaesthetized using a mixture of Ketamine (56 mg/kg) and Xylazine (5.0 mg/kg). Each rat was then shaved on the back and was stabbed with 2 full thickness incision (2 cm in length) or/ and excision (1cm in diameter) wounds on the dorsal thoracic region using a surgical scalpel.

The rats were treated with Teucrium polium honey twice a day from day 0 . Change in the wound length and area was measured to see the wound healing activity of honey, every 3 days. Wound contraction is expressed as reduction in original wound size [9].

4.4 In vitro wound healing evaluation : Elia Ranzato, Simona Martinotti, Bruno Burlando has performed this experiment under in vitro conditions to check the wound healing effects of monofloral honeys like Acacia, Buckwheat and Manuka on human fibroblasts.

All the reagents required for this experiment were collected from Sigma-Aldrich and the human fibroblast cell line was collected from European Collection of Cell Cultures (ECACC). The cell line was collected from the skin of an individual having hypo-gammaglobulinemia . The cells were maintained at 37 degree Celcius , 5% carbon dioxide , in DMEM supplemented with 10% fetal bovine serum (FBS)and 1% antibiotic mixture.

a) Scratch wound assay

To perform this experiment Fibroblasts were added to the 12 – well plates and were allowed to settle and grow. Then the scratch wounds were created in the cell monolayers using a sterile micropipette tip. The suspended cells were then washed away and the cultures were refed with medium containing different concentrations of honey for 24 hours.

The cells were then fixed using formaldehyde in PBS or 30 minutes and stained using toluidine blue for 30 minutes at room temperature.

To see the wound healing effect of honey under in vitro conditions wound area was measured just after wounding and after treatment and then the cells were observed using inverted microscope equipped with a digital camera.

b) Cell migration assay

Cell migration assay was performed using trans well plates. The cells were seeded in the upper compartment of the filter and the medium containing 0.1% honey was added to the lower compartment of the filter. After 24 hours the filter was removed and stained with Crystal Violet for 10 minutes and then washed thrice with water.

Then the upper side of the filter was cleared by scraping with a cotton swab to remove the cells which just attached but did not migrate and then the filter was washed with PBS ,dye was eluted from cells using 33% acetic acid and was measured at 540 nm in a plate reader [11].

5. RESULT ANALYSIS :

5.1 Anti- microbial activity

a) The result of the test performed to check the anti-bacterial activity of 4 different honey samples has shown that ,all the bacterial isolates were capable of growing at concentrations of honey ranging from 1% to 10% (v/v).

The MIC (minimum inhibitory concentration – minimum concentration of honey that can prevent the growth of bacterial isolates) values for all the 4 honey samples varied greatly, with Manuka , Heather and local honey having 20% MIC value against all the strains of *P.aeruginosa* while Khadikraft honey having 11% MIC value , showing that it has the best anti- microbial activity. The ATCC strain of *P.aeruginosa* gave 10% to 11% MIC value. Chloroxylenol was used as an antiseptic control and the MIC of chloroxylenol was found to be 15% for all the strains of *P. aeruginosa* [1].

b) 29 honey products were taken to test their anti-microbial activity against 10 bacterial isolates collected from different sources . All the 29 samples were tested to check any kind of contamination with aerobic bacteria and fungi. Out of 29 , 18 were found to be contaminated and were eliminated from the further process.

15 honey products were found to have *Bacillus* species , single supermarket honey had *Proteus* species , commercial North African honey had an unidentified

Enterobacteriaceae organism and second commercial North African honey had an unidentified fungus.

MIC ANALYSIS :

Out of 29 honey samples taken , 18 were contaminated with aerobic bacteria and fungi , thus the remaining uncontaminated 11 honey samples were tested for anti-microbial activity against 10 bacterial isolates.

Out of 11 uncontaminated products , 8 were found to be effective against all the 10 bacterial isolates , at concentrations ranging from <2% to 16% (v/v).

Out of these 8 effective products , Scottish Heather honey had the best anti-microbial activity and inhibited the growth of all the 10 isolates at concentrations ranging from <2% to 6% (v/v).

Sugar solution had failed in inhibiting 5/10 bacterial isolates at 45% concentration and below and hence had the worst anti-microbial activity.

Acinetobacter baumanii and *P. aeruginosa* had shown most resistivity, but at the same time all the 10 honey samples were able to inhibit their growth at concentration as low as 4%.

Enterococcus faecalis was the most resistant bacterial strain , although 8/10 honey samples were able to inhibit its growth at concentration ranging from 6% to 16% (v/v).

The MIC values for brand one medical grade honey in irradiated and non-irradiated form were the same[2].

c) When 13 different honey samples were tested for their anti – microbial activity against *E.coli* and *P.aeruginosa* , the result has shown that, the artificial honey had no effect on the growth of *E.coli*. but inhibited the growth of *P.aeruginosa* at 10% and 5% concentration.

All the other honeys had greater anti-microbial activity against *E.coli* than artificial honey t 10% and 5% concentration.

At 2.5% concentration , only Paterson's curse honey had significant growth inhibitory activity on *E.coli* while the growth of *P.aeruginosa* was inhibited by Lavender 2, Red stringy bark 2 and Rosemary honey only.

The growth of *P. aeruginosa* was also significantly inhibited by Bulgarian lavender, Lavender 2, Red stringy bark 2, Rosemary honey and Medihoney.

At 1% concentration , there was no anti-microbial activity against any of the bacterial isolates.

At 10 % concentration , Rewa Rewa honey had shown the greatest inhibition of E.coli with zones of inhibition significantly larger than Manuka, Red stringy bark 1, Lavender 1, L.x allardii and lavender 2 honeys.

Paterson's curse honey had the most significant effect only on *E.coli*, at 2.5% concentration but not on *P. aeruginosa*. But its zone of inhibition was not significantly larger than that of the other honeys showing some anti-microbial activity.

Bulgarian Lavender honey, at 10% concentration produced largest zone of inhibition against *P.aeruginosa*, but it was not significantly different from Rosemary or Lavender 2 honey .But at the same time was significantly greater than all other honeys.

Out of the 3 honeys labelled as anti-bacterial honeys , Rewa Rewa, Medihoney and Manuka honey, Medihoney at 10% concentration had the best anti-bacterial activity and produced significantly larger zone of inhibition against *P.aeruginosa* than rewa rewa, manuka, red stringy bark 1, Paterson's curse, L.x allardii, Llavender 1 and Lavender 3 honeys. Medihoney was not that different from Red stringy bark 2, Rosemary, Bulgarian lavender, L. angustifolia or Lavender 2 honeys [3].

5.2 Anti- oxidant activity

a) Total phenolics :

Polyphenols are important compounds that not only influences the appearance but also the functional properties of honey. 4 different Malaysian honeys (Gelam, Longan, Rubber tree and Sourwood honey) were checked for anti-oxidant property.

The total phenolic content of the honey samples ranged from 144.51 and 580.03 mg GA / kg of honey.

The highest phenolic content was observed in Sourwood honey having a value of 580.03 mg/kg and second highest phenolic content was observed in Longan honey having a value of 563.55 mg/kg. So , Sourwood honey and Longan honey were observed to have better anti-oxidant potential and also their phenolic content was greater than the phenolic content in Manuka honey which was 429.61 mg/kg.

The phenolic content of Sourwood and Longan honey were greater than,

Slovenian fir (242 mg/kg), Forest honey (234mg/kg), Algerian honey (411 to 498.15 mg/kg), Indian rain forest honey (455 mg/kg), Bangladeshi honey (152.4 to 688.5 mg/kg), Morning glory honey from Cuba (348 mg/kg), Black mangrove honey (233.6 mg/kg), Christmas vine honey (214 mg/kg).

Higher the phenolic content of Sourwood and Longan honey , higher is the amount of these substances in Sourwood and Longan trees.

Sourwood honey was observed to have greater phenolic content as compared to other Malaysian honeys like, Tualang honey (252+ or – 7.9 mg/kg), Pineapple honey (278 mg/kg) and Manuka honey (52.63 + or – 1.21 mg/100g) [4].

To test the total phenolic content Gallic acid was used as standard. The total phenolic content of all the honey samples used (5 different monofloral honeys and 3 different multifloral honeys) ranged from 152.4 to 688.5 mg Gallic acid /kg .

The mean total phenolic content in our honey samples (44mg GA/kg) was greater than Manuka honey and this shows that the Bangladeshi honey samples were equally good ,if not superior to Manuka honey.

The anti-oxidant potential of all the Bangladeshi honey samples used were greater than honey samples from Cuba , Slovenia and Burkina Faso.

Highest total phenolic content was observed in BDH-8 (688.5 + or – 5.9 mg GA /kg) [5].

4 different Indian honey samples (cotton, coriander, Dalbergia and Murraya) were used to test the anti-oxidant potential. The total phenolic content in these honey samples were small. The mean of total phenolic content in our honey samples were found to be – Coriander honey (24.98 + or – 0.13 mg), Murraya honey (26.28 + or – 0.17 mg) , Dalbergia honey (31.29 + or – 0.23 mg) and cotton honey (19.28 + or – 0.15 mg) [6].

b) Total flavonoids :

Flavonoids are low molecular weight phenolic compounds that enhances the aroma and the anti-oxidant property of honey. The total flavonoid content of the honey samples used, ranged from 14.20 to 156.82 mg catechin/kg. The highest flavonoid content was observed in Sourwood honey (156.82 mg/kg) and the second highest flavonoid content was observed in Longan honey (142.63 mg/kg). Both Sourwood and Longan honey had greater flavonoid content than Manuka honey (97.62 mg/kg). The flavonoid content of these 2 honeys (Sourwood and Longan) were greater than Algerian honey, Indian forest honey, fir, Lavender , ivy and Acacia from Cuba , Bangladeshi honeys, Tualang, Gelam and Pineapple honey from Malaysia[4].

All the Bangladeshi honey samples used for testing the anti-oxidant property had lower flavonoid content than polyphenols . Bangladeshi honey samples had slightly lower flavonoid content value than Manuka honey (85.05 mg catechin /kg). The total flavonoid content of the honey samples ranged from 36 to 155 mg catechin/kg which was similar to the Malaysian honey samples but greater than Cuba samples. Highest flavonoid content was observed in BDH-8 (155 + or – 6.9 mg Catechin /kg), which was a multifloral honey [5].

The total flavonoid content obtained were different for all the honey samples.

Highest flavonoid content was observed in Coriander honey (7.18 + or 1.53).

Minimum flavonoid content was observed in Cotton honey (4.79 + or – 1.78) [6].

c) DPPH radical scavenging activity :

DPPH is a nitrogen based radical that is used for testing free radical scavenging activity of various substances. For determining the free radical scavenging activity of all the 4 Malaysian honey samples , DPPH Assay was used. Higher the DPPH scavenging activity of the honey samples , higher is the anti-oxidant activity. All the 4 honey samples were tested for anti-oxidant activity at different concentrations (10, 20, 40 and 60 mg/ml). Maximum DPPH inhibition was observed at the concentration of 60mg/ml for all the honey samples.

Maximum DPPH radical scavenging activity was seen in Sourwood honey (59.26%) . DPPH scavenging activity and anti-oxidant potential of all the 4 Malaysian honey samples were greater than Gelam and Borneo tropical honeys, Indian honey and Algerian honey [4].

Higher the DPPH radical scavenging activity , higher is the anti-oxidant property of the sample. DPPH radical scavenging activity of the Bangladeshi honey samples ranged from 33.6% to 97.5 %, which was similar to Indian honeys. The highest DPPH scavenging activity was seen in 4 of the samples , BDH- 2, 4, 7 and 8 and this shows that these 4 samples had good anti-oxidant potential. Highest DPPH value and highest anti-oxidant potential was observed in BDH-8 sample. Second highest DPPH value and good anti-oxidant potential was seen in a monofloral honey (BDH-4) sourced from G. abyssinica , which had greater anti-oxidant potential than BDH-2 [5].

The highest DPPH value was observed in Coriander honey (52.49 + or – 0.45).

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The second highest DPPH value was observed in Murraya honey (43.98 + or – 0.76) and much difference was not observed between Murraya (43.98 + or – 0.76) and Dalbergia honey (42.36 + or – 0.39).

The lowest DPPH value was observed in Cotton honey (39.89 + or – 0.82)[6].

5.3 Wound healing property :

a) 6 honey formulations were prepared to check the wound healing property. 10 Albino mice were taken and were divided into 2 equal groups of males and females and were wounded using hot metallic rod on their backs. All the wounds were then treated with these 6 formulations to see their wound healing property.

The maximum burn diameter contraction was reached by F3 (honey 76 % - chitosan formula) and had shown best wound healing property than pure honey and commercial products [7].

b) To check the wound healing property of Tualang honey, 36 Sprague Dawley rats were used and were divided into 3 equal groups , each having 12 rats. 3 full thickness burn wounds were made on the dorsum of each rat and these three wounds were treated with tualang honey, hydrofibre and hydrofibre silver to see which one has better wound healing property.

Tualang honey had shown the maximum wound size reduction while in case of hydrofibre silver , wound size reduction was smaller. In case of Tualang honey , wound size significantly reduced on days 3, 9 and 15. There was 12.86% reduction in wound size from the original 100 mm square wound on day 3 under the treatment of tualang honey and further this wound size reduction had increased to 33.94% by day 9. Hydrofibre silver had shown only 2.20 % wound size reduction on day 3 and 13.74 % on day 9. Wound healing activity was observed till 21 days to see the effect of Tualang honey on wounds. On 21st day ,wounds inoculated with *P.aeruginosa* and the wounds inoculated with *A.baumannii* healed completely under the treatment of tualang honey while the other wounds inoculated with other microorganisms did not heal completely [8].

c) To determine the wound healing property of Teucrium polium honey ,36 Sprague Dawley rats were taken and were divided into 4 equal groups , each having 9 rats . Out of the 4 groups , 2 were incision wound groups and 2 were excision wound groups. 2 full-thickness wounds were made on the dorsal thoraic region of each rat. Then these wounds were treated with Teucrium polium honey twice a day to see its effect.

In case of incision wound groups , the mean healed wound area percentage increased significantly on days 6, 9, 12 and 15 as compared to control and also in case of excision wound groups , the mean healed wound area percentage increased on days 6, 9, 12 and 15 as compared to control. On days 6, 9 and 12 ,there was a significant increase in mean healed wound area in excision wound model than the control while on day 6 and 9 , the mean healed wound area increased significantly in incision wound model as compared to excision wound model [9].

d) Scratch wound assay :

During this experiment , a monolayer of fibroblast cells were wounded by scratching and were incubated with or without honey. A series of positive controls were exposed to platelet lysate (PL) which was known to promote healing of scratch wounds. Platelet lysate was obtained from blood samples . In 24 hours, the cells exposed to Acacia and Buckwheat honey has shown higher wound closure rates than controls but in case of Manuka honey , wound closure rate was significant but of lower effect [11].

Cell migration assay :

To check the effect of honey on cell migration rates, Chemotaxis Assay was performed .In the presence of 0.1 % honey , the number of cells migrating was much higher for all the honeys with respect to control.

Acacia honey had shown stronger effect than that of platelet lysate while the effect of Buckwheat honey was similar to platelet lysate. In case of Manuka honey the effect was lower but significant and also it had lower potential than the other two honeys [11].

6. DISCUSSION :

Honey contains different compounds which are responsible for its different properties including anti-microbial, anti-oxidant and wound healing properties. Different honey samples from different origins and sources have been tested and many of them differed from each other based on their nectar sources .

6.1 Anti-microbial activity of the honey samples have been tested against different microorganisms including *P. aeruginosa* , wound bacterial isolates and *E. coli* and all of them has shown different anti-microbial activities. The anti-

microbial activity of the samples have been tested using Agar dilution method.

Anti-microbial activity is basically the ability of the honey samples to prevent the growth of microorganisms. The growth of unwanted or harmful microorganisms can lead to many diseases. Different compounds present in honey like Glyoxal, 3 – deoxyglucosulose and Methylglyoxal are responsible for its anti-bacterial activity and also the sugar naturally present in honey has the ability to draw water from the bacterial cells , thereby preventing them from multiplying . If taken in consideration the Manuka honey , it has shown a great anti-microbial activity because it contains the compound Methylglyoxal that is cytotoxic in nature and is a small molecule that may easily pass into the skin and kill the bacteria. Hence , different honey samples have been tested to check their anti-microbial activities. Studies on honey has also shown that , commercially available Dabur honey also has anti-bacterial activity, but when compared to manuka honey it was less significant and effective.

6.2 Honey is a natural product that is produced by honeybees and it consists of a complex mixture of sugars in which glucose and fructose are the main ingredients. Honey has various properties and one of them is its anti-oxidant property. Anti-oxidant property has been observed in honey due to the presence of polyphenolic compounds like phenolic acids, flavonoids , flavonols , Catechin and Cinnamic acid derivatives. Anti-oxidant activity is basically the ability of the honey samples to scavenge free radicals or to prevent the formation of free radicals. Different honey samples collected from different sources and origins have been tested for their anti-oxidant properties by measuring the total phenolic content, total flavonoid content and the DPPH free radical scavenging activity of the honey samples and all of them has shown different levels of anti-oxidant property at different concentrations. Studies has also shown that commercially available Patanjali honey and Dabur honey has anti-oxidant property.

6.3 Like many other properties honey also contains wound healing property. Honey has an acidic pH ranging from 3.2 to 4.5 which when applied to wounds, the acidic pH stimulates the blood to release oxygen which is very important for wound healing. Also this acidic pH prevents or reduces the presence of proteases that impairs the wound healing process.

Sugar which is naturally present in honey has the ability to draw water out of the damaged tissues , thereby reducing the swelling and encouraging the flow of lymph to heal the wound.

Different honey samples have been tested for wound healing property using Albino mice and Sprague Dawley rats . Full thickness wounds have been created on the back and the dorsal thoraic region of the rats and then treated with different concentrations of honey to check their wound healing property. Also the honey treatment has been compared with the treatment using silver sulphadiazine, normal saline, honey based hydrogel formulation , hydrofibre and hydrofibre silver. In the first case honey 76% - chitosan formula has shown the maximum wound healing property and in the rest of the two cases honey has shown the maximum wound healing property.

Also the wound healing effect of honey on human fibroblast was tested under in vitro conditions using scratch wound healing model. Honey samples tested were Acacia, Buckwheat and Manuka. These honey samples were tested using Scratch wound assay and cell migration assay.

Acacia and Buckwheat honey has shown best results in case of Scratch wound assay and also in case of cell migration assay Acacia honey has shown the stronger effect.

7.CONCLUSION :

We studied several properties of different honeys from different geographical areas.

7.1 a) One of them is anti-microbial activity that was studied for 4 honey samples including Manuka honey, Heather honey , locally marketed Indian honey and the honey that was procured from local beekeepers in a South Indian village , amongst which Khadikraft honey (locally marketed Indian honey) has shown the best anti-microbial activity against *P. aeruginosa* with 11% MIC (Minimum Inhibitory Concentration) value .

b) When 29 honey samples were tested for anti-microbial activity against 10 wound bacterial isolates , Scottish Heather honey has shown the best anti-microbial activity at concentrations ranging from <2% to 6%.

c) When 13 different honey samples were tested against *E. coli* and *P. aeruginosa* , at 2.5% concentration only Paterson's curse honey has shown significant growth inhibitory activity on *E. coli* while the growth of *P. aeruginosa* was inhibited by Lavender 2,Red stringy bark 2 and Rosemary honey.

7.2 a) We studied the anti-oxidant potential of 4 Malaysian honey samples including Gelam , Longan , rubber tree and Sourwood by measuring the total phenolic and total flavonoid content and by analysing the DPPH radical scavenging activity of the honey samples. Highest phenolic

content was observed in Sourwood and Longan honey and highest flavonoid content and highest DPPH radical scavenging activity was observed in Sourwood honey.

b) Also the anti-oxidant potential of 5 different monofloral and 3 different multifloral honey samples have been studied . The mean total phenolic content in our honey samples was greater than Manuka honey and many other honey samples from Cuba , Slovenia and Burkina Faso. Highest total phenolic content was observed in BDH-8. But the flavonoid content in these honey samples were lesser than Manuka honey and the highest flavonoid content and highest DPPH radical scavenging activity was observed in BDH-8 , a multifloral honey.

c) We also studied the anti-oxidant potential of 4 different Indian honey samples including cotton, coriander, Dalbergia and Murraya. The mean value of total phenolic content was seen to be highest in Dalbergia honey.

The highest flavonoid content was observed in Coriander honey and the lowest was in Gossypium honey . The highest DPPH radical scavenging activity was shown by Coriander honey and the second highest was Murraya honey.

7.3 Another very important property of honey is its wound healing property.

a) To analyse this wound healing property of honey , an experiment was performed using 10 Albino mice and they were then wounded on their backs. These wounds were then treated with 6 honey formulations to check their wound healing property. Effectiveness of honey was determined by measuring the wound area and the maximum burn diameter contraction was reached by F3 which was honey 75% chitosan formula which was much efficient than pure honey and commercial products.

b) Another experiment was performed to check the wound healing property of Tualang honey and compare it with hydrofibre and hydrofibre silver. To test this 36 female Sprague Dawley rats were taken and 3 burn wounds were made on the Dorsum and the wounds were inoculated with *P. aeruginosa, Klebsiella pneumonia* or *Acinetobacter baumannii.* And these 3 wounds were treated with Tualang honey , hydrofibre and hydrofibre silver. The maximum wound size reduction was observed in case of Tualang honey. While the wound size reduction for hydrofibre silver was smaller .

c) Another experiment was done using 36 Sprague Dawley rats and they were divided into 4 groups of 2 excision and 2 incision. Animals were wounded on their dorsal thoraic region and then the treatment was started using Teucrium polium honey twice a day. On day 6, 9, 12 and 15 , the mean healed wound area % increased for incision groups. On day 6, 9 and 12 ,the mean healed wound area % increased significantly for excision group than the control group. While on day 6 and 9 , the mean healed wound area of incision wound model increased significantly in comparison to excision group.

d) Another experiment was performed to analyze the wound healing effect of honey on human fibroblast under in vitro conditions. Scratch wound assay and cell migration assay was performed to analyze the honey samples and in both the cases Acacia honey has shown highest wound healing property.

There are many commercially available honey products which can be tested using one of the above mentioned protocols , to check if they also contain anti-microbial, anti-oxidant and wound healing properties and whether they can be used for medicinal purposes or not. They can also be tested to check if they contain any harmful compounds or antibiotics which may lead to various health problems.

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REFERENCE :

1. Venkatachalam Mullai, M.Sc., and Thangam Menon, Ph.D., 2007,Bactericidal activity of different types of honey against clinical and environmental isolates of *Pseudomonas aeruginosa,* Volume 13, Number 4.

2. R.Carnwath, E.M.Graham, K. Reynolds, P.J.Pollock,2013, The antimicrobial activity of honey against common equine wound bacterial isolates,110-114.

3. Jenny M. Wilkinson and Heather M.A. Cavanagh,2005, Antibacterial Activity of 13 Honeys Against *Escherichia coli* and *Pseudomonas aeruginosa*, J Med Food 8 (1).

4. Mohammed Moniruzzaman, Siti Amrah Sulaiman, Md Ibrahim Khalil and Siew Hua Gan, 2013,Evaluation of physicochemical and anti-oxidant properties of Sourwood and other Malaysian honeys: a comparison with Manuka honey,7:138.

5. Asiful Islam, Ibrahim Khalil, Nazmul Islam, Mohammed Moniruzzaman, Abdul Mottalib, Siti Amrah Sulaiman and Siew Hua Gan, 2012,Physicochemical and antioxidant properties of Bangladeshi Honeys stored for more than one year, 12:177.

6. Rajni Kamboj,1 Manav Bandhu Bera2 & Vikas Nanda2*,2013, Evaluation of physico-chemical properties, trace metal content and antioxidant activity of Indian honeys, 578–587.

7. Reham F. El-Kased1, Reham I. Amer2,3, Dalia Attia4 & M. M. Elmazar5, 2017, Honey-based hydrogel: In vitro and comparative In vivo evaluation for burn wound healing, 7: 9692.

8. Yan-Teng Khoo1, Ahmad Sukari Halim1*, Kirnpal-Kaur B Singh2, Noor-Ayunie Mohamad1,2010, Wound contraction effects and antibacterial properties of Tualang honey on full-thickness burn wounds in rats in comparison to hydrofibre, 10:48.

9. *1Ali Mohammad Alizadeh, 2Hamid Sohanaki, 3Mahmod Khaniki, 1Mohammad Ali Mohaghgheghi, 4Giti Ghmami, 1Maryamsadat Mosavi ,2011, The Effect of Teucrium Polium Honey on the Wound Healing and Tensile Strength in Rat , 499-505.

10. Natalia G Vallianou1*, Penny Gounari1, Alexandros Skourtis1, John Panagos1 and Christos Kazazis2,2014, Honey and its Anti-Inflammatory, Anti-Bacterial and Anti-Oxidant Properties, 2:2.

11. Elia Ranzato, Simona Martinotti, Bruno Burlando, 2013,Honey exposure stimulates wound repair of human dermal fibroblasts, Vol 1, Issue 1.

12. Tahereh Eteraf-Oskouei 1,2, Moslem Najafi*2, 2012, Traditional and Modern Uses of Natural Honey in Human Diseases, 731-742.

13. Sultan Ayoub Meo a,* , Saleh Ahmad Al- Asirib, 2016, Role of Honey in Modern Medicine, 975–978.

14. Manisha Deb Mandal1, Shyamapada Mandal2*, 2011, Honey: its medicinal property and antibacterial activity, 154-160.

15. Van den Berg AJ, van den Worm E, van Ufford HC, Halkes SB, Hoekstra MJ, Beukelman CJ; 2008; An in vitro examination of the antioxidant and anti-inflammatory properties of buckwheat honey; J Wound Care;17(4):172-4.34.

16. Bashkaran K, Zunaina E, Bakiah S, Sulaiman SA, Sirajudeen K, Naik V; 2011; Anti-inflammatory and antioxidant effects of Tualang honey in alkali injury on the eyes of rabbits: experimental animal study. BMC Complement Altern Med;11:90.35.

17. Park DV. Antioxidants in human health and tissue: Nutritional antioxidants and disease prevention: Mechanism of action; 1999; CABI Publishing.

18. Aljadi AM, Kamaruddin MY;2004; Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys; Food Chem;85(4):513-8.37.

19. Storz G, Imlay JA.,1999, Oxidative stress. Cur Opin Microbiol ; 2:188–194.

20. Bergman A, Yanai J, Weiss J, Bell D, David MP; 1983; Acceleration of wound healing by topical application of honey; An animal model. Am J Surg. ;145(3):374-6.

21. Sherlock O, Dolan A, Athman R, Power A, Gethin G, Cowman S, et al.; 2010; Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. BMC Complement Altern Med. ;10:47.

22. Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KN, Salleh MS, Gurtu S. ;2010;Antioxidant protection of Malaysian tualang honey in pancreas of normal and streptozotocin-induced diabetic rats; Ann Endocrinol (Paris);71(4):291-6.38.

23. Schramm DD, Karim M, Schrader HR, Holt RR, Cardetti M, Keen CL; 2003; Honey with high levels of antioxidants can provide protection to healthy human subjects; J Agric Food Chem;51(6):1732-5.33.

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