International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 03-12-2020; Accepted: 19-12-2020: Published: 04-01-2021
www.botanyjournals.com
Volume 6; Issue 1; 2021; Page No. 18-22

Proximate analysis of honey for its possible role as a natural preservative

Khushbu Verma[1*], Anoop M[2], Shveta Dhankhar[1]
[1] Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India
[2] Bhagwant Ayurvedic College and Bhagwant Hospital, Muzaffar Nagar, Uttar Pradesh, India

Abstract
In the present study, honey was used to as natural preservative in citrus fruit juice. *Citrus limetta* fruit juice samples were taken for study which is mostly consumed by the common people for their health issue. Different samples S1, S2, S3, S4, S5 and S6 of citrus fruit juice were made by adding honey in different concentration. Samples of citrus fruit juice stored at 4ºC for 15 days in the presence of honey were shown to inhibit opportunistic bacterial growth better compared to the sample stored without honey. Honey can be used in the citrus fruit juice for good taste, prevent the spoilage and boost an immune system. The following judgments suggest that organic honey can be used as an antibacterial agent as well as a preservative for Citrus fruit juice (*Citrus limetta*).

Keywords: honey, natural preservative, antibacterial agent, *citrus limetta*, citrus fruit juice

Introduction
Citrus is a genus of flowering trees and shrubs in the rue family, Rutaceae. Plants in the genus produce citrus fruits, including important crops like oranges, lemons, grapefruit, pomelo and limes. The most recent research indicates an origin in Australia, New Caledonia and New Guinea (Liu *et al*., 2012) [13]. The juice is commonly available at mobile road stalls, where it is freshly pressed. In Iran it is used to treat influenza and common cold. Citrus fruits have long been valued as part of a nutritious and tasty diet. Preservatives are chemicals used to keep food fresh. Although there are a number of different types of food preservatives, antimicrobials, antioxidants, and products that slow the natural ripening process are some of the most common. Despite their important function, preservatives can pose a number of serious health risks. Honey a sweet food made by bees using nectar from flowers. In early times, honey has been used as folk medicine (Ghisalberti 1979; Zumla and Lulat 1989) [7, 21]. In number of publications use of honey in wound ulcers and skin burns has been indicate. (Efem, 1988; Gupta *et al*, 1992; Willix *et al*, 1992) [5, 8, 20]. Krushna *et al*., (2007) [12] demonstrate that the role of both peroxide and the non-peroxide components in honey, in antibacterial activity confirmed its role as a safe and effective preservative of milk samples.

Material and Methods
Phyto-chemical analysis of honey
Organic honey was used in this study as preservative. Honey is well known for its valuable constituents. Different test were performed to analyze Carbohydrate, proteins, and terpenoids.

Molisch's test for carbohydrates
Two drops of Molisch's reagent were added to 2ml of honey sample. It was mixed well with the help of glass rod. Test tube was inclined and added about 1 ml of conc. H_2SO_4 (Foulger 1931) [6].

Xanthoproteic test for proteins
5ml of honey sample was taken and added 1ml of conc. HNO_3 followed by boiling process. After cooling, excess 40% NaOH was added (Nigam and Omkar 2003) [15].

Salkowski test for Terpenoids
2ml of chloroform was added to 2ml of honey sample. Then carefully addition of conc. H_2SO_4 was done (Rahman *et al* 2010) [17].

Flavonoids
1-2 ml of dilute NaOH was added with 5ml of the honey sample. Then slowly HCl wass added drop by drop (Kokate 1997) [11].

H_2O_2 Test for honey
0.5ml honey sample was dissolved in 0.1% (W/V) TCA (Trichloroacetic acid). Then 0.5ml K-phosphate buffer and 2ml reagent (1M KI w/v in fresh D/W) was added in reaction mixture. This mixture was incubated in dark place for 1hr. Optimum density of the mixture was measured at 390nm. Sample and blank were analyzed in triplicates (Aljadi and Yusoff 2003) [1].

Sample preparation for proximate analysis of citrus fruit juice
In the present study, fresh, matured, and ripe fruits were used. Fruits were properly washed and cut into two halves and then juice was extracted from sterile juice squeezer and seeds were discarded. Different concentrations of juice were formed with addition of honey and natural fresh juice used as control. The detailed for various combinations are given in Table 1. Prepared juice combinations were poured into the sterile container and stored at refrigerator at 4º C in refrigerator for 15 days.

Table 1: Juice formulation

Sample	Citrus fruit Juice (%)	Honey (%)
Control	100 %	0.0 %
S_1	95 %	5.0 %
S_2	90 %	10 %
S_3	85 %	15 %
S_4	80 %	20 %
S_5	75 %	25 %

These 15 days old six fruit juice samples were used for proximate analysis. The proximate analysis of the fruits juices sample was done to determine presence of tannins, flavonoids, sugars, titratable acidity, pH of sample, total soluble solids (TSS), colour grading of juices, and sensory evaluation.

Proximate Analysis Juice for honey as an natural Preservative

Qualitative analysis of secondary metabolites

Tannins

2ml of juice sample and 2ml of H_2O in a clean test tube were added. Afterwards, 3-4 drop of 5% solution of $FeCl_3$ was added. Flavonoids- 5ml of the juice sample was taken and 1-2ml of dilute NaOH were added. Then HCl was added drop by drop (Kokate 1997) [11].

Titratable Acidity (TA)

3ml of fruit juice sample was mixed with 2ml distilled water. Further, 3-4 drops of phenolphthalein indicator was added and stirred. Content was rapidly titrated with 0.1 N NaOH solutions in a burette, continued to add alkali drop by the drop and stirred the content till first definite change to pink color. Practical was performed in triplicates for all the samples and readings were noted (AOAC, 2000) [2].

%acid= (Normality) (ml titrated) (equivalent weight of citric acid= 64) (Volume of sample taken for estimation) (1000)

Total Soluble Solid (TSS)

Small amount of fresh juice was placed onto the prism of the refractometer. Analysis was done through the eyepiece while pointing the prism in the direction of good light. Reading was taken of where the base of the blue color sits on the scale and recorded the % percentage sugar (°Brix) (AOAC, 2000) [2].

Measuring Of pH

The electrodes of pH meter were calibrated with distilled water. 30ml of juice sample was taken in the beaker and then the electrode was dip into it. Readings on digital display of the pH meter were noted (IS: 1479-1960).

Colorimetery Test

Meter was adjusted according to the color of the sample. Then it was calibrated with distilled water. Cuvette was filled with sample and fitted into the colorimeter. The reading was noted down from the digital display (AOAC, 2000) [2].

Sensory Evaluation

25 members were selected for taste of each of the given samples. Glass of water was putted for rinsing mouth between tasting each sample. Given samples were rated for quality attributes as per a point hedonic scale and overall acceptability (Peryam and Girardot 1952) [16].

Microbial Analysis

Nutrient Agar media and Petri plates are autoclaved at 121^0C and 15 lbs for 15-20 minutes. Then the autoclaved media and Petri plates placed in the Laminar Air Flow. The media was poured into the respective sterile Petri plates and then allow these for 5-10 minutes to solidify. Inoculate 15 days old juice samples by using cotton swab and covered with parafilm. Incubate at 37 ºC for 24hrs. Then, Petri plates were checked for bacterial colonies.

Statistical analysis

Statistical analysis for Honey based experiments was done by using the software Sigma Plot version 11. All the experiments were carried out in triplicates (n=3). All values in the text, table and figures indicates mean values ± SD. Differences between control and Honey Treated Juice samples were statistically examined by student's *t-test* and the level of significance was taken $P \leq 0.05$.

Result and Discussion

Phytochemical analysis of honey

Organic honey was used in this study as an antibacterial agent as well as preservative. Honey is well known for its valuable constituents. Different tests were performed to analyze Carbohydrate, proteins, terpenoids, flavonoids and hydrogen peroxide. The presence of Carbohydrate was confirmed in honey sample by a red cum violet ring appearing at the junction of the two liquids shown in Fig.1 (A). The presence of protein was determined in honey sample by adding acid and yellow color will be noticed. When NaOH is added deep orange color will develop (Fig.1B) In honey sample, presence of terpenoids was confirmed with addition of concentrated H_2SO_4 by appearance of reddish brown color at the interface shown in Figure 1 (C). Flavonoids are the natural antibacterial agents present in honey, determination of flavonoids in honey by yellow color precipitates are formed in the bottom of test tube and colorless solution appears on top of solution Figure 1 (D).

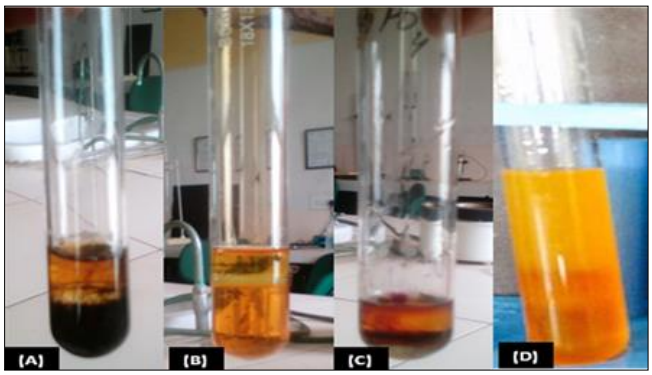

Fig 1: (A) Molisch's test (B) Xanthoproteic test (C) Salkowski test and (D) Flavanoid test

Hydrogen peroxide is the main antibacterial agent present in honey. In honey sample H_2O_2 concentration was determined by UV Spectrophotometer Shimadzu and the result was 5.135±0.1904 mM content/ml of fresh weight as shown in table 2.

Table 2: Hydrogen Peroxide concentration in honey

H_2O_2 Concentration in Honey sample (mM content/ml of fresh weight)	5.135 ± 0.1904

Qualitative analysis of secondary metabolites in Juice Sample

The presence of flavonoids in citrus fruit juice sample was determined by yellow color precipitates are formed in the bottom of test tube and colorless solution appears on top of solution Figure 2 (A). In citrus fruit juice sample tenin is also determined by green colour precipitate formation shown in Figure 2 (B).

Fig 2: Qualitative analysis of (A) tenin and (B) flavonoids in citrus fruit juice sample

Titratable acidity of Juice Sample

Titratable acidity was determined by neutralizing the acid present in a known quantity (weight or volume) of food sample using a standard base. The end point was pink colour appeared in the juice sample and triplicate readings are note down with all the six juice samples. Highest Significant decrease in citric acid contect was detected in S2 sample (10% honey) in comparison to control sample. No significant changes were recorded in rest of juice samples. So, S2 sample is best sample in context of citric acid content because citric acid content can be low in the citric juices according to a report (Figure 3).

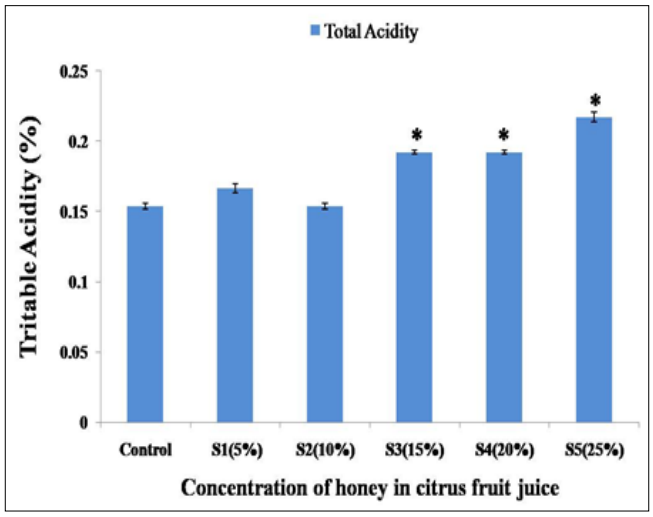

Fig 3: Titratable acidity

Total Soluble Solid (TSS)

By using refractometer sugar content of sugar solutions e.g., juices samples in which sugar is the major component is measured and Total soluble solids content of solution is determined into degree brix as shown in Table 3.

Table 3: Total soluble solids of citrus fruit juices sample

S.no.	Samples	Readings in degree brix
1.	Distilled water	0.0%
2.	Control	8.8%
3.	S1 (5% Honey)	14.0%
4.	S2 (10% Honey)	19.0%
5.	S3 (15% Honey)	22.0%
6.	S4 (20% Honey)	26.8%
7.	S5 (25% Honey)	30.6%

Colorimetry test

A colorimetery method was used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light. Through colorimeter results 5% and 10% honey mixed juice showed the similar absorbance at yellow 620 nm with control sample. But in other treated juice sample variations was observed in Abs. as shown in Figure 4.

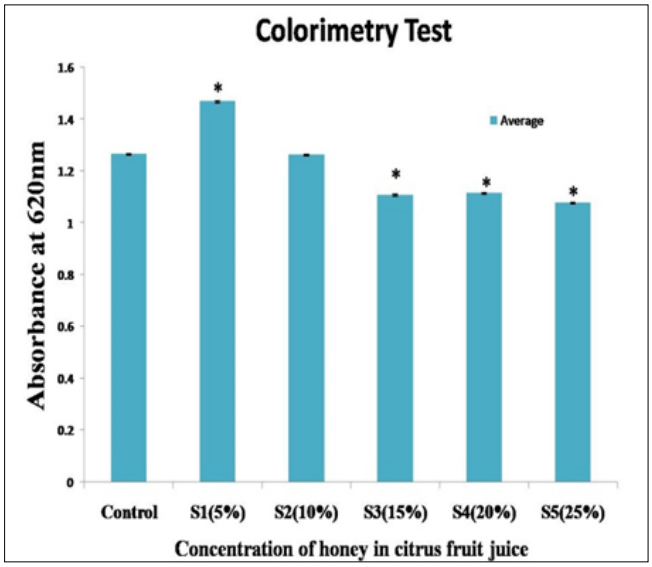

Fig 4: Colorimetry test

Measuring of pH

The pH of the all the six citrus fruit juice samples was ranging between 4.1-4.5 slightly acidic in nature as shown in Figure 2.2. S1 (5%) and S2 (10%) samples are less acidic. Highest Significant decrease in citric acid content was detected in S5 sample (25% honey) in comparison to control sample. No significant changes were recorded in S1 (5%) and S2 (10%) juice samples. So, according to studies less acidic juice sample was good for digestion. S2 and S3 samples were best sample in all the six samples (Figure 5).

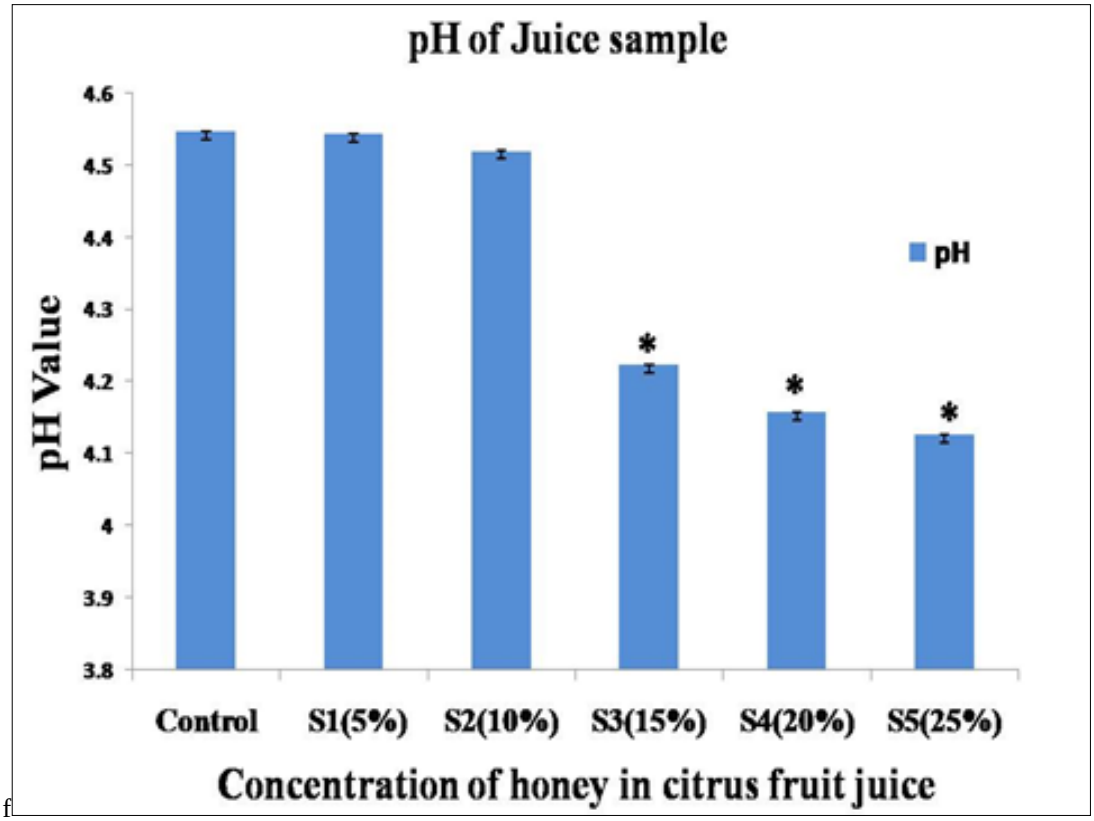

Fig 5: pH of the citrus fruit juice sample

Sensory evaluation

The panelists mean the scores for the six citrus fruit juice samples. Sensory evaluation result showed a significant difference in taste and overall acceptability of the citrus fruit juice sample whereas no significant difference was observed in their colors. Overall, the 5% added honey juice sample (S1) was the most preferred and accepted to the panelists than the other juice samples as shown in Figure 6.

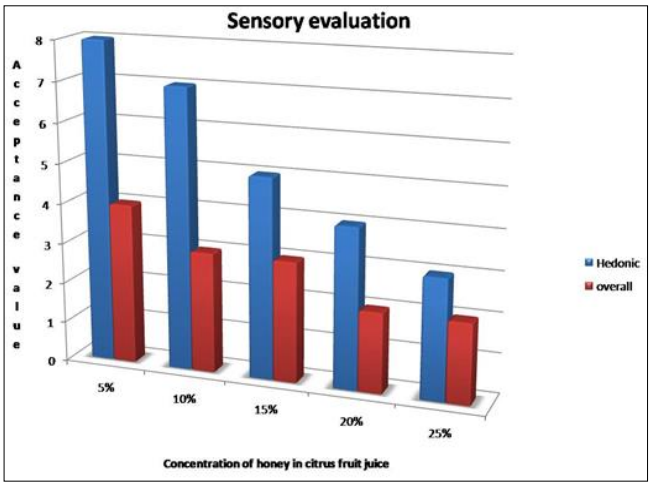

Fig 6: Sensory evaluation

Microbial analysis for citrus fruit juice samples

Microbial analysis of fruit juice sample was used to determine the growth of food borne pathogen in Fifteen days old juice samples.

In microbial analysis, it was found that as the concentration of honey increases, the rate of the inhibition of microbes also increase in comparison to control with no addition of honey as shown in figure 7.

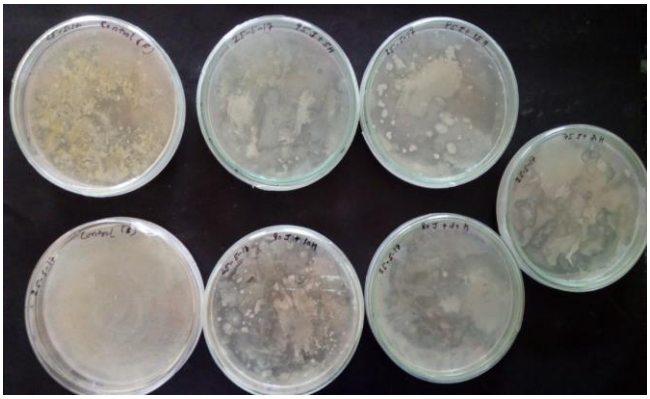

Fig 7: microbial analysis of juice sample at different concentration

The result of this study clearly suggests that Honey can be alternative source for preservation of Juice sample. These might be due to the osmotic effect, low pH, phytochemicals that are present in honey. Dustmann, (1979) [4]; Molan, (1992) [14, 20] and White *et al* (1963) [19] have been reported that antimicrobial activity of honey is mainly due to its acidity, osmolarity, and hydrogen peroxide (H_2O_2). They had attained interest in honey since the identification of antimicrobial phytochemicals in it. Aromatic acids or phenolic compounds are additional honey components, which contribute in antimicrobial activity. The antibacterial activity of honey is all depends on the concentration used and the nature of bacterial strain suggested by Blair *et al* (2009) [3]. Food borne pathogens becomes more pathogenic as time passes so honey is best for inhibition of food borne pathogens by its antibacterial activity explained by Taormina *et al* (2001) [18]. Molan (1992) [14, 20] showed comparable studies of honey in the mode of antibacterial action. Present data shows that organic honey was used against food borne pathogens and it shows good results.

Current study is the first report on the antimicrobial activity of organic honey for finding honey as natural preservative. There are many factors in the honey that effect on the growth of food borne bacteria. As shown in previous studies (Huttunen *et al*., 2013) [9] honeys have varying and diverse effects on the growth of each bacterial strain and each organism have unique response profile to different honeys. This gives rationale for further studies on antimicrobial activity of organic honeys against food poisoning bacteria including characterization of the antimicrobial components. In the current study, different samples are formed with addition of honey at different concentration and pure raw Cirus fruit juice was taken as control. After 15 days titratable acidity, pH, total soluble solids, sensory evaluation and microbial analysis was done and the results were good. Considering honey as preservative in food products especially in organic food it would be important to characterize the antimicrobial components and evaluate the effect of heat treatments on antimicrobial activity.

Conclusion

Organic honey is natural, eco-friendly, having no side effects and good for health so it can be used rather than antibiotics. The exact explanation is clear that the higher the concentration of honey the greater its usefulness as an antibacterial agent. It is also evident that the antibacterial effect decreases over time and that different species of bacteria differ in their susceptibility to honey. It has been concluded that pH of fruit juices was slightly acidic and able to drink. Keeping time period for 15 days doesn't affects the titratable acidity, pH, and total soluble solids of S1 and S2 fruit juice samples. Inhibited growth of Microbes was recorded in S1 and S2 the honey mixed juice samples significantly. So, the present study concluded that organic honey can be used as natural preservative and it improves the digestion, immunity and health problems.

References

1. Aljadi AM, Yusoff KM. Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. Turk J Med Sci. 2003; 33:229-236.
2. AOAC. Official method of analysis. Association of Official Analytical Chemist, Washington, D.C., U.S.A, 2000.
3. Blair SE, Cokcetin NN, Harry EJ, Carter DA. The unusual antibacterial activity of medical-grade Leptospermum honey: antibacterial spectrum, resistance and transcriptome analysis. Eur J Clin Microbiol Infect Dis. 2009; 28:1199-1208.
4. Dustmann JH. Antibacterial effect of honey. Apiacta. 1979; 14:7-11.
5. Efem SEE. Clinical Observations on the Wound Healing Properties of Honey. British Journal of Surgery. 1988; 75(7):679-681.
6. Foulger JH. The User of the Molisch (α-Naphthol) Reactions in the Study of Sugars in Biological Fluids" J. Biol. Chem. 1931; 92:345-353.
7. Ghisalberti EL. Propolis: a review. Bee Word. 1979; 60:59-84.
8. Gupta SK, Singh H, Varshney AC. Therapeutic efficacy of honey in infected wounds in buffalos. Ind J Anim Sci. 1992; 62:521-523.
9. Huttunen S, Riihinen K, Kauhanen J, Tikkanen-Kaukanen C. Antimicrobial activity of different Finnish monofloral honeys against human pathogenic bacteria. APMIS. 2013; 121:827-834.
10. IS : 1479 (Part 1) – 1960 (Reaffirmed 2003)
11. Kokate KC. 4th ed. Delhi: Vallabh Prakashan. Practical pharmacognosy, 1997, 218.
12. Krushna NSA, Kowsalya A, Radha S, Narayanan RB. Honey as a natural preservative of milk. Indian Journal of Experimental Biology. 2007; 45:459-464.
13. Liu Y, Heying E, Tanumihardjo S. History, Global Distribution, and Nutritional Importance of Citrus Fruits. Comprehensive Reviews in Food Science and Food Safety, 2012, 11: 6.
14. Molan PC. The Antibacterial Activity of Honey. 1. The Nature of the Antibacterial Activity. Bee World. 1992; 73:5-28.
15. Nigam SC, Omkar. Experimental Animal Physiology and Biochemistry. New Age International Pvt. Limited. New Delhi, 2003.
16. Peryam DR, Girardot NF. Advanced taste test method. Food Engineering. 1952; 24:58-61. 194.
17. Rahman A, Sitepu IR, Tang SY, Hashidoko Y. Salkowski's Reagent Test as a Primary Screening Index for Functionalities of Rhizobacteria Isolated from Wild Dipterocarp Saplings Growing Naturally on Medium-Strongly Acidic Tropical Peat Soil. Biosci. Biotechnol. Biochem. 2010; 74(11):2202-2208.
18. Taormina PJ, Niemira BA, Beuchat LR. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. Int Jour Food Microbiol. 2001; 69:217-225.
19. White JW, Subers MH, Schepartz AI. The identification of inhibine the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochim. Biophys. Acta. 1963; 73:57-70.
20. Willix DJ, Molan PC, Harfoot CJ. Acomparison of the Sensitivity of Wound Infecting Species of Bacteria to the Antibacterial Activity of Manuka Honey and other Honey. Journal of Applied Bacteriology. 1992; 73:388-394.
21. Zumla A, Lulat A. Honey – a remedy rediscovered. Journal of Royal Society of Medicine. 1989; 82:384-385.