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Antimicrobial Potential Of Honey Against Microbes Isolated From Packed Fruit Juices Of *Citrus Sinensis* And *Punica Granatum*

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Abstract **:** Fruit juices are a fantastic source of nutrients that are good for people, it compressed of many nutritional substances like water, fibre, vitamins, natural sugars, proteins and phytochemicals. In addition to regulating the digestive system, their high cellulose and high fibre concentration also helpful for digestion. Although it contains different nutritional values, but it results in many outbreaks due to improper washing of fruits, poor sanitation of cutting utensils like unwashed knives, microorganisms contaminated jars, etc. The ideal aim of the study was to determine the antimicrobial activity of honey against the microbes isolated from the packed fruit juices. Severe outbreaks of food borne illness are brought on by the ingestion of unsafe and unhygienic fruits. The honey was taken as an antimicrobial agent, which is then screened for their physiochemical analysis by performing various tests like $p^{H,}$ free acidity, moisture content, ash and sugar. The microbes isolated from the packed fruit jucies were *E. coli*, *Staphylococcus*, *Pseudomonas*, *Mucor* and *Aspergillus*. The antimicrobial activity of honey against the isolated microbes was determined by using agar well diffusion and disc diffusion method. In which the honey was taken in two concentrations which are diluted honey and undiluted honey. They possessed a great zone of inhibition against the microbes. In disc diffusion the undiluted honey showed excellent impact compared to the diluted honey. And in agar well diffusion the undiluted honey again result in great zone compared to diluted honey. Through this we state that undiluted honey has great impact on the isolated microbes and can be used as an alternative source of sweetner in the fruit juices which can minimize the bacterial contamination which result in low infection.

Keywords: Honey, Packed fruit juices, Physiochemical Screening, Antimicrobial activity.

INTRODUCTION

Fruits are a crucial part of a balanced diet. They contain many phytonutrients, which have been associated with a number of positive health effects, includes boosting the immune system. Consumption options include eating them raw or turning them into juice. To increase the shelf life of the juice, it can either be drunk right away or put through additional local or commercial processing and packaging.

Vitamins A, B, C and folate, minerals including iron, copper, potassium, iodine, zinc, selenium, manganese, boron, molybdenum and magnesium are found more in fruit juices and also soluble fibre and phytonutrients like antioxidants, amino acids and bioactive compounds are believed to be abundant in fruit juices. These nutrients are crucial for healthy nutrition and prevention of illness as well as providing a delectable taste and positive health effects.

Foods and drinks are sterilized using a procedure called heat pasteurization. It makes them safe for human eating and slows down the rate of deterioration of goods compared to when they aren't pasteurized. In this procedure, items are typically heated for a short period of time to a temperature of about 135°F (57°C), killing any potentially harmful bacteria or microbes that may be present. Additionally, pasteurization is frequently used on eggs, honey, wine, beer, milk, cheese, other dairy goods and even juices.

Fruit juice that has just been squeezed may not always be as wholesome as it seems because of microbial contamination. *Salmonella Typhi*, *Pseudomonas* sps., *Staphylococcus* sps., *Vibrio cholerae*, *Escherichia coli*, and other microbes are frequently identified in street juice. Factors that could lead to fruit juice being contaminated by microbe's, includes from contaminated fruits from the farm, handling, processing, packing, and transportation. Contamination during the juice production process may result from unclean conditions, food-borne illnesses and airborne dust, prolonged storage without sufficient pasteurization and refrigeration, and contaminated water sources, particularly for locally produced ready-to-drink and packaged fruit juice.

Microbial contamination occurs in the production process of fruit juices itself, it includes the factory environment, equipment, the raw materials, packages, and also lack of hygiene. The source of contamination can be caused by the packaging materials like glass and bottles. Primary spoilage is due to yeasts in carbonated products, due to their high carbonation resistance and low pH levels.

Honey has a cherished role in the human diet because of its distinctive flavor, nutritional benefits, and health-promoting qualities. About 80% of its weight is made up of sugars, primarily fructose and glucose, and trace amounts of oligosaccharides. As a result, it is a food product that is both easily digestible and high in energy. 100 g of honey has roughly 320 kcal, which the body may use. However, the presence of ingredients other than sugar, such as enzymes, peptides, free amino acids, vitamins, organic acids, flavonoids, phenolic acids, and other phytochemicals and minerals, is what primarily gives this product its health-promoting characteristics.

Numerous germs are prevented from growing or persisting by the antimicrobial properties of honey. Yeasts and bacteria that produce spores make up the majority of the microorganisms that can be discovered in honey. In honey, bacterial spores have been discovered. Since bacteria cannot multiply in honey, the presence of a lot of vegetative bacteria could be a sign of secondary contamination.

METHODOLOGY

Collection of Samples

The packed fruit juice samples were collected from the reputed super markets of Thiruvannamalai. The pH of the sample was noted and they were processed immediately after that. The pure honey was collected from the traditional medical shop.

Physiochemical Analysis of Honey

The physiochemical analysis of honey was determined by the following factors which are determination of p^H, free acidity, moisture content, ash and sugar.

Determination of pH

The procedure outlined by the International Honey Commission was followed to determine the pH of each of the honey samples. Briefly, 10 g of the honey sample was dissolved in 75 ml of distilled water in a 250 ml beaker. The solution was stirred until an even mixture was attained and the pH reading was recorded with a pH meter.

Determination of Free Acidity

Five gram of the honey sample was weighed and diluted with 37.5 ml distilled water in a 250 ml conical flask. The honey solution was titrated against 0.1 M sodium hydroxide (NaOH) whereas phenolphthalein was used as the color indicator. The endpoint was recorded and free acidity was determined as;

$$Free\ acidity = \frac{Titre \times N \times 196}{Weight\ of\ sample} \times 100$$

Where N = Normality of the base NaOH.

Determination of Moisture Content

Five grams of the sample was weighed and dried in a hot air oven at 105 ℃ for 4 h to get a constant weight. This is according to the method described by the Association of Analytical Chemists. The percent moisture content was calculated on a dry basis as

$$Moisture\ content = \frac{\text{Weight of wet sample} - \text{weight of dry sample}}{Weight\ of\ wet\ sample} \times 100$$

Determination of Ash

The determination of ash content was carried-out by following the procedures inwhich to remove moisture that would cause foaming of the samples at the early stages of ashing, the sample was kept in a previously weighed porcelain crucible and dried in an oven at 105 ℃ for 4 h. Upon removing the crucibles from the oven, they were then cooled in a desiccator. The materials were then ashed and dried in a hot air oven at a temperature of 600℃ for 2 h. The ash content was calculated on a dry basis according to the equation:

$$Ash\ content = \frac{Weight\ of\ ash - Weight\ of\ empty\ crucible}{Weight\ of\ the\ sample} \times 100$$

Determination of Sugars

Total sugars and reducing sugars were determined by weighing 5 g of the sample and adding it to 100 ml of warm distilled water in a beaker. This solution was mixed by stirring until all the soluble matters got dissolved and then filtered through a Wattman filter paper into a 250 ml volumetric flask. Then 100 ml of the solution were transferred by pipetting into a conical flask. Afterwards, 10 ml of 0.1 M diluted hydrogen chloride and 2–3 drops of phenolphthalein indicator was added and the

solution was boiled for 3 min. On cooling, 100 ml of the sample solution was pipetted and prepared into a burette. This solution was used for titration against Fehling's solution and the interpretation was calculated:

$$Reducing\ sugar = \frac{Factor \times Dilution}{Titre \times Weight\ of\ sample \times 10} \times 100\%$$

$$Total\ Sugar = \frac{Factor \times Dilution \times 2.5}{Titre \times Weight\ of\ sample \times 10} \times 100\%$$

Non-Reducing sugar (NRS) was estimated by subtracting reducing sugar from total sugar, That is

$$NRS\% = \text{Total sugar} - \text{Reducing sugar}$$

Isolation And Enumeration of Bacteria

The microorganisms are isolated from thee packed fruit juices by following the serial dilution and then pour plate method. Different types of selective media used for isolation and identification of bacterial and fungal colonies by streak plate method.

Identification of Microorganisms

The morphological characteristics of bacteria were examined by the gram staining, capsule staining, motility test and biochemical test. The fungi are isolated by inoculating and incubating the sample in the sabouraud dextrose agar plates and the morphological characters were examined by Lactophenol Cotton Blue test.

Determination Of Antimicrobial Activity

The prepared cultures were kept ready for antimicrobial activity. The antimicrobial and antifungal activity of honey was performed by disc diffusion assay and agar well diffusion assay method respectively.

RESULT

Antimicrobial activity of any substance is defined as its ability to either kill bacteria or inhibit the growth of bacteria. Antimicrobial activity is significant with respect to the organisms by preventing their growth. Honey act as an antimicrobial activity against the microbes isolated from the packed fruit juice sample and it has various benefits in it.

Physical and chemical properties remain one of the key indicators of assessing the quality of honey. The honey sample undergoes for the determination of pH, viscosity, Total soluble solids, acidity, moisture content, total ash and reducing sugars.

The fruit juices were serially diluted and the respected dilutions were poured on the nutrient agar for isolating the microbes. The isolation of bacteria by means of gram staining and motility was done and 3 bacterial isolates were obtained from that. In which 2 were showing Gram negative rods and motile in nature. The remaining 1 shown gram positive cocci and it is non-motile. The organisms were further screened for biochemical characterization. The isolation of fungi by means of Lactophenol Cotton Blue staining for microscopic observation and 2 fungal isolates was obtained.

The colony morphology of the bacterial isolates was studied by streak plate method, on cetrimide agar, EMB agar, Mannitol salt agar, SS agar and SDA. On cetrimide agar the organism shows that flat yellow fluorescent green colonies. On EMB agar the organism shows the metallic sheen colonies. On MSA the organism shows that golden yellow color colonies. On SS agar the organism does not show any colonies and on the SDA plate the fungal colonies shows small scanty white colonies

The growth of the respected bacterial colonies in plate and their Gram stain, biochemical test result shows *E.coli* isolates tested positive for catalase, indole, and methyl red but negative for oxidase, Voges-Proskauer, Simmon citrate, urease test and the TSI test revealed A/A with gas but no H_2S production in it, *Staphylococcus* isolates tested positive for Voges-Proskauer and negative for catalase, indole, methyl red, oxidase, Simmon citrate, urease test and the TSI test revealed A/A with no gas and no acid production in it and Pseudomonas isolates shows positive for methyl red and citrate test and negative for catalase, indole, oxidase, Voges-Proskauer, urease test and the TSI test revealed K/K with no gas and no acid production**.** And the fungal colonies grown well in their plates and the LCB result shows insertion of columella and the lack of stolons and rhizoids and another one showed conidia head and vesicle shape**.**

The antimicrobial activity of honey was done by taking diluted honey and undiluted honey against the isolated microbes from the packed fruit juices, which are *E.coli, Staphylococcus, Psuedomonas, Mucor and Aspergillus.* The diluted and undiluted honey sample posses great zone of inhibition in disc diffusion assay in which the diluted honey sample shows 20.5, 21.3, 12.6, 20.1, 11.2 and undiluted honey sample shows 21.1, 18.5, 14.3, 22.5, 14.5 and agar well diffusion method in which the diluted honey sample shows 11.1, 14.6, 10.4, 13.8, 12.1 and the undiluted honey sample shows 13.7, 22.9, 21.8, 18.7, 15.3**.**

The concentration of honey has an impact on antibacterial activity, with larger concentration it to be more effective. It has been demonstrated that honey possesses antibacterial properties at concentrations between 3% and 50%. It has been established that the concentration of honey and the kind of bacteria have an impact on the bactericidal effect of honey.

The present study of diluted and undiluted honey was found to be effective against the Gram positive organism, Gram negative organism and the fungal isolates. It showed a great zone of inhibition.

For the microbiological standards for fruit juices, it is suggested that packed fruit juices which are not pasteurized is the potential source for common food borne contamination. The investigation stated that, by mixing honey with juices can prevent the growth of some organisms. Those organisms are sensitive to honey. So, it can be used as an alternative for antibiotics. The antibiotics usage has been rapidly growing in the modern society and produce harmful effect for human health.

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