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ISOLATION OF FOOD BORNE MICROORGANISM FROM CITRUS FRUIT JUICE

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ABSTRACT: In the present study, honey was used to observe its antibacterial activity against food borne microorganisms. There was four bacterial strains *Leuconostoc sp., Micrococcus roseus, Proteus sp.,* and *Streptococcus sp* are isolated from the citrus fruit (*Citrus limetta*) juice which are identified by performing some biochemical tests like citrate test, catalase test, Gram reaction and Macconkey agar media. The antibacterial action of honey was showed by Disc diffusion method. Bacterial strain *Streptococcus sp* was more sensitive and *Micrococcus roseus* was found less sensitive against honey. The 100% concentration of the honey was found to be the best concentration which kills all the bacteria. The major antibacterial components of honey are hydrogen peroxide, low pH, high osmolarity, and other non peroxide components like flavonoids, phenolic compounds, defensin-1 etc. The more sensitive strain *Streptococcus sp* is catalase negative and inhibited by enzymatically production of hydrogen peroxide which cause suffocation in the bacteria and killed it completely. The other bacterial strains were inhibited by low pH and high osmolarity. Honey can be used in the citrus fruit juice for good taste, prevent spoilage and boost immune system.

Key words: Antibacterial activity, Dabur Honey, Streptomycin, Bacterial strains, Inhibition zone and Hydrogen peroxide.

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INTRODUCTION

Honey is a naturally flavorful, sweet and already known for its antimicrobial properties since the 19th century. The antibacterial action of honey depends on the origin and processing because it produced from various sources (Molan, 1992). Since long times ago the antimicrobial activity of honey used against many bacterial and fungal species which are highly responsible for causing food spoilage and bacterial, fungal infections Molan, 1992a; Wilkinson and Cavanagh, 2005. Molan, 1992 demonstrate that honey shows an inhibitory effect against 60 species of bacteria which are gram-negatives and gram-positives, anaerobes and aerobes. Dustmann, 1979 and Molan, 1992 have been shown that honey obtained antimicrobial activity and the mainly antimicrobial factors are high osmolarities, acidic, and hydrogen peroxide. Snowdon and Cliver, 1996 reported that nonperoxide factors like flavonoids, phenolic acids, lysozymes and aromatic acids also contribute to the antibacterial action of honey. Hydrogen peroxide, which is the main antimicrobial factor and enzymatic production of glucose oxidase naturally present in honey bees described by White et al, 1963. It is also used as an antiseptic demonstrated by Turner, 1983. A naturally low pH range from 3.2 and 4.5 is mildly acidic to inhibit the microbes but if honey is diluted then it's not enough to inhibit microbes to grow described by Cooper et al., 2002; Molan, 1992b. Kwakman and Zaat, 2012 demonstrate the strong osmotic effect of honey which is responsible for the antibacterial action.

Honey is a highly saturated sugar solution (fructose and glucose) which is the main constituent and inhibits the growth of microbes by an osmotic effect. Kwakman et al., 2010 in recent times found defensin-1 which is also responsible for an antibacterial action. So it may work as a natural preservative for food products due to its antimicrobial qualities. By using the natural antimicrobials foodborne pathogen can be easily eliminated and worked as an inhibitor for harmful pathogens which are responsible for causing various diseases. The purpose of this study was to determine the inhibitory effect of an organic Honey sample against foodborne pathogens which are isolated from citrus fruit juice.

MATERIALS AND METHOD

Collection and preparation of samples

Pure organic Dabur honey and Fresh citrus fruit commonly mousambi (*Citrus limetta*) were used in this study which is collected from local market of Jaipur (Rajasthan). Honey was stored at room temperature (25-30ºc) in dark. Fruits were properly washed and cut into two halves and then juice was extracted from sterile juice squeezer and seeds were discarded and juice poured into the sterile container.

Isolation and identification of bacterial strains from citrus fruit juice

To isolate the bacterial strains in citrus fruit juice sample 1ml of juice sample was serially diluted in sterile distilled water and 200µl of a sample were poured on the nutrient agar plate and spread by sterile cotton swab and then incubated at 37ºC for 24hrs. The randomly picked colonies were re-inoculated in 20ml nutrient broth and incubated at 37ºC for 24hr. Individual colonies are identified from obtained culture by using Gram reaction test, catalase test, citrate test and by using selective media Macconkey agar media. Four bacterial strains are identified through using these tests *Leuconostoc sp., Micrococcus roseus, Proteus sp.,* and *Streptococcus sp.* These bacterial strains are highly responsible for causing food spoilage in citrus fruit juice.

Inhibitory activity of Honey by disc diffusion method

The antibacterial action of organic honey was estimated by Disc diffusion method against *Leuconostoc sp., Micrococcus roseus, Proteus sp.,* and *Streptococcus sp.* Whatman No.1 Filter paper was used to prepare the discs of 6 mm diameter demonstrated by Bauer et al (1996). Nutrient agar media Petri plates and discs were sterilized by autoclave at 15lbs pressure and 121ºC for 15–20 minutes. Discs were impregnated with the 25%, 50%, 75% and 100% concentration of honey for 1-2hrs after autoclaved. Nutrient agar media was poured into the sterilized Petri plates and allow to cool or solidifying for 10-15min. Bacterial strains sample were spread over the agar plates by L-Spreader and after few minutes discs of different concentrations of honey were placed over the plates with respective bacterial strain. Similarly, discs of antibacterial drug (streptomycin) were used to place over the agar plates of bacterial strains. All the Petri plates were covered with parafilm and incubated at 37 ºC for 24hrs. The diameter of inhibitory zones of *Leuconostoc sp., Micrococcus roseus, Proteus sp.,* and *Streptococcus sp.* were measured in millimeter.

RESULT AND DISCUSSION

In this study, freshly prepared juice sample was used for microbiological analysis. There are several antibacterial drugs are easily available in the market but due to their harmful side effects, long duration of treatment, and high prices are not successfully kill the food borne pathogens. So in the present study, organic honey was used due to its antibacterial and strong antioxidant properties. Honey is the natural sweetening agent as well having antimicrobial properties which make it highly useful product and easily available in the market. Four bacterial strains *Leuconostoc sp., Micrococcus roseus, Proteus sp.,* and *Streptococcus sp.* were found in juice sample which are the main cause of food spoilage. It can be observed that catalase negative *Streptococcus sp.* and catalase positive *Leuconostoc sp., Micrococcus roseus, Proteus sp.* were inhibited by the organic honey. Organic honey sample shows maximum antibacterial activity especially against *Streptococcus sp.* which is 18.5mm in diameter at 100% concentration whereas the *Micrococcus roseus* shows the less inhibitory zone against honey sample which shown in Table 2. The Antibiotic drug (Streptomycin) shows the inhibition zone of 23.7 mm in diameter. By using catalase test, Gram reaction test, citrate test and selective media Macconkey agar *Leuconostoc sp., Micrococcus roseus, Proteus sp.,* and *Streptococcus sp* were identified. Biochemical and morphological characteristics of these four bacterial strains are explained in Table 1.

Table 1:- Morphological and biochemical characteristics of test strains

S.NO.	Bacterial strains	Colour on NA	Shape	Gram reaction	Citrate Test	Catalase test
A	*Leuconostoc sp.*	Light yellow	Cocci	positive	positive	positive
B	*Micrococcus roseus*	pink	Cocci in tetrad	positive	positive	positive
C	*Streptococcus sp.*	Creamy white	Coccus	positive	negative	Negative
D	*Proteus sp.*	Creamy white	Rod shape	negative	positive	positive

Table 2:- Inhibition zones of the test strains at different concentration of Honey

S.NO.	Isolated Bacterial Strains	IZ of honey at 25%	IZ of honey at 50%	IZ of honey at 75%	IZ of honey at 100%	IZ of Streptomycin
A	*Leuconostoc sp.*	7.2mm	11.5mm	12.0mm	13.0mm	23.7 mm
B	*Micrococcus roseus*	4.6mm	5.2mm	9.1mm	11.1mm	23.7 mm
C	*Streptococcus sp.*	9.2mm	11.7mm	16.5mm	18.5mm	23.7 mm
D	*Proteus sp.*	5.5 mm	5.9mm	6.8mm	8.2mm	23.7mm

The present study based on antibacterial action of honey it is due to the capability of honey to inhibit the bacterial strains which are antibiotic resist it‘s credit goes to its high osmotic effect, high pH, enzymatically production of hydrogen peroxide and other components, like flavonoids, benzoic acid phenolic compound and ascorbic acid etc Molan (1992a), Bogdanov S. (1984) and Heerng W. (1998). Catalase negative species *Streptococcus sp.* most sensitive bacterial strain shows a marked inhibition due to glucose oxidase naturally present in honey enzymatically produce hydrogen peroxide neither by low pH, high osmolarity or any other non peroxide component Lusby et al(2002), Namias (2003), Molan (1992a). The antibacterial action of Honey is all depends on the concentration used and the nature of bacterial strain Basualdo C et al (2007), Adeleke O.E et al (2006). Weston et al., (2000) demonstrate that antimicrobial activity on *Leuconostoc sp., Micrococcus roseus* (gram-positive) and *Proteus sp.* (gram- negative) is due to non-peroxide constituent of honey. World Health Organization (1999) shows that the effectiveness of the antibiotics has been reduce due to bacterial strain turning resistance and these resistances become a serious problem in most of the regions of the world demonstrated by Assefa A. and Yohannes G. (1997), and Shears P. (2000). 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 PJ et al (2001). Molan PC (1992a), Kingsley A. (2001), Nzeako and Hamdi (2000) shows comparable studies of honey against *Streptococcus pyogenus, , Staphylococcus flexneri, S. aureus, P. aeruginosa, Salmonella typhi, Proteus mirabilis and, Escherichia coli* in the mode of antibacterial action. Several reports show that the antimicrobial action of honey against multi-antibiotic resistance bacterial species likes *Shigella dysenteriae* Adebolu, (2005); Wahdan, (1998); Mavric et al., (2008); Atrott and Henle, (2009) and Snow, (2008). has attained interest in honey since the identification of antimicrobial phytochemicals in it. Honey shows antibacterial as well as antifungal activity, especially anti-*Candida* action proved by Ahmad *et al*., (2012), Koc *et al*., (2008) and Irish et al., (2006). All of these judgment shows that honey can be used as an inhibitor in citrus fruit juice due to its antimicrobial actions.

CONCLUSION

From this study, it is concluded that the organic dabur Honey can be used as inhibitor for the food borne pathogens *Leuconostoc sp., Micrococcus roseus, Proteus sp.,* and *Streptococcus sp* which are isolated from citrus fruit (*Citrus limetta*) juice. Honey at 100% concentration effectual in prohibits the growth of all the bacterial strains which are isolated from citrus fruit juice. Fresh citrus fruit juice contains many harmful bacteria which are dangerous for the health of peoples. By the use of natural antimicrobials spoilage and pathogenicity can be prevented. Organic honey is natural, have no side effect and good for health so it can be used rather than antibiotics.

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