

Quality Assessment of Ice-cream & In Vitro Antibacterial Effect of Bitter Gourd Extract on the Isolated Pathogens.

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ABSTRACT

The study is performed to assist the microbial quality of different ice-cream & ice pops of different brands, to analyze the presence of contaminants that will cause pathogenesis. Antibacterial efficiency of Bitter Gourd is also studied against these isolated pathogens. Microbial count of ice cream estimated by performing serial dilution & spread plate technique, while further microbes isolated performing biochemical Test & confirmation test. The effectivity of Bitter Gourd was observed after the preparation of its aqueous extract & tested by performing antibacterial assay. Both Gram positive & negative groups of bacteria were isolated including *Bacillus*, *Lactobacillus*, *S.aureus*, *S.epidermidis*, *E.coli*, *Klebsiella*, *Enterobacter*, *Shigella* and *Pseudomonas*. Among these isolates, *Pseudomonas*, *Klebsiella* & *S.epidermidis* has shown sensitivity against the extract of bitter gourd, while all others are resistant to it. These contaminants can be avoided & the quality of Branded ice cream & ice pops can be improved if the industries set up under appropriate Food & Hygiene practices & Bitter Gourd can be used as the treatment or pre-treatment agent against the pathogens for which it is effective. As per being a natural product, it does not contain any side effects.

Keywords

Ice cream, sanitary measures, pathogens, food borne illnesses, *Momordica Charantia*, phytochemicals, antibacterial activity.

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INTRODUCTION

Ice – creams can also be referred as a frozen dessert. It is produced by combining a variety of ingredients including milk, sugar, stabilizers, emulsifiers, flavoring agents and water (1). Being nutritionally rich, it favors the growth of various micro-organisms that may be pathogenic and causes diseases like diarrhea, dysentery, etc. These are usually Psychrophiles, as in *Listeria monocytogens*, *Staphylococcus aureus*, *Salmonella spp.*, *E. coli*, *Moraxella*, *Serratia*, *Streptococcus*, *Pseudomonas*, *Aeromonas*, *Enterobacter*, *Klebsiella*, *Bacillus*, *Chromobacterium*, *Citrobacter*, *Clostridium* and many more. Primary sources of contamination if these micro-organisms in ice cream samples may include milk,

and other ingredients while the secondary sources of contamination can be the processers and utensils used in their production (1,2).

An antimicrobial is a compound that kills or inhibits the growth of microbes such as bacteria (antibacterial activity), fungi (antifungal activity), viruses (antiviral activity) or parasites (antiparasitic activity) (3).

There is a wide field of herbal medicine in which plants stem, roots, leaves, fruits etc, can be used in the treatment of many diseases. *Momordica charantia* is a medicinal plant belonging to the family Cucurbitaceae, found in tropical and subtropical regions of the world such

as India, Asia, and South America. *M. charantia* is commonly known as “bitter gourd”, “bitter melon”, “balsam pear” or “karela” and is cultivated throughout the world for its use as food (vegetable) as well as medicine (4, 5).

The health benefits of bitter gourd have been well documented, especially its anti-diabetic, anti-rheumatic, anti-ulcer, anti-inflammatory & anti-tumor properties. Bitter gourd also shows a significant antimicrobial activity (6). The medicinal value of the plants lies in some chemical substances. These chemicals are termed as phytochemicals. Phytochemicals are those compounds present in plants that play significant role in the prevention of any disease. These phytochemicals are grouped as flavonoids, alkaloids, glycosides, saponins, tannins, terpenoids, carbohydrates, and sterols. The isolation of these phytochemicals from the plant depends as the solvent varied. Being aqueous as the solvent, the phytochemicals with antimicrobial activity, isolated from the fruit of bitter gourd included as Saponins, Flavonoids & Terpenoids (4,7,8).

Microbial quality determination is used to find out the hygienic quality in production of any food product (9). Beside this, increasing resistance of pathogens towards the Antimicrobial drugs due to over usage of antibiotics is also one among the serious issues. Therefore, the principal on the basis of which this study is carried out is to analyze the microbial quality of different ice-pops & ice-creams supplied in Karachi city (Pakistan) & to analyze the antimicrobial efficacy of *Momordica charantia* aqueous fruit extract on the contaminants/pathogens that were isolated. It may further be help full in the development of new drugs against these food pathogens.

MATERIALS AND METHODS

Collection Of Ice-Cream Samples: 12 different samples of ice-creams were collected from the retail stores of the Karachi-city, comprising of two different brands. 6 samples of each brand were taken among which, 3 were the ice-pops while the other 3 were milk-based ice-creams.

Microbiological Analysis: For microbiological Analysis of the taken samples, Total bacterial Count was performed in which serial dilutions were made up to 10^{-4} . For an enumeration of bacterial colonies, diluted sample

along with molten Nutrient agar, in sterile petri-plates, incubated at 37°C for 24h. (10) The following day the total numbers of colonies were counted and their morphology was observed, for their identification. Further identification was based on Gram Reaction and Biochemical. For the confirmation of the Gram Negative isolates, the isolated organisms were sub-cultured on Eosin-Methylene Blue (EMB) Agar and Salmoella-Shigella (S.S) Agar. To differentiate among *Staphylococcus spp.* They were sub-cultured on Mannitol-Salt Agar (MSA). While for further differentiation of *S.epidermidis* & *S.saprophyticus*, Novobicin-sensitivity Test was performed on Muller-Hinton Agar (MHA). Bromothymol blue Lactose Broth & Sulfide, Indol, Motility medium were used for the confirmation of *Lactobacillus*.

Collection of Momordica charantia: The fruits were purchased from the local vegetable market of city, Karachi & were brought to the laboratory rinsed with water, were cut into small circular shapes, then dried in the sunlight and crushed to small pieces using pestle and mortar and further finely powered in an electric grinder. 8 g of powder was mixed with 17ml of distilled water & aqueous extract was prepared. (7)

Method to Obtain Fruit Extract: A method that was adopted for the preparation of the fruit aqueous extract was Maceration. In maceration the prepared powder of *M. charantia* fruit was kept in contact with solvent at 30°C and 120 rpm for 8 h. in a contain with stopper until the whole matter get mixed. After this, extract with solvent is collected and concentrated using a rotary evaporator for about 40min. (7)

Antibacterial Assay & Antimicrobial Activity: Different Microbes that were isolated used to analyze the antimicrobial activity of *M. charantia*. The experiment was performed under strict sterile conditions. Isolated cultures were purified using Nutrient agar Slant. Each culture streaked over the separate MHA plate after matching it with Macfarland tube 0.5 & then wells were made using a sterile borer. The concentrated extract was introduced into the well and the plates were incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the

diameter of the zone of inhibition. Along with the pure extract, antimicrobial activity was also checked at different concentration (40µl, 80µl, 120µl) (7).

RESULTS

These results show a wide range in the number of bacteria per ml and determined by the plate count and the direct microscopic method and confirmation test on specific mediums. According to the prescribed limit of the Food Safety and Standards Regulations, Total Bacterial Count of industrial ice cream should not exceed 2, 50,000 /ml (11). In this study, two of the samples are under the prescribed limit (76,000 to 1,40,000 cfu/ml) while the other ten of the samples contains as much Total Bacterial Count that is not under the prescribed limit, among which seven samples have a countable number (3,62,000 to 15,68,000 cfu/ml) and the other three samples were uncountable. All studied samples have shown the heavy contamination of pathogens. These include 4 species of gram positive bacteria that are *Bacillus spp.*, *Lactobacillus spp.*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* from 7 samples that constitute about 33.33%. While 5 species of gram negative bacteria including *E.coli*, *Shigella spp.*, *Pseudomonas spp.*, *Klebsiella spp.* and *Enterobacter spp.* Were isolated from 7 samples, i.e. 41.66%. The antibacterial activity of bitter gourd was observed over the isolated pathogens with the pure aqueous extract and also with the concentrations of 40µl, 80µl & 120µl of the extract as well. Except the 3 isolates that include *Klebsiella*, *Pseudomonas* and *S.epidermidis*, all other isolates (*Bacillus*, *Lactobacillus*, *Staphylococcus aureus*, *E.coli*, *Shigella* and *Enterobacter*) have shown resistance against it.

DISCUSSION

In this study, the percentage of gram negative that are usually predominantly occurring is 41.66% that are not in concurrence with the other studies done, that have occurrence of 66.7% of gram negative bacteria. They are heat sensitive & destroyed by high temperatures. Therefore, if they are present dominantly, it may be because of contamination after processing either during, packaging, transporting or selling of the products (1) while the percentage of gram positives is in concurrence with the study of Mathews et al., 2013 i.e. 33.3%.

Among all 5 isolated gram negative species of bacteria in 12 samples, occurrence of each species is 16.66%, but in another study of (Mathews *et al.*, 2013), the estimation of *E.coli* is 6.66%, *Klebsiella* is 4%, *Pseudomonas* is 1.33% while *Shigella* & *Enterobacter* are 0% in 150 samples. In the study of (Okojoh, 2006), *Shigella spp.* was isolated i.e. 60% among 5 samples. *Enterobacter spp.* was isolated with the percentage of 10.34% among 29 samples (12). The presence of fecal coliform indicates the post-treatment contamination which may either come from water, lack of personal hygiene of the ice cream manufacturer, utensils used for ice cream and distribution environment. A study conducted in which it was reported that 640-683 *E. coli* colonies /100ml were isolated from drinking water. The ice cream manufacturers may use the same water for the preparation of ice cream as well as for washing of their hands and utensils. Once the ice cream becomes contaminated, freezing temperature later could not make the product fit for consumption (13, 14).

The 4 species of gram positive that were isolated are in the percentage of occurrence; *Bacillus spp.* is 16.66%, *Lactobacillus spp.* is 33.33%, *Staphylococcus aureus* is 8.33% & *Staphylococcus epidermidis* is 16.66%. In concurrence with the study of (Mathews, Ngoma, Gashe, Mpuchane, 2013) *Bacillus* isolated were 10% among 150 samples. Its presence is usually because they are spore forming organisms and therefore survive in the harsh conditions, while *Lactobacillus spp.*, *Staphylococcus aureus* & *Staphylococcus epidermidis* are 0%. But *S.epidermidis* occurrence is approximately 2% among 150 open ice cream samples (15). 50% of *S.aureus* was isolated among 10 samples. The possible sources of this organism in ice cream could be from a human nose where it is commonly found; hands, skin and clothing of handlers (16). As a result of its transmission, staphylococcal food poisoning occur due to the growth the organism and release of enterotoxin into the food. Enterotoxin production and secretion occurs especially when ice cream products are not hygienically prepared and stored. (17).

The sensitivity of *Klebsiella* (pure extract=29mm, 40µl=20mm, 80µl=20mm, 120µl=25mm), *Pseudomonas* (pure extract=20mm, 40µl=5mm, 80µl=10mm, 120µl=10mm) & *S.epidermidis* (pure extract=35mm, 40µl=25mm, 80µl=29mm, 120µl=30mm) against the

Table I: Antimicrobial Activity Analysis of Aqueous Extract for Gram Negative Isolates

Solvent	Concentrations	Zone Diameter In mm				
		<i>E.coli</i>	<i>Enterobacter</i>	<i>Klebsiella</i>	<i>Shigella</i>	<i>Pseudomonas</i>
Aqueous	Pure Extract	0mm	0mm	29mm	0mm	20mm
	40µL of Pure Extract	0mm	0mm	20mm	0mm	5mm
	80 µL of Pure Extract	0mm	0mm	20mm	0mm	10mm
	120µLPure Extract	0mm	0mm	25mm	0mm	10mm

Table II: Antimicrobial Activity Analysis of Aqueous Extract for Gram Positive Isolates

Solvent	Concentrations	Zone Diameter In mm			
		<i>Bacillus</i>	<i>Lactobacillus</i>	<i>S.aureus</i>	<i>S.epidermidis</i>
Aqueous	Pure Extract	0mm	0mm	0mm	35mm
	40µL of Pure Extract	0mm	0mm	0mm	25mm
	80 µL of Pure Extract	0mm	0mm	0mm	29mm
	120µLPure Extract	0mm	0mm	0mm	30mm

Extract of bitter gourd observed in the way of zone of inhibition around the wells containing extract and its concentrations. In comparison with one of the related study, sensitivity was observed over *E.coli*, *Klebsiella*, *Bacillus* & *S.aureus* within the aqueous extract, that's zone diameter are: *E.coli* (pure extract=12±2.25mm, 40µl=no zone, 80µl=no zone, 120µl=10mm); *Klebsiella* (pure extract=10±0.00mm, 40µl=2mm, 80µl=4mm, 120µl=8mm); *Bacillus* (pure extract=6±1.12mm, 40µl=no zone, 80µl=no zone, 120µl=8mm); *S.aureus* (pure extract=8±1.40mm, 40µl=no zone, 80µl=7mm, 120µl=8mm) (18).

CONCLUSION

Ice cream is the popular frozen dessert among all. Being highly nutritious, it is the favorable medium for the growth

of micro-organisms. These micro-organisms spoil it after contaminating it & may cause certain food borne pathogenesis and out breaks. Its contamination followed by disturbance in extrinsic & intrinsic factors. Alteration in these factors will lead to spoil the quality of even Industrial based or Branded Ice cream products. To improve it, pre & post processing, storage of the product, cleaning of utensils & quality of the ingredients in the industries must be setup under the influence of proper Food & Hygiene practices. While bitter gourd or bitter melon has its medicinal values including anti-diabetic & anti-microbial importance & its extract has shown to be effective against some of the isolates. Therefore, it can be use as the treatment of the pathogenesis cause by these pathogens as being natural product, it do not have any

side effects. And may its consumption will act as a pre-treatment agent.

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