ROLE OF CAPSAICIN IN ORAL HYGIENE

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ABSTRACT

Chili peppers are one of the most consumed spices in all parts of the world, more prominently in Asia. They are the berry fruits of the plant from Genus *Capsicum* and the family Solanaceae. They are well-known for adding "heat" to the dishes. The pungent and spicy nature of chilies is due to the presence of an alkaloid, Capsaicin. It is marked for its physiological effects. In the world of continuous exposure to diseases and mutational pathogens, we should always be ahead with various antimicrobials. The best sources are the plants that have been providing us with medications for a long-time and an advantage is that they are a readily available natural source. The antimicrobial effectiveness can be compared to the existing antibiotics[1]. Continuous screening of microbes under the influence of capsaicin has concluded their role of being not only a bio-preservative but also a very efficient antibiotic against various microbes. Through a set of experiments, the effectiveness of capsaicin being an antibiotic agent against one of the most common conditions "Tooth Cavities" caused by the Gram-positive bacteria *"Streptococcus mutans*", is recorded. The conclusions of the results can ascertain that chilies should be an integral part of a balanced diet and are a great addition to products that deal with oral hygiene.

INTRODUCTION

Capsaicin is an alkaloid extracted from chilies primarily as crystals. Capsaicin contributes to 1% of the total body mass of pepper fruits and is biosynthesized in the placental epidermal cells of the seeds where it accumulates as "Blisters". The burning sensation when consumed is a defense mechanism against herbivores. This property of capsaicin is exploited by humans in cooking. The flavor of capsaicin in a dish is not recognized as one of the standard flavors (sweet, salt, bitter, sour, and umami) because it doesn't interact with the taste buds. It interacts with TRPV1(Transient Receptor Potential Vanilloid 1) which induces a physical irritation on the tongue contributing to the noxious heat we feel when we eat spicy food. Therefore it is considered a neurotoxin. Many experiments were conducted over some time to understand the physiological and chemical properties of capsaicin and its extensive use in other non-culinary fields. Inferring from the results of experiments conducted, capsaicin was discovered to be a great asset for antimicrobial, anti-inflammatory, and anticancer treatments. Capsaicin is also a great asset in the field of painkillers due to its analgesic properties.

Oral Hygiene

The oral cavity is the first exposure area to the food we take in, it's not always the food alone that is entered but it also brings various microbes with it therefore the mouth is exposed to a lot of microbes and it's a great niche for various microbes as the nutrients are quick and easy to acquire. The oral cavity consists of approximately 600 species of prokaryotes which includes a lot of Gram-Positive and Gram-Negative bacteria. Dental caries caused by one of the Gram-positive bacteria *Streptococcus mutans* is the most common condition seen in almost all age groups. They were first isolated by J. Clarke from the plaques of a victim. Lactobacillus bacterium also aids streptococcus in progressing the condition. The bacterium possesses the ability to convert incoming saccharides into various organic acids. They rely on glycolysis as a mechanism for their food breakdown. *Streptococcus* follows multiple pathways to progress their development in the niche. They break down sucrose into the sticky extracellular substance that aids in attachment of the bacterium to the hard enamel forming biofilms (Glycosyltransferase enzymes(GTF's) aid in the process)and have other pathways to break down sucrose into acids. GTFs are also involved in inducing a low-pH matrix that progresses the demineralization of the tooth and the low-pH, in turn, presents a favorable environment for the growth of acid-tolerant bacteria.

All the residing bacteria due to the favorable conditions start proliferating and carrying out their cell process which also includes fermentation. This process of fermentation is caused by the microbes inside the biofilm resulting in the production of various organic acids such as propionic, and lactic acids that dissolve the enamel leading to structural dysmorphia and cavity formation. The condition worsens periodically by degrading the collagen fibers due to the enzymatic action of bacteria in the cavity lesions. The act of dissolution of tooth enamel is not credited to only one type of bacteria a lot taking part in it which are the members of the genera

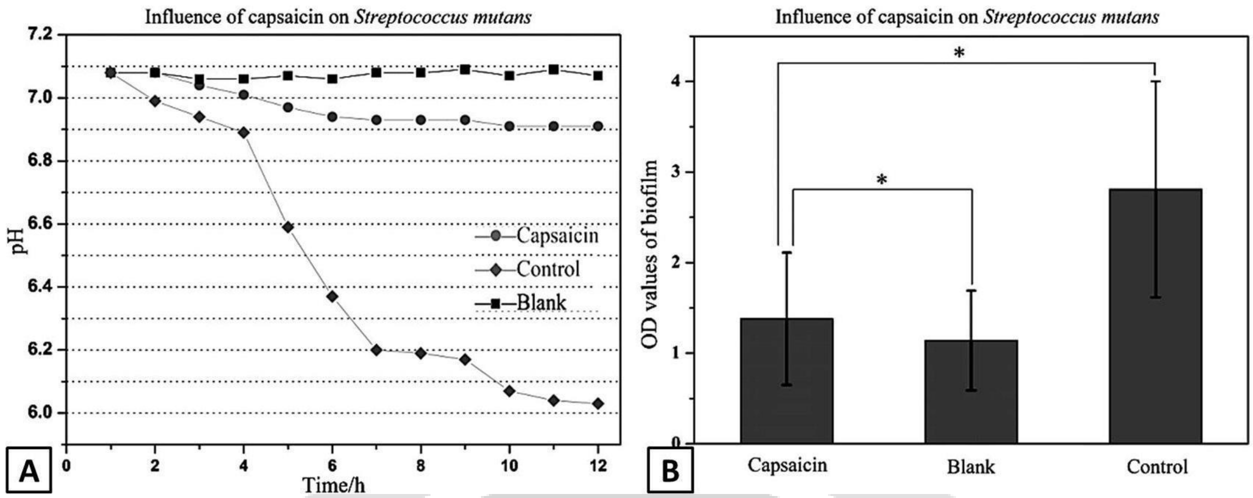
Streptococcus, Actinomyces, Bifidobacteria, and *Lactobacillus* are some of the most prominent causatives. The adverse effects of these biofilms and dental plaques are even hazardous as they induce cancer. Lipoteichoic Acid (LTA) and Lipopolysaccharide(LPS) are two components that are produced by the bacteria harbored on the tooth enamel which are known components that play a major role in cancer development[3]. WHO (World Health Organization) stated that every year an average of 650,000 new oral and pharynx cases are recorded and half of them are passed away. Even though we brush our teeth and use mouthwashes the numbers are still not significantly altered and the chemical contents of them are abrasive which also leads to the degradation of tooth enamel, therefore we need more natural sources of antimicrobials. As stated earlier capsaicin is a great source for replacing some antibiotics as its effectiveness is superior, Through some previously conducted experiments and conclusions let's discuss how Capsaicin can be a great inclusion as an antibiotic for dental plaque and therefore a necessary component of a normal diet and oral hygiene products.

EXPERIMENTAL PROOFS

Capsaicin was assumed to be an efficient antibiotic therefore, there were several experiments carried out to prove its effectiveness specifically against oral bacteria that form biofilms and induce degradation of dentine enamel. One such experiment was carried out by Hao Gu and Zhi Yang[2]in which they evaluated the antibacterial activity of capsaicin by the MBC, the change in pH in the first 12 hours, and the OD values of biofilms. The bacteria that were experimented on are *Streptococcus mutans, Actinomyces viscosus, Lactobacillus,* and *Streptococcus sanguis.* The process carried out by them involved collecting and growing clinically isolated bacterial strains on a BHI flat medium and cultivating them in the anaerobic incubator(80% Nitrogen, 10% Carbon dioxide, 10 % Hydrogen, 37℃). The standard microbial inoculum concentrations were adjusted to 10^8CFU/ml. Subsequently, three test samples were prepared (i) Capsaicin with 95% BHI and 5% alcohol with microbial inoculums (ii) a Control group with the solution of 95% BHI and 5% alcohol with microbial inoculums but no capsaicin (iii) Blank solution with capsaicin, 95% BHI and 5% alcohol. To find out the MIC by measuring OD values, 0.4 mg/ml of capsaicin solution was diluted from 1:2 to 1:32 and separately added 200**µ**l to 96-well plates with 40**µ**l of microbial inoculum and were incubated at 37℃ for two days (pH was recorded for every 12 hours). After the incubation the supernatant was discarded and the adhered cells were rinsed twice with a cleanser that is PBS solution. Cells were later fixed with the addition of 200**µ**l of methanol for 15 min followed by a staining step of adding 0.1% of Crystal violet dye and were incubated for 20 minutes at 37℃. The dye was later discarded and the tubes were rinsed with distilled water until they became colorless. 200**µ**l of 75% ethanol was added to release the complexes of Crystal violet bound to biofilms. They were vibrated for 30 minutes and the OD values were measured at an absorbance of 595 nm.

After recording the pH changes in 3 batches , it was shown that the pH in the control decreased to 6 which is mild acidic pH whereas the cultures with capsaicin showed a very less decrease in pH comparatively to the control which is 7.09 to 6.91 and the blank did not show any change in pH due to absence of microbes in it. The OD values of capsaicin included were 1.38$\pm$0.38 and were very lower than the control which was 2.81$\pm$1.19 and the MIC value of capsaicin on *Streptococcus mutans, Actinomyces viscosus, Lactobacillus,* and *Streptococcus sanguis* was 50**µ**g/ml, 50**µ**g/ml, 50**µ**g/ml and 25**µ**g/ml respectively. The pH changes and OD values of other bacteria were as follows-

BACTERIA	MIC	CONTROL pH	CAPSAICIN GROUP pH	CONTROL OD	CAPSAICIN GROUP OD
Actinomyces	50**µ**g/ml	7.08 to 6.79	7.08 to 6.82	1.35$\pm$0.28	0.97$\pm$0.14
Lactobacillus	50**µ**g/ml	7.05 to 6.4	7.05 to 6.67	1.10$\pm$0.30	0.53$\pm$0.17
S.sanguis	25**µ**g/ml	7.05 to 6.37	7.05 to 6.65	2.12$\pm$1.07	1.47$\pm$0.62


Influence of capsaicin on Streptococcus mutans
pH
Time/h
Capsaicin
Control
Blank
A
Influence of capsaicin on Streptococcus mutans
OD values of biofilm
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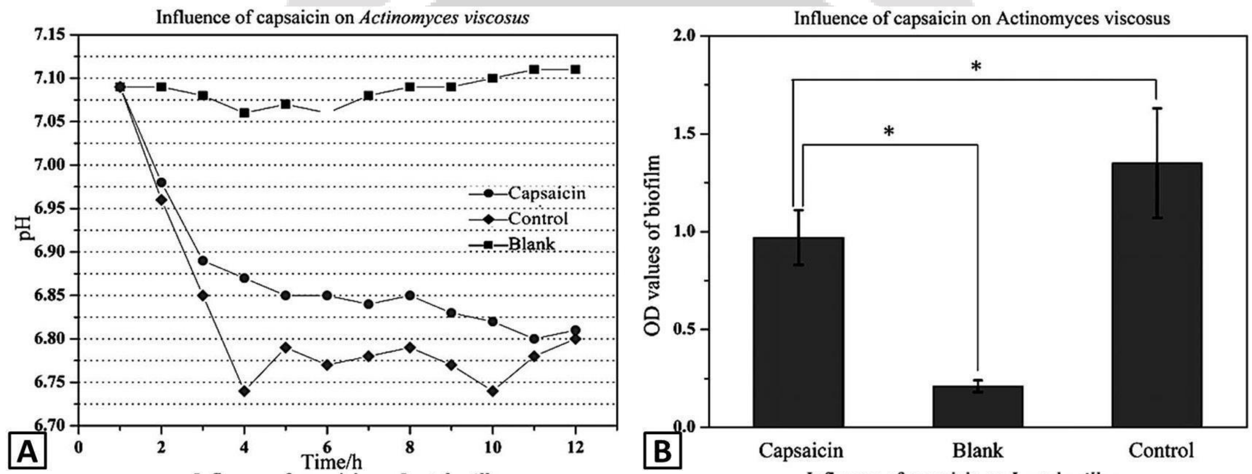
Influence of capsaicin on Actinomyces viscosus
pH
Time/h
Capsaicin
Control
Blank
A
Influence of capsaicin on Actinomyces viscosus
OD values of biofilm
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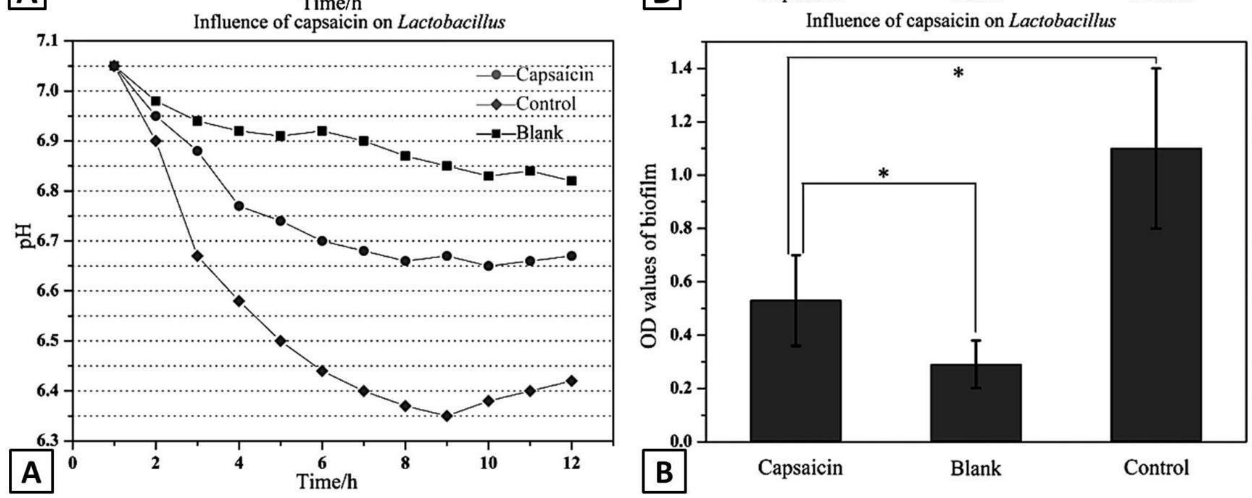
Influence of capsaicin on Lactobacillus
pH
Time/h
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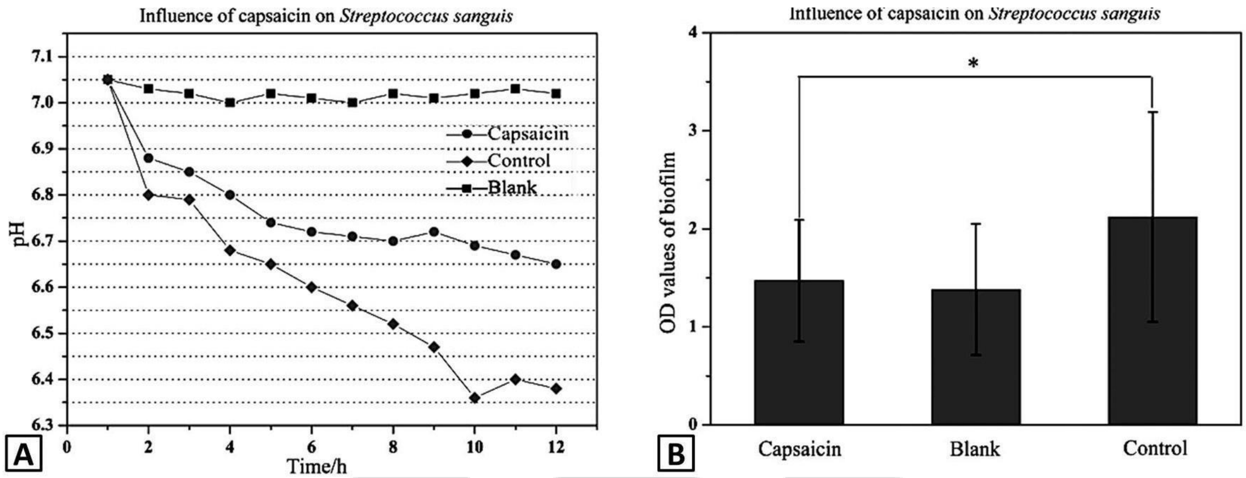

The charts above represent capsaicin's effectiveness as a significant inhibitor of cariogenic microbial growth. The pH maintenance wasn't much of a variation from the control as the concentrations of the inoculum were very less but on a large scale such as in the buccal cavity maintaining 6.9 pH is effective against the growth of cariogenic bacteria that proliferate more in acidic pH and the pH being neutral proves that the chemicals that degrade the enamel were not released due to the inhibitory actions of capsaicin. Conspicuous lessening of OD of bacterial biofilms is indirectly proportional to the potency of capsaicin. Biofilm in the oral environment forms a network with all the bacteria providing them shelter and nutrients. Biofilms are made up of Extracellular polymeric substances(EPS) that form the mesh for communication and adhesion of bacteria therefore destroying the biofilms or inhibiting their formation can hinder the growth of bacteria, therefore we can say that capsaicin is an excellent antibiotic against dental caries.

MECHANISM OF ACTION

Capsaicin naturally being an alkaline substance maintains the pH of the mouth by reacting with the acid produced by oral bacteria which is a neutralization reaction. The above set of experiments proved the antibacterial properties of capsaicin and it is an excellent inclusion for the maintenance of oral hygiene. But the experiments did not specify the mechanisms through which the capsaicin works, therefore other articles were studied. There were a series of experiments conducted to learn the mechanism of capsaicin using *S.typhimurium* (*Ayariga, Joseph A.*, et al., 2022), SYBR 1 or SYT0-9 and propidium iodide live/dead assay was conducted with *S.typhimurium* incubated with capsaicin. The assay gives green fluorescence with bacteria of the intact plasma membrane and gives red fluorescence with damaged cell membranes. The assay proved the efficient bactericidal effect of capsaicin by disrupting the cell membrane. Similar tests were done using the fluorescent dye ethidium monoazide which covalently binds only to nucleic acids but the limitation is it is cell membrane impermeable therefore the cell membrane should be ruptured or desensitized to allow the dye to interact with nucleic acids. Capsaicin's potential cell membrane disruption was tested using this experiment which proved that capsaicin at higher concentrations led to cell lysis allowing the dye to interact with the nucleic acids.

Capsaicin was also believed to change the biophysical properties of the membrane making it more fluid. Capsaicin caused a thermal-induced excess area that promoted fluctuation of liposomes more on temperature increase. This instability of the membrane can cause functional shortcomings leading to the death of the cell. Capsaicin itself being a lipophilic substance can insert itself into the phospholipid bilayer and can cause structural descent leading to senescence of the cell. The bactericidal effects of capsaicin may also be due to reactive oxygen products. Inducing a membrane potential can affect the influx of ions leading to cellular discordance may also be a way capsaicin affects a cell. Being a lipophilic substance capsaicin can act as a lipid oxidizing agent therefore oxidization of the phospholipid layer might cause a series of events leading to bacterial cell death.

As an alkaloid, capsaicin may be able to affect bacterial metabolism. Alkaloids may also exhibit bactericidal activities by interfering with energy and primary metabolisms to inhibit their growth [4]. Adenosine triphosphate (ATP) is the potential target. As ATP plays a major role in respiration, and primary metabolisms and is the main source of energy for a lot of enzyme catalysis inhibition of ATP synthase is lethal for bacteria. They can also inhibit the activity of bacterial functional proteases and topoisomerase. Improper functioning of topoisomerase is directly linked with gene expression which is protein production, a very critical process in cell metabolism. It is interfering with transcription and translation in the cell which disturbed the functioning of the cell and leads to senescence.

CONCLUSIONS

Antibiotics of microbial origin are considered to be more potent than Phyto products but continuous exposure to the existing antibiotics such as Penicillin has made the bacterias gain resistance to them leading to a gradual decrease in functional applications of these antibiotics therefore we should opt for more discoveries regarding multi-potent natural components that can help us fight against the pathogens. Capsaicin is one such alkaloid with antibacterial efficiency comparable to existing antibiotics, and a low tendency to drug resistance. The components of toothpaste and mouthwashes we use are mostly synthetic and have very low inclusions of natural components. So I conclude that the addition of capsaicin in products of oral hygiene can help by maintaining the pH, clearing the biofilms, and inhibiting bacterial growth. This will potentially decrease the victim's oral cancer caused by secretions of bacterial biota and help prevent a lot of oral infections. As capsaicin can affect microbes of a wide range it will help in controlling food-borne diseases. Extensive research should be done on the mechanisms of action of capsaicin so that we can increase the range of applications in human health. A generous amount of inclusion of chilies in the usual diet can be an excellent way to prevent oral infections. Capsaicin can be tagged with antibiotics and should be investigated for the increase in bactericidal effects; it will help against microbes with antibiotic resistance. There is a great need for a wide range of antibiotics therefore extensive research on capsaicin by simulating environments of buccal cavities and other tracts that are exposed to microbes should be done to acquire an in-depth analysis of bactericidal effects, efficiency on a wide range of microbes and reduced tendency to induce drug resistance.

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