Research & Reviews in Biotechnology & Biosciences
Website: www.biotechjournal.in
Volume: 7, Issue: 2, Year: 2020 PP: 28-33

Research paper

Response of gut microbe to the chilli extract and processed chilli sauce

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Article History

Received: 25/08/2020
Revised: 30/08/2020
Accepted: 18/09/2020

http://doi.org/10.5281/zenodo.4308438

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Abstract

Chilli is an economically important, high valued spice worldwide. The main objective of this study is to determine the antibacterial effects of aqueous chilli extract and processed chilli sauces by well diffusion Method. In this aspect, a study for antibacterial effects of three locally available chiliesandsixchilli sauces collected from Mangaloreregionhas been carried out against *Bacillussubtilis*and*E. coli*.The chillies used for this study wereGadhari chilli –red, green and long green chilli. Following the standard procedure the aqueous chilli extracts and chilli sauce were processed against test organism on Muller Hinton Agar and EMB agar for *E.coli*. The results indicated that aqueous extract of red bird eye chilli was more antibacterial against Bacillus than *E.coli*.Amongthe chilli sauces, all the six brands showed antibacterial activity against both the organisms than aqueous extract.Redchillisauce ofBrand D was more effective against both *Bacillus subtilis*and*E. coli*.It clearly indicates that preservativesin the processed chilli sauce might beantibacterialagainst*E.coli*,in addition to the active component of chillies.*E.coli*, is essential to condition the gut and for digestion.Natural aqueous chilli extract is safe for the *E.coli*thereby restoring the gut normal flora on its consumption. Processed chilli sauce can be negative to the gut flora due to its inhibitory action probably also due to its preservatives.

Key word-Chilly, *E.coli, B.subtilis,* chillisauce,gut flora, preservatives

Introduction

Chilli imparts unique taste to the food due to itsspicy nature. It is also known for many medicinal properties.Various studies have beenreported about its active antimicrobial compounds and its action on various microbes in addition to its health benefits.Dominant chemicals in chili are capsaicinoids, carotenoids, phenolic, and vitamins, which are phytochemicals. The capsaicinoids, capsaicin and dihydrocapsaicin are powerful free radical scavengers and also are antimicrobial agents on a wide range of bacteria and fungi. Chilli has a good antimicrobial activity against *Staphylococcus aureus, Salmonella typhimurium, Bacillus cereus, Listeria monocitogene, Helicobacter pylori*. Other medicinal purpose involves pain relief, potent anti-inflammatory agent, treatment

for sensory nerve fibre disorders, including pain associated with arthritis and psoriasis and lowers the risk for diabetes (1). In the present study, an attempt has been made to investigate the antimicrobial effect ofthree types of aqueouschili extract and 6 types of processed chilli sauces against Gram positive *Bacillussubtilis*and Gram negative gut microbe, E.*coli*.A Gandharichilli,bird eye chilli (*Capsicum frutescens*) is small, but it is very hot and pungent. Have wide range of uses, including pharmaceutical, natural colouring agents and as the active ingredient in most defense repellents. The capsaicin is the active component. It can control cholesterol and blood pressure and helps in reducing unnecessary fat. It shows antimicrobial activity against *Bacillus species, Escherichiacoli*, *Salmonellatyphimurium, Pseudomonas aeruginosa, Staphylococcus aureus and Vibrio.* (2)TheCapsaicin is an active component of green chilli(*Capsicum annuum*).Capsaicin and capsaicinoids found in green chilli, has demonstrated a high degree of biological activity affecting the nervous, cardiovascular, and digestive systems. Medical preparations were applied for a variety of ailments including respiratory problems, bowel complaints, earaches, and sores (3). It also contains different defence proteins and different AMP's (4). It inhibits the growth of *Bacillus*, *Clostridium*, *Pseudomonas*, *Staphylococcus*, *Salmonella*and*Streptococcus species* (5).

Studies have reported that chilli sauce is a processed food product containing preservatives to inhibit the microbes and to increase the shelf life of sauce.In some bacteria, these capsaicin of the sauces cause s membrane damage in killing the cell.Sauces show antibacterial activity against Gram-positive and Gram-negative bacterial strains. High concentrations of capsaicin retarded the growth of *Escherichia coli* and *Pseudomonas solanacerum*, whereas growth of *Bacillus subtilis*was strongly inhibited. This suggests that capsaicin has a variable effect on differenttypes of microbes (6).

Methodology:

Sample collection: The fresh bird eye chilli (red and green), long green chillies were collected directly from the plant from Mangalore region. The chilli sauces of various brands were collected from nearby restaurants of Mangalore in sterile tubes and named as A, B, C, D, E and F. The chillies and sauces used in the study are shown in the figure 1.

Test microorganisms: These were *Bacillus subtilis*a common contaminant isolated from the environmental samplesand*E. coli* was isolated from the Coliform positive water samples isolated by the Multiple Tube Test (MPN).The turbidity of the 18 hour old bacterial culture was adjusted to 0.5 McFarland standards (equivalent to 1.5 × 108 CFU/ml). For Bacillus study, Muller Hinton Agar and for *E. coli E*osin methylene blue Agar was used. The selective medium EMB agar was used for the selective action on *E.coli.*

Preparation of chilli extract: 5g of local chilli varieties were weighedandsurface sterilized with alcohol, dried and aqueous extract was prepared using 5 ml of sterile water in sterile mortar and pestle. The aqueous extract was stored in clean sterile tubes and preserved in the refrigerator until use.

Wells of 1 cm diameter were created on the media.The bacterial culture was spread on sterile nutrient medium. 0.5 ml of aqueous chilli extract and chilli sauces were added in to the respective wells using sterile pipettes. Sterile distilled water was added in the

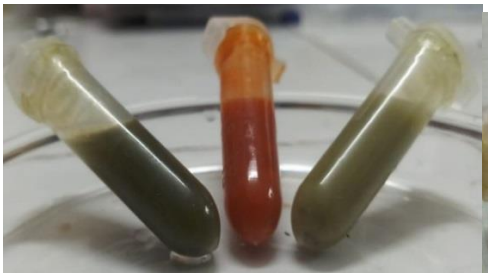
control well. Incubated the plates at 37^{0}C for 24 hours.After incubation the inhibition zones were measured and recorded.

Result:

The inhibition zone for *Bacillussubtilis*and*E.coli*against aqueous chilli extracts and processed chilli sauces were given in the Figure 2 and 3 and in Table 1 and 2, after 24 hours of incubation. *E. coli*did not show any inhibition zone whereas Bacillus has shown moderate inhibition zone against aqueous chilli extract.

Gandhari chilli (red and green)

Green chilli



Aqueous chilli extracts Chilli sauce

Figure 1: Red, green chillies and sauces used for the study

Figure 2. Antibacterial effect of aqueous chilli extract against *Bacillus subtilis*and *E. coli*

Figure 3: Antibacterial effect of processed chilli sauce against *Bacillus subtilis*and *E. coli*

Tables

Table 1: Antibacterial effect of aqueous chilli extract

Aqueous chilly extract	Inhibition zone (diameter in cm)	
	Bacillus subtilis	***E. coli***
Red Bird Eye chilly	2	No zone
Green Bird Eye chilly	1.8	No zone
Green chilly	1.4	No zone

Table 2: Antibacterial effect of processed chilli sauces

Chilli sauce	Brand	Inhibition zone (diameter in cm)	
		Bacillus subtilis	***E. coli***
Green chilli sauce	Brand A	1.6	3.5
Red chilli sauce	Brand B	2.2	4
Red chilli sauce	Brand C	2.1	4.3
Red chilli sauce	Brand D	2.3	4.6
Red chilli sauce	Brand E	2.1	3.4
Red chilli sauce	Brand F	2	4.3

Discussion

Inhibition zone of aqueous chilli extract shows moderate antibacterial effect against Bacillusthan*E. coli*. As chilli extract is not effective against *E. coli*, it would not harm the Gram *negative gut flora.*Against*B.subtilis*Red bird eye chilli extract developed maximum inhibition zone, followed by green bird eye chilli and green chilli. Therefore, aqueous extract of red bird eye chilli is more antibacterial against Gram positive *Bacillussubtilis.* This may be due to the additional active compounds in ripened red chilli and the susceptible Gram positive cell wall structure. Our result does not correlate with JharnaDas'sreportof2018where green and red chilli extract is antibacterial against *E.coli*too.This may be due to the EMB agar that we have used for antibacterial study for *E.coli*and the active antibacterial compounds present in the chilli aqueous extract vary from place to place a due to the difference in the soil composition and climate.Also Lukas*et.al* stated that Habanero chilli sauce had weaker antibacterial activity on *E.coli* and *Bacillus thuringensis.*But our study shows good inhibition for *E.coli* and less on *B.subtilis.* This may be due to the varied compositions of sauces and its preservatives.

Chilli sauces of all six brands developed inhibition zones against B.subtilisand*E. coli*. For Bacillus, red chilli sauce of Brand D developed maximum inhibition zone and green chilli sauce of Brand Adeveloped minimum inhibition zone. Order of effectiveness of chilli sauce brands against *B.subtitlis*.is – Brand D, B, C, E, F and A. For *E. coli* red chilli sauce of Brand D developed maximum inhibition zone and Brand E developed minimum inhibitionzone.The order of antibacterial effect of chilli sauce brands against *E. coli* is – Brand D, C, F, B, A and E. Therefore, chilli sauce Brand D is more effective against both *Bacillus subtilis*and*E. coli*.

The comparative antibacterial effect of chilli extract and sauce clearly shows that Bacillus is more susceptible to the active compounds of chillies especiallyto red chillies extracts and sauces. But chilli sauce showed the antibacterial effect slightly more on *E.coli.*than*B.subtitlis.*Muangkote*et al* reported that after heating, roasting the antibacterial activity of the chilli has reduced. Whereas in our study all the chilli sauces have shown inhibitory activity against both the organisms. This effect can be attributed to the preservatives and other ingredients added in the sauce to increase the taste and shelf life of the sauce. Thus red chilli sauce being more effective due to its duel action is more harmful to the Gram negative gut microbeand Gram positive bacteria. It may disturb the gut environment.Bacillus is a common contaminant hence the action of chilli extracts on it is not much significant from the health point of view.

Thus consuming raw chillies though hot to the tongueis not harmful to the gut microbial health.The antimicrobial effect of sauces may be due to the preservatives like sodium benzoate, sodium bisulphite, potassium sorbate and vinegar are added in it to prevent spoilage. Hence eating excessive processed chilli sauce can be negative for the gut micro flora. However similar experiment can be tried on the clinical gut isolates to apply the effect on gut flora on the whole.

Conclusion

All the chilli extracts and6 processed chilli sauce brands have exhibited varied antibacterialeffectagainst*Bacillussubtilis*and *E. coli*.Natural aqueous chilli extract is safe for the *E.coli*thereby restoring the gut flora on itsconsumption.Processed chilli sauce may not be beneficial to the gut flora due to its preservative in addition to capsaicin an active antibacterial component in chillies.

Acknowledge: Authors would like to thank Department of Microbiology, University College, Mangalore University, Mangalore,Karnataka.

Conflict of interest: Authors declares no conflict of interest.

Compliance with ethical standards: Authors declare that this study does not involve animal or human participation.

Author'scontribution:A corresponding author designed the study and prepared the manuscript. Faculties have given useful suggestions in improving the study. Other authors were involved in the project research work.

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