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Antimicrobial property of Capsaicin

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Abstract

Antimicrobial agents have always been of clinical significance. The effectiveness of antibiotics in the near future is unpredictable due to increasing antibiotic resistance among common pathogenic microbial strains. Capsaicin, the active ingredient in chillies has numerous biological properties which are yet to be explored. Due to its characteristic pungent nature, it has attracted interest in the field of antimicrobial studies, especially in the past two decades. In the current study, pure capsaicin at different concentrations and the Soxhlet extract of Bhut jolokia (India's 'hottest' chilli) were screened for their antimicrobial effects on the common pathogenic bacterial strains Salmonella paratyphi A, Salmonella paratyphi B, Proteus mirabilis and Micrococcus luteus as an extensive literature study revealed no screening performed on these strains. Antimicrobial activity was observed in Salmonella paratyphi A, Salmonella paratyphi B and M. luteus with M. luteus showing the maximum susceptibility. In this age of antibiotic resistance and emergence of pathogenic microbial mutants, it is of significant importance to have knowledge about secondary antimicrobial compounds apart from the currently known antibiotics. In a different aspect, a genetic relationship can be established between eukaryotes and prokaryotes which respond to capsaicin due a common receptor gene.

Keywords: Antimicrobial activity, Antibiotic resistance, Capsaicin, Pathogenic.

Introduction

Antimicrobial agents have always carried major clinical significance, especially in this age of growing antibiotic resistance amongst common disease causing pathogens. In a country like India where no clinical prescription is required to purchase antibiotics, resistance to antibiotics is promoted significantly.

The growing trend of antibiotic resistance is becoming increasingly difficult to counter with emergence of pathogenic, antibiotic-resistant bacterial strains like MRSA (Methicillin resistant *Staphylococcus aureus*).

Among human population, nutritional agents are preferred over conventional therapeutics by the former's virtue of being safe, cost-effective and regularly consumed in the given geographic area. Edibles like Manuka honey are a known traditional remedy against skin infections caused by *Staphylococus aureus*[1].

Capsaicin (8-methyl-N-vanillyl-6-nonenamide)[2] is an active component present in Capsicum plants (chili peppers) and is responsible for 'burning' sensation caused by chillies. It is a part of the secondary metabolite family 'Capsaicinoids' that has been known to evolve as a defence mechanism in plants against mammals, whose characteristic grinding teeth repress seed dispersion in several plants.

It is noted that members of class Aves lack the TRPV1 receptor and facilitate effective seed dispersal in plants[3].

Capsaicin has been mainly employed in the past for its analgesic purposes[4]. Due to its characteristic pungent nature, it has attracted interest in the field of antimicrobial studies in the recent times.

A bactericidal effect has been identified against food borne pathogens *Escherichia coli*, *Helicobacter pylori*, *Pseudomonas aeruginosa* and *Salmonella typhi*[5,6].

India is reported to be the world's biggest producer, consumer and exporter of chilli peppers[7]. Bhut jolokia is the 'hottest' known chilli in India and the third hottest in the world according to the Guinness world records[8,9].

It is commonly cultivated in Assam and is known to have high capsaicinoid content along with other plant metabolites.

Materials and methods

Test microorganisms: Non-pathogenic bacterial strains of *Salmonella paratyphi* A (SpA), *Salmonella paratyphi* B (SpB), *Proteus mirabilis* (*P. mirabilis*) and *Micrococcus luteus* (*M. Luteus*) were obtained (Dept. of Microbiology, K.C. College, Mumbai) and cultured in Nutrient Agar slants at 37°C for 24 hrs.

Capsaicin: Pure Capsaicin (HiMedia Labs, Mumbai, India) was dissolved in dimethyl sulphoxide (DMSO) and dilution was performed to obtain the following concentrations: 5μgml^{-1}, 10μgml^{-1}, 50μgml^{-1}, 100μgml^{-1} and 500μgml^{-1}.

Table-1: Bacterial strains assayed for antimicrobial property.

Pathogen	Basics	Sources	Symptoms	Incub-ation	Antibiotic resistance
Salmonella paratyphi A[10]	Pathogen responsible for causing paratyphoid fever.	Food and water contaminated with faeces of an infected individual.	Diarrhoea, high fever, vomiting, headache, nausea and stomach cramps.	6-30 Days	Multidrug-resistant strains (resistant to chloramphenicol, trimethoprim-sulfamethoxazole and fluoroquinolone) have been commonly observed[11].
Salmonella paratyphi B[10]	Another pathogen which causes the enteric fever - paratyphoid.	Food and water contaminated with faeces of an infected individual.	Diarrhoea, high fever, vomiting, headache, nausea and stomach cramps.	6-30 Days	Multidrug-resistant strains (resistant to chloramphenicol, trimethoprim sulfamethoxazole and fluoroquinolone) have been commonly observed[11].
Proteus mirabilis[12]	A gram-negative, facultatively anaerobic bacterium that commonly causes urinary tract infections. It increases the alkalinity of urine due to its high level of urease production which hydrolyses urea to ammonia.	Soil, sewage, birds, reptiles and as normal flora of human intestines.	Kidney stones, painful urination, obstruction in the urinary tract.	16-24 Hours	Insusceptible to tetracycline and nitrofurantoin. Strains resistant to first generation cephalosporins and ampicillin have also been observed[13].
Micrococcus luteus[14]	A spherical, gram-positive bacterium that contributes to body odour.	Found in water bodies, soil and in the skin microflora.	Skin infections in immunocompromised patients.	16-18 Hours	Resistant to Penicillin. Inhibition of 24mm with tetracycline[14].

Chilli extract: Chilli extract (C.E.) was obtained by the Soxhlet extraction of 10g *Bhut jolokia* peppers with methanol as solvent[15]. Methanol was completely evaporated in a hot air oven and the extract obtained (0.477g) was dissolved in 10 ml of DMSO.

Susceptibility Tests: Susceptibility was studied by the methods of agar disc diffusion (Kirby-Bauer method) and agar well diffusion on bacteria cultured by spread plate and bulk seed methods on solid nutrient media. Zones of inhibition were measured for each concentration. Wells were created immediately after culturing with a sterile cork borer and 100 µl of test sample was poured in each well by means of a micropipette.

Concentration of bacterial suspension was adjusted by the use of colorimeter to obtain a mat growth on spread plate culture and 10^8 cfu ml^{-1} for bulk seed culture. i. All the data is representative of minimum three replicates for each test. ii. All tests were performed in a Laminar Air Flow to avoid contamination. iii. Negative controls were prepared using plain DMSO in the same method. Microbial growth was found to be unaffected.

Results and discussion

Kirby-Bauer method: No zone of inhibition was observed in any of the organisms in Kirby Bauer method. Lower volume of test sample provided by disc was postulated to be the reason for negative result.

Table-2: Results of Kirby-Bauer method.

Test organism	Zone of inhibition (mm)
SpA	Neg
SpB	Neg
P. mirabilis	Neg
M. luteus	Neg

Agar well diffusion method: i. Spread plate culture, ii. Bulk seed culture.

Table-3: Zones of inhibition in spread plate culture.

Test organism		Concentration of Capsaicin (µgml^{-1})					
		5	10	50	100	500	C.E.
SpA	Mean zone of inhibition (mm)	12.66±1.15	14.33±0.57	13.33±1.10	14.66±0.57	15.33±0.57	19.66±0.57
SpB		12.33±0.57	13.33±0.57	13.00±1.73	14.66±0.57	15.33±0.57	16.00±0.00
P. mirabilis		Neg	Neg	Neg	Neg	Neg	Neg
M. luteus		15.66±2.08	13.33±2.88	15.66±0.57	17.33±1.15	18.00±3.00	27.33±2.51

Table-4: Zones of inhibition in bulk seed culture.

Test organism		Concentration of Capsaicin (µgml^{-1})					
		5	10	50	100	500	C.E.
SpA	Mean zone of inhibition (mm)	10.66±1.15	13.00±1.73	11.33±1.15	10.66±1.15	10.66±1.15	neg
SpB		11.66±2.08	10.33±0.57	9.33±1.15	10.66±1.15	10.66±2.30	neg
P. mirabilis		Neg	Neg	Neg	Neg	Neg	Neg
M. luteus		10.66±1.15	11.66±1.52	12.00±1.73	11.66±1.52	15.33±0.57	23.33±2.88

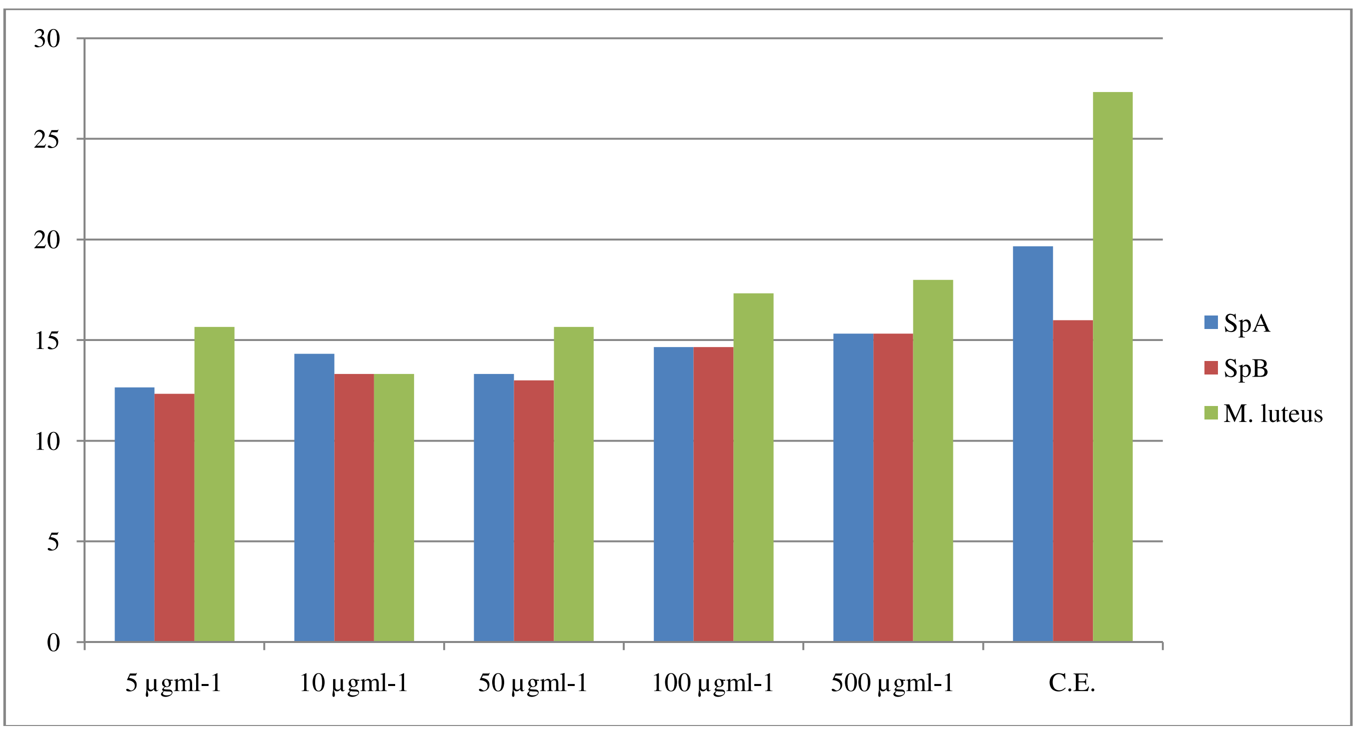

Figure-1: Comparative analysis of zones of inhibition in spread plate culture.

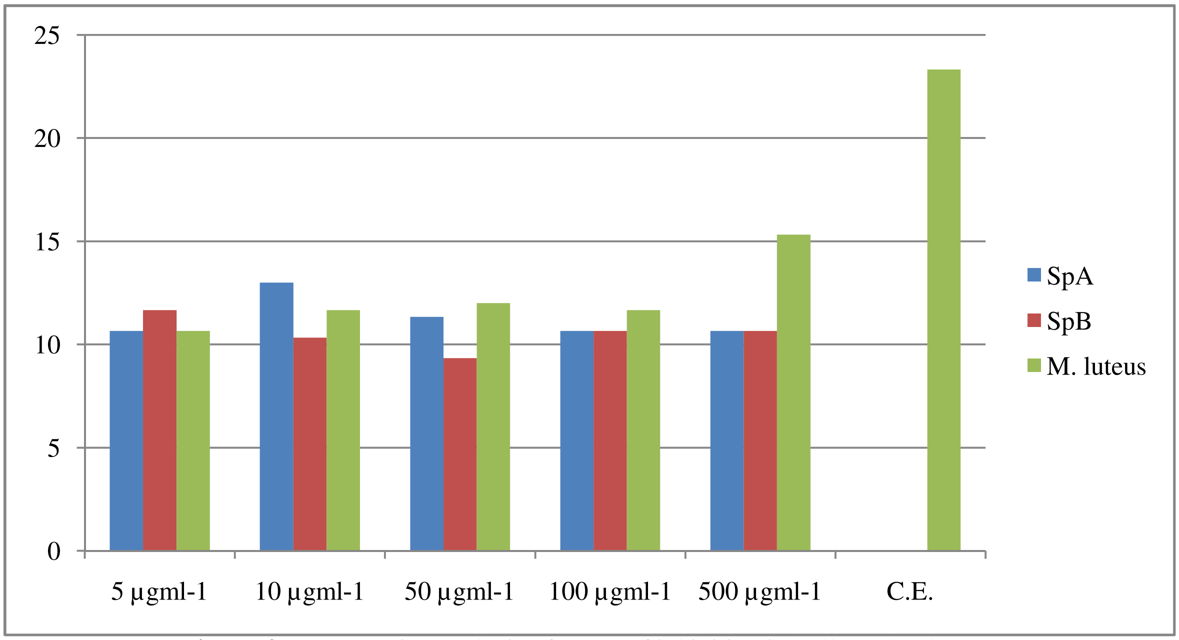

Figure-2: Comparative analysis of zones of inhibition in bulk seed culture.

Salmonella paratyphi A and Salmonella paratyphi B: The causative agent of paratyphoid fever did not present with any strikingly significant susceptibility to Capsaicin in both, pure and chilli extract forms as compared to the currently available antibiotics employed for treatment.

The field of inhibition observed only displayed a reduction in number of colonies and not a completely clear zone. The result is consistent with a similar analysis previously performed on *Salmonella typhi*[6] where no significant inhibition was observed with pure Capsaicin and chilli extract.

It was noted that a lower zone of inhibition was observed when culturing was done with bulk seed method. This reason suggested for this effect has been described that the colonies present in the bulk of the medium may have affected the diffusion of the sample in the medium; however, further analysis has to be performed to get any conclusive explanation.

Micrococcus luteus: *M. luteus* is resistant to penicillin. Mean zone of inhibition obtained with C.E. (Spread plate method) was 27.33 mm which is greater than 24 mm (zone of inhibition with tetracycline) and strikingly close to 34 mm, the zone of inhibition with chloramphenicol. A significant zone of inhibition was observed with both, standard capsaicin and C.E.; however, a striking observation was made - susceptibility towards C.E. was higher than that towards standard capsaicin even at its highest concentration. The other components in chilli namely carotenoids, chlorophyll and other plant metabolites may have contributed to the inhibitory effect; however, further detailed analysis is required for a specific conclusion.

Proteus mirabilis: A negative result was obtained i.e. *P. mirabilis* grew normally in the presence of Capsaicin and was hence concluded to be resistant to Capsaicin.

Conclusion

The organisms of genus *Salmonella* have been majorly discussed in the subject of antibiotic resistance. Significant evidence is available to suggest the antibiotic resistance among *Salmonella* species[18]. The lookout for alternative antimicrobial agents must continue to prepare for worst case scenarios relating to typhoid and paratyphoid fever, especially in developing countries like India or underdeveloped countries of Africa where hygiene standards are low as compared to developed countries.

M. *luteus* is abacterial isolate from the normal micro flora of the skin; this organism is known for causing body odour due to its products of metabolism. It is also known for causing skin infections in immunocompromised/immunodeficient patients. *M. luteus* is resistant to penicillin. Mean zone of inhibition obtained with C.E. (Spread plate method) was 27.33 mm which is greater than 24 mm (zone of inhibition with tetracycline) and strikingly close to 34 mm, the zone of inhibition with chloramphenicol. These findings suggest promising prospect of chilli as a natural antimicrobial agent. Topical ointments containing capsaicin are currently employed for analgesic purposes. Modified ointments can be formulated to tackle skin infections caused by *M. luteus* to replace the use of antibiotics.

This study can be of significant importance in the field of ayurveda. Topical ointments can be prepared to counter skin infections instead of administering antibiotics which are known to have side effects. Capsaicin ointments are currently used as analgesics.

Further testing can be performed to amplify the data in order to meet the requirements for commercialization of an idea. Tests like bactericidal versus bacteriostatic effect, analysis with different species of chillies coupled with HPLC, testing with more microorganisms are beneficial to obtain the required data.

It has been observed that even eukaryotes are susceptible to high doses of capsaicin. High dose of Capsaicin is toxic to humans and low doses cause symptoms like irritation, sneezing and a burning effect in the superficial layers of the body. This suggests that a common gene may be present in eukaryotes and susceptible prokaryotes responsible for generating a similar response. Thus, with further investigation a genetic relationship can be established and the theory of common ancestry can gain further solidity.

There is a possibility of existence of genetic orthologs between eukaryotes and prokaryotes which are responsible for generating susceptibility towards Capsaicin (which evolved as a defense mechanism in plants).

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