International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
February - March, 2016, Vol. 5, No.2, pp 2045-2055
ISSN (P): 2393-932X, ISSN (E): 2278-0238

Research Article

THE USE OF TURMERIC *CURCUMA LONGA* AS A FOOD SAFETY INTERVENTION AGENT IN PRAWNS

Femeena Hassan, Reena Nelson Anthikat*, Nija. K.V.

Quality Assurance and Management Division, Central Institute of Fisheries Technology, Cochin, Kerala, India.

***Corresponding author's Email:** reenarn@yahoo.com

(Received: December 04, 2015; **Accepted:** January 17, 2016**)**

ABSTRACT

In this study, the use of turmeric (Curcuma longa L) extract as a food safety intervention agent has been investigated. Five taxonomically different varieties of prawns shown to be different by SDS-PAGE were used to isolate bacteria. The selected six prawn isolates were also studied for their biochemical characteristics. Bacterial isolates were identified as both gram positive rods and gram positive cocci. These prawn isolates were sensitive to raw rhizome aqueous extract. The rationale being S aureus ATCC 6538 was shown to be sensitive to 200 µl aqueous extract. Anti oxidant property of turmeric is a bonus as a sanitization agent in Post harvest intervention in the processing of shrimps. Commercially available Curcumin powder showed 50 percent scavenging of DPPH radicals at 9.124 µg/ml. With proper research & development, turmeric extract may be an alternative for cleaning fresh shrimps & vegetables to eliminate microbial contamination. The present study concluded that the six prawn isolates which showed sensitivity to discs of Ampicillin 10 mcg, Tetracycline 30 mcg, Colistin 10 mcg and Gentamycin 10 mcg were sensitive to 200µl raw rhizome aqueous extract of Curcuma longa L from 100,000 µg/ml stock. Positive antioxidant dose dependant scavenging of DPPH radical gives impetus to its use as a food safety intervention agent.

Keywords: Turmeric, food safety intervention, SDS-PAGE, prawn.

INTRODUCTION

Foods often provide ideal environment for microbial survival and growth. Microbial growth in foods involves successional changes, with intrinsic or food-related, and extrinsic or environmental factors, interacting with microbial community over time. The food industry is interested in controlling pathogenic bacteria from "farm to fork" or "gate to plate". There have been promising results [1]. The use of antimicrobial treatments with chlorinated compounds added to chill water to reduce the risk of pathogens circulating in the chilling tank water is a common practice for poultry as a mode of Post-harvest intervention. The use of Chlorine in Fish processing plants to wash PUD (Peeled Undeveined) shrimps at 10-20 ppm chlorinated water is prevalent. Hence the use of an alternate plant compound as a suitable disinfecting agent is the need of the hour.

SDS PAGE was used to validate morphological and genetic variations among different species of prawns. TPC (Total Plate Count) or Total Viable Count or Total Bacterial count gives information about the number of aerobic bacteria present in any sample. Gram's characteristic is a technique in the staining reaction studies. Biochemical tests are differential characterization tests to identify the unknown bacterium.

Curcuma longa L. (Turmeric) has anti-oxidant, anti-inflammatory, anti-viral and anti-fungal actions.[2] The most important compounds responsible for the anti-oxidant activity of turmeric are phenolic compounds such as curcuminoid dyes and essential oils[3] (Burke. 1994, Neghetini. 2006)

C. longa shows anti-oxidant, hepato-protective, anti-inflammatory, anti-carcinogenic, anti-microbial,

cardiovascular, and gastrointestinal effects along with enhanced immunity due to curcumin which is the active constituent. Several studies have been done in Kerala, South India to look into the effect of arecanut extract on anti-oxidative stress in rats as well as in its potential antioxidant activity in-vitro[4] Catechin has been shown to have potent anti-oxidant activity that efficiently scavenges free radicals [5]. Calendula offcinalis (marigold) flower extract is reported to have antioxidant potential both in vitro and in vivo[6].

The present study aimed to scientifically support the addition of turmeric, an antimicrobial and antioxidant, as an effective control measure into a process to reduce and ultimately prevent or eliminate food safety risks in terms of microbiological control. It may be applicable as a Pre-harvest intervention or a Post-harvest intervention.

MATERIALS AND METHODS

Fresh prawns viz. *Fenneropenaeus indicus*, *Penaeus monodon, Metapenaeus dobsonii, Macrobrachium idella, Metapenaeus monoceros* were procured from KUFOS (Kerala University of Fisheries and Ocean Studies, Panangad, Cochin), washed with potable water and kept in ice in the ratio 1:1. They were beheaded, washed clean of adhering dirt and slime and kept in ice. The extraction of sarcoplasmic and myofibrillar protein content was carried out by the method of Sankar and Ramachandran (2000). The estimation of protein content was determined by Biuret method (Gornall, A.C., Bradwill, C.J. and David, M.M (1949). Electrophoresis of protein was done according to Laemmli (1970) by SDS-PAGE. Molecular weights of the protein bands were calculated by measuring the relative mobility of standard molecular weight marker.

METHOD

Determination of Total Plate Count by Spread Plate Technique

Total Plate Count (TPC) or total viable count or[7] total bacterial count gives information about the number of aerobic bacteria present in the sample. The TPC can be determined by microscopic or cultural method. Plate Count Agar (PCA) was used in the Spread Plate Method (Surface Plate Method) to check for plate viable cells (Colony Forming Units or CFU).

In the procedure, 5g of the prawn sample were macerated well with a known volume of 45ml phosphate-Buffered dilution water, pH- 7.2. Decimal dilutions of the sample were taken.

For Spread Plating: The plates with Plate Count Agar were arranged and spread plate technique was carried out by inoculating 0.1ml of each 10-1, 10-2, 10-3 and 10-4 dilutions on the surface of the agar plates. A sterile bent glass rod was used to spread the inoculum uniformly well on the surface of the plates.

Morphological Study of the colonies was done by Gram's Staining Test

Morphological study of the cells was done by staining reactions based on Gram's characteristics discovered by Christian Gram in 1884. The fixed bacterial smear was treated with the following staining reagents in the order of the sequence listed below.

Crystal violet ☐ Iodine solution ☐ Alcohol ☐ Safranin ☐

Physiological Characteristics of the microorganisms were done using biochemical tests namely:

Biochemical characterization was done as a part of differential tests done to identify the unknown bacterium. Indole Production Test, Methyl Red Test, Voges Proskauer Test, Citrate Utilisation Test, Triple Sugar Iron Agar Test, Urease Test and Carbohydrate Fermentation Test for lactose and sucrose were done.

The culture was inoculated in peptone water and kept overnight at 37°C and used as inoculum for the biochemical tests.

Anti-microbial activity

Kirby-Bauer Method of Disk Diffusion Test[8] Anti-microbial activity by the disk diffusion test or the Kirby Bauer Method, which was developed in the early 1960's at the University of Washington Medical School by William Kirby, A. W Bauer and their colleagues, was done using Ampicillin, Tetracycline, Colistin and Gentamycin. Turmeric aqueous extract was also used for the diffusion method, by cutting out wells to fill the plant extract. The other alternative is to perform impregnated paper disks. A. Bondi in 1947 reported the use of filter paper disks containing specified concentrations of the test extract.

A sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of Mueller-Hinton agar plate (MHA). After the agar surface had dried for about 5 minutes, the appropriate

antibiotic test disks were placed on it, either with sterilized forceps or with a multiple applicator device. The plates were incubated at 37°C for 16-18 hours. The diameters of the zones of inhibition were measured to the nearest millimeter.

Raw Material Preparation

40 g of blended Rhizomes of Curcuma longa L were boiled in 400 ml of distilled water for 3 hours. The boiled suspension was filtered using filter paper. The extract was concentrated by evaporation. The yield of the extract was found to be 8.6815%.

Turmeric aqueous extract at different concentrations were used for Diffusion Method.

Wells were cut into the Mueller Hinton Agar (MHA) using a sterile cork borer, for the purpose of holding a specific quantity of a particular anti-microbial agent.[9] The petri-plate contained an agar medium seeded with the test organism or the isolate. Five wells were cut out and filled with the plant extract. The plates were kept for incubation at room temperature for 48 hours. The vehicle sterile water was loaded into one well. Positive control Chlorine at 3.43962% was loaded into another well. The plates were incubated at room temperature for 48 hrs.

Determination of Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Activity

Antioxidant activity was measured in terms of Hydrogen donating or radical scavenging ability using the stable radical 2,2 diphenyl – 1- picrylhydrazyl (DPPH)

DPPH in its radical form has an absorption peak at 515 nm, which disappears upon reduction by an antioxidant compound 250µg/L [10] (Aquino R et al, 2001). Different concentrations of the extract (0.25µg – 3µg/ml), were added to the 1.5ml freshly prepared DPPH solution (0.25g/L in methanol). After 20 minutes the absorbance was measured at 515nm. The percentage in addition was calculated by comparing with the control. The DPPH concentration in the reaction medium was calculated from the calibration curve of percentage of DPPH scavenged vs. concentration of the standard antioxidant (L- Ascorbic Acid).

RESULTS

Protein fractionation studies

Protein fractions of different varieties of prawns collected from different farms of KUFOS (Kerala University of Fisheries and Ocean Studies) showed marked dissimilarities among them (Table 1.0, Fig 1.0).

Table 1: Protein content in different species of prawn collected from Cochin farm

Species	Sarcoplasmic protein content (%)	Myofibrillar protein content (%)
Fenneropenaeus indicus	36.38	58.56
Penaeus monodon	36.03	60.41
Metapenaeus dobsonii	31.96	53.63
Macrobrachium idella	31.96	45.04
Metapenaeus monoceros	34.84	58.85

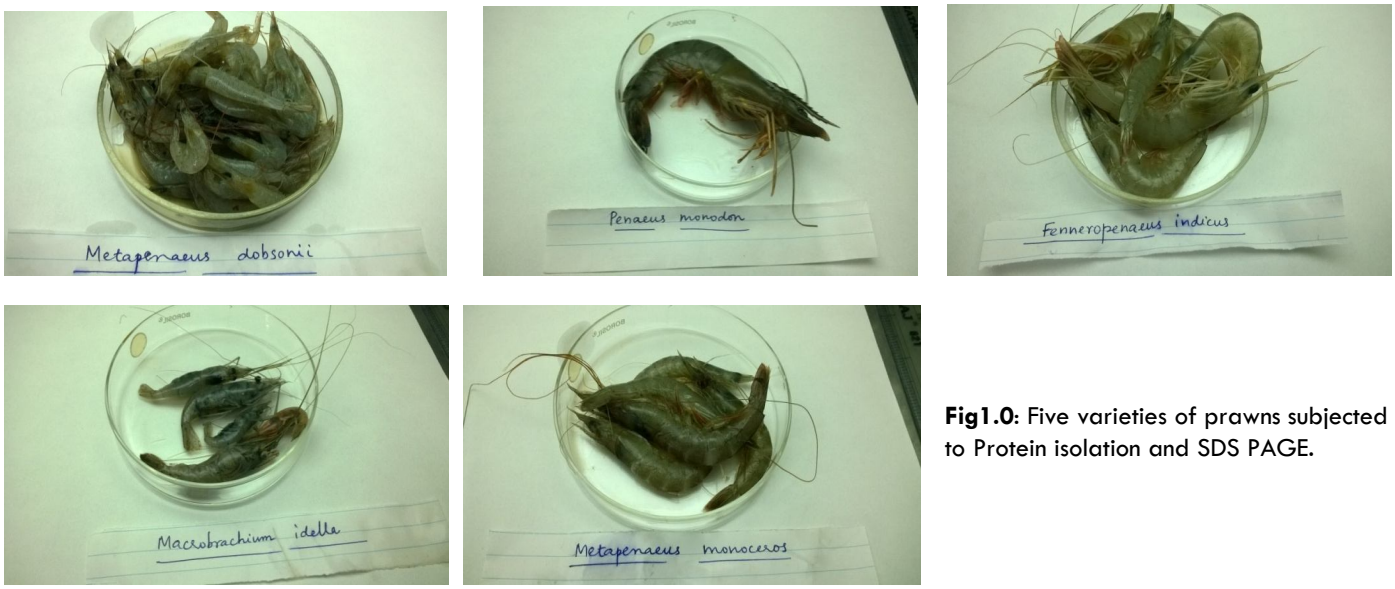

Fig1.0: Five varieties of prawns subjected to Protein isolation and SDS PAGE.

Table 2: Sarcoplasmic proteins: Relative fronts and Molecular weights

***Fenneropenaeus indicus* (Lane 6)**		***Penaeus monodon* (Lane 5)**		***Metapenaeus dobsonii* (Lane 4)**		***Macrobrachium idella* (Lane 2)**		***Metapenaeus monoceros* (Lane 3)**	
MW	**Rf**	**MW**	**Rf**	**MW**	**Rf**	**MW**	**Rf**	**MW**	**Rf**
97.4	0.104	97.4	0.042	97.4	0.091	97.4	0.019	97.4	0.091
81.2	0.206	97.4	0.094	82.5	0.198	97.4	0.042	82.2	0.200
77.0	0.235	83.1	0.193	76.1	0.241	97.4	0.091	77.0	0.235
66.7	0.312	76.4	0.239	56.0	0.422	94.8	0.123	51.8	0.472
56.0	0.422	51.3	0.478	41.7	0.624	81.5	0.204	41.9	0.620
41.3	0.632	41.1	0.636	31.0	0.875	75.5	0.245	-	-
31.5	0.857	31.3	0.861	-	-	56.8	0.414	-	-
-	-	31.0	0.942	-	-	51.3	0.478	-	-
-	-	31.0	0.971	-	-	45.9	0.549	-	-

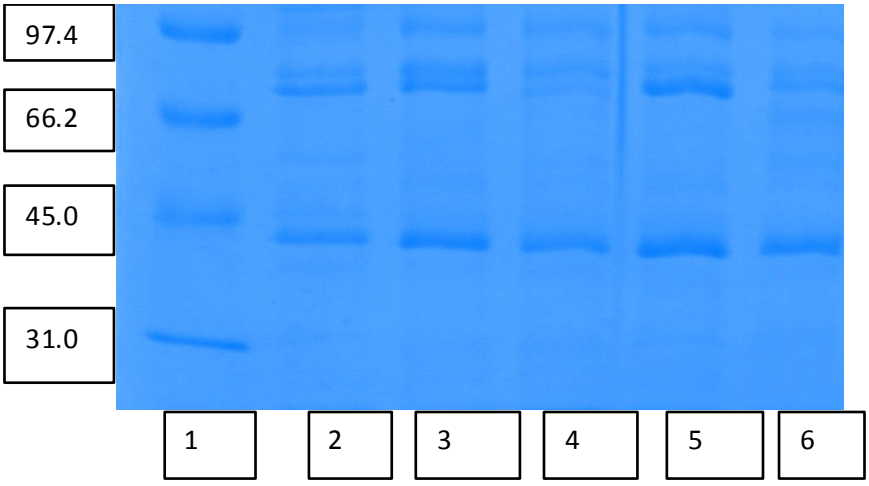

Fig 2.0: Sarcoplasmic Protein SDS PAGE.

1-protein marker

2-*Macrobrachium idella*

3-*Metapenaeus monoceros*

4- *Metapenaeus dobsonii*

5-*Penaeus monodon*

6-*Fenneopenaeus indicus*

Table 3: Myofibrillar proteins: Relative fronts and Molecular weights

***Fenneropenaeus indicus* (Lane 5)**		***Penaeus monodon* (Lane 2)**		***Metapenaeus dobsonii* (Lane 4)**		***Macrobrachium idella* (Lane 3)**		***Metapenaeus monoceros* (Lane 6)**	
MW	**Rf**	**MW**	**Rf**	**MW**	**Rf**	**MW**	**Rf**	**MW**	**Rf**
177.6	0.093	191.2	0.038	199.4	0.007	194.3	0.026	200.0	0.005
166.0	0.143	151.7	0.210	166.6	0.141	148.9	0.224	186.4	0.057
149.3	0.222	106.4	0.487	148.4	0.227	126.0	0.348	117.0	0.403
126.8	0.344	102.5	0.520	116.2	0.408	112.3	0.439	104.2	0.506
115.6	0.413	96.9	0.568	106.7	0.484	106.4	0.487	98.4	0.556
100.9	0.535	72.8	0.690	102.2	0.523	102.8	0.518	72.4	0.692
96.9	0.568	59.2	0.776	98.4	0.556	92.1	0.589	58.9	0.778
77.4	0.663	54.6	0.809	68.8	0.714	69.2	0.711	51.8	0.831
72.0	0.695	45.0	0.974	62.4	0.754	57.9	0.785	45.0	0.969
63.2	0.749	-	-	53.3	0.819	52.7	0.823	-	-
54.9	0.807	-	-	46.1	0.878	47.7	0.864	-	-
45.0	0.928	-	-	45.0	0.979	45.0	0.979	-	-
45.0	0.959	-	-	-	-	-	-	-	-

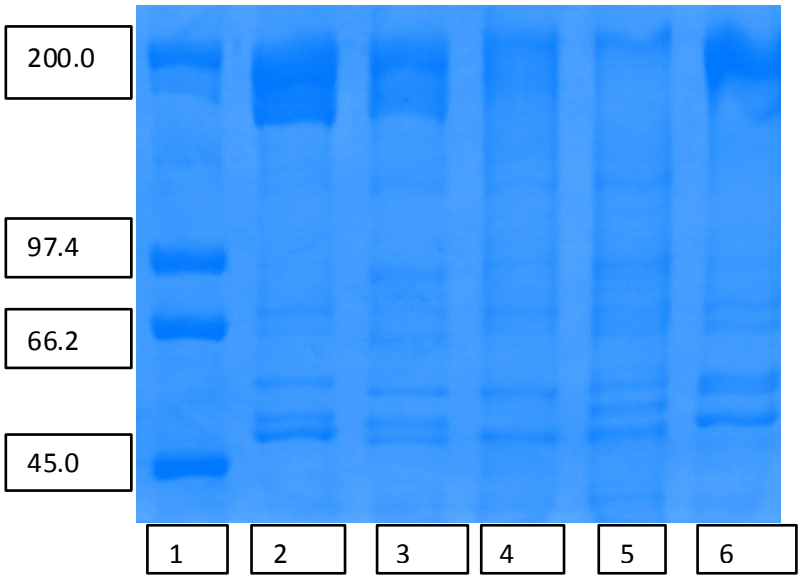

Fig 3.0: Myofibrilar Protein SDS PAGE.

1-protein marker

2- *Penaeus monodon*

3- *Macrobrachium idella*

4- *Metapenaeus dobsonii*

5- *Fenneopenaeus indicus*

6- *Metapenaeus monoceros*

Table 4: Cultural characteristics

Morphological Study: Gram's characteristics

1 10^{-1} :C	G-ve rods	White concentric dry, smooth margined, flat elevation
1 10^{-2} :A	G+ve cocci in bunches	White large, round smooth margined, raised elevation
2 10^{-1} :C'	G+ve cocci in bunches	Golden yellow irregular and spreading, irregular margined, flat elevation
2 10^{-2} :D'	G+ve rods; spindle shaped	White dry concentric, irregular margined, raised elevation
2 10^{-4} E'	G-ve short cocobacillus	White irregular and sreading mucoid, wary margined, umbonate elevation
TCBS	Gram positive cocci,oairs	Small yellow convex colonies Fig 4

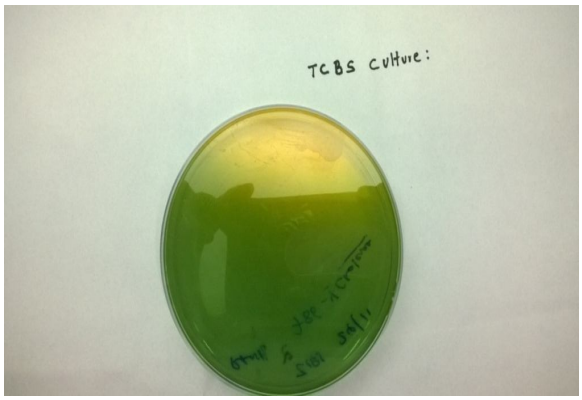

Fig4.0: Cultural characteristics of *Vibrio* isolated on TCBS

Table5: Physiological Characterisation: Biochemical Tests, Fig 5.0.

Culture	I	MR	VP	C	TSI	Urease	Lac	Suc	Oxidase	Lys	Orni	Arg
1 C	-ve	-ve	-ve	+ve	NG, NH_2S A/AK	-ve	-ve	+ve	+ve	-ve	-ve	-ve
1 A	-ve	-ve	-ve	-ve	NG, NH_2S A/AK	-ve	+ve	+ve	-ve	-ve	-ve	-ve
2 C'	-ve	-ve	-ve	-ve	NG, NH_2S A/A	+ve	-ve	+ve	-ve	-ve	-ve	-ve
2 D'	-ve	-ve	-ve	-ve	No Change	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2 E'	-ve	-ve	-ve	-ve	NG, NH_2S A/AK	+ve	-ve	-ve	-ve	-ve	-ve	-ve
TCBS	-ve	-ve	-ve	-ve	NG, NH_2S A/A	-ve	+ve	+ve	+ve	+ve	-ve	+ve

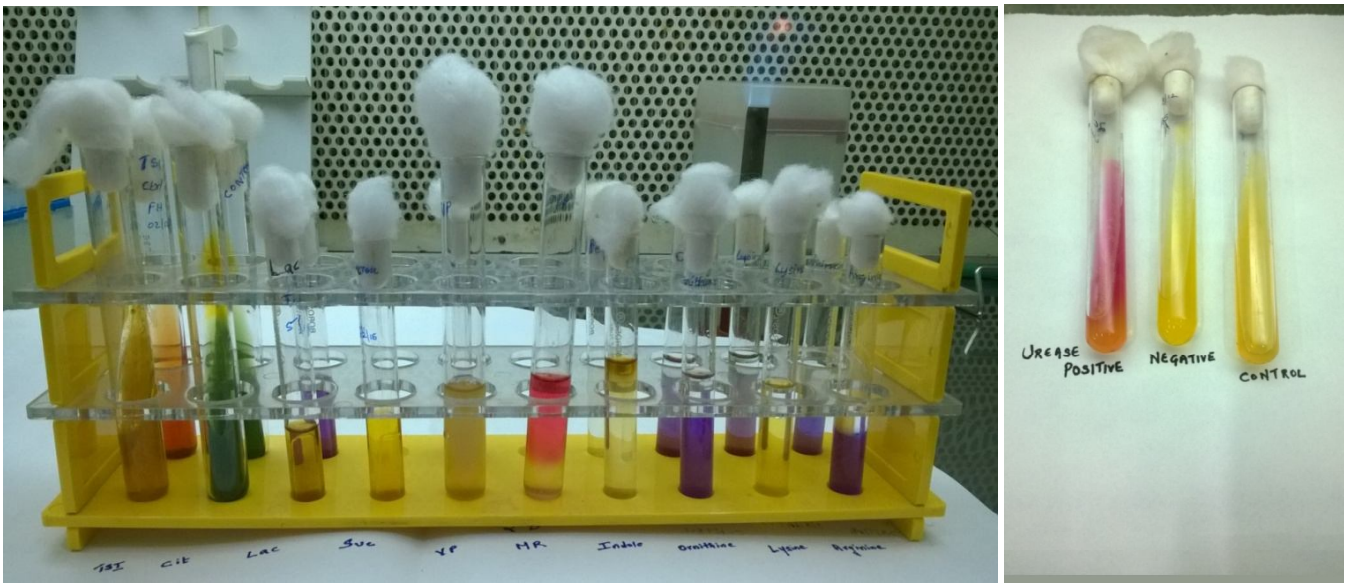

Fig 5.0: Biochemical tests for characterization

Table 6: Anti-microbial activity

Disk-Diffusion Method: Kirby Bauer Method Fig 6.0,7.0,8.0 and 9.0.

Culture	A	TE	Cl	Gen
1 C	R	22mm	R	23mm
1 A	R	26mm	R	22.5mm
2 C'	7 mm	26mm	7mm	23mm
2 D'	R	R	R	R
2 E'	R	16.5mm	10mm	19mm

Table 7 i : Aqueous Turmeric powder: Fig 10.0 and 11.0 Stock:100µg/ml of commercial turmeric powder

190 l	200 l	100 l	Culture
-	-	-	**1 C**
-	-	-	**1 A**
-	-	-	**2 C'**
-	-	-	**2 D'**
-	-	-	**2 E'**
-	-	-	**TCBS**

Table 7 ii: Stock: 100,000µg/ml of raw rhizome aqueous extract

Isolate	200µl	20µl Chlorine	Control :Sterile water
1C	No zone	40mm	No zone
1A	No zone	29mm	No zone
2C'	12mm	R	No zone
2D'	No zone	23mm	No zone
2E'	10mm	30mm	No zone
TCBS	No zone	12mm	No zone

Table 8: DPPH Radical Scavenging by different concentrations of turmeric

Concentration (g/ml)	% Inhibition
0.25	2.75
0.5	5.95
1.0	11.85
1.5	9.21
2.0	13.03
2.5	13.7
3.0	12.07

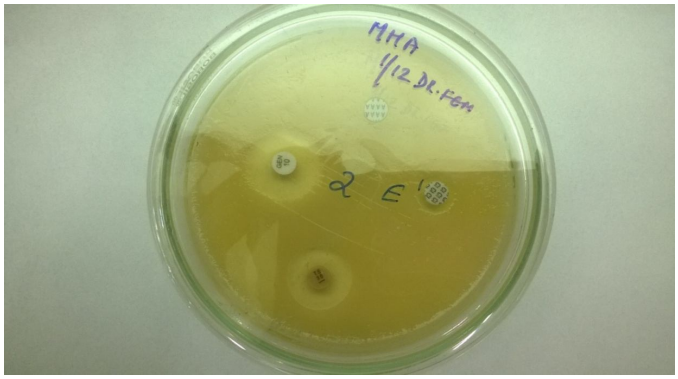

Fig6.0: Isolate 2E' against antibacterial antibiotics

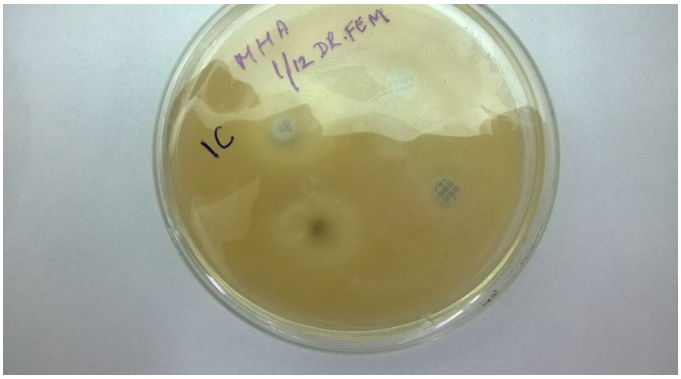

Fig7.0: Isolate 1C against antibacterial antibiotics

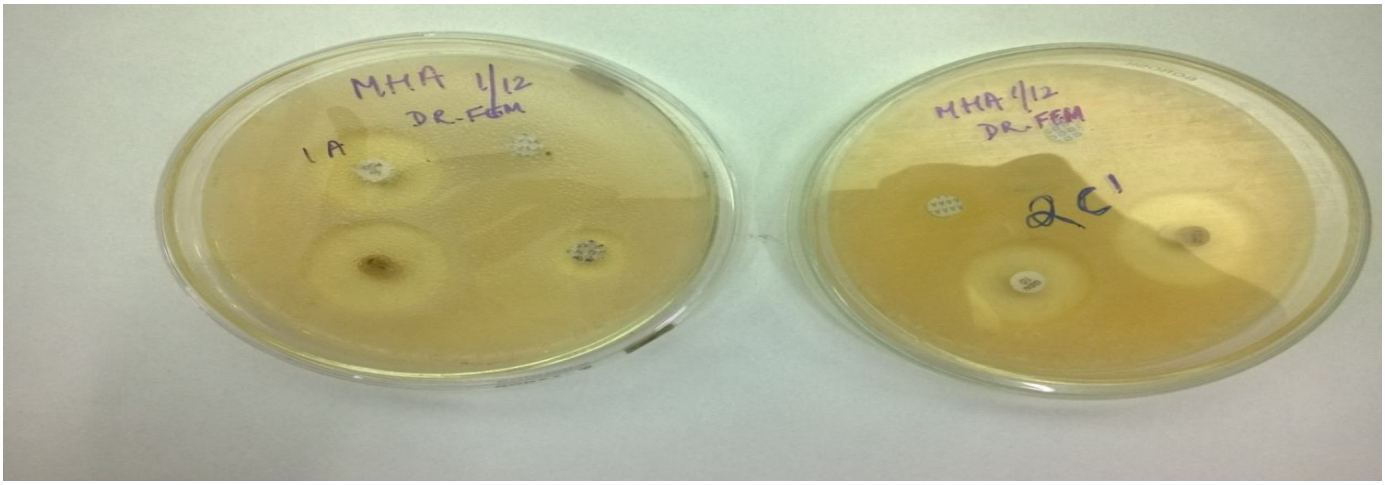

Fig8.0: Isolate 1A and 2C' against antibiotics

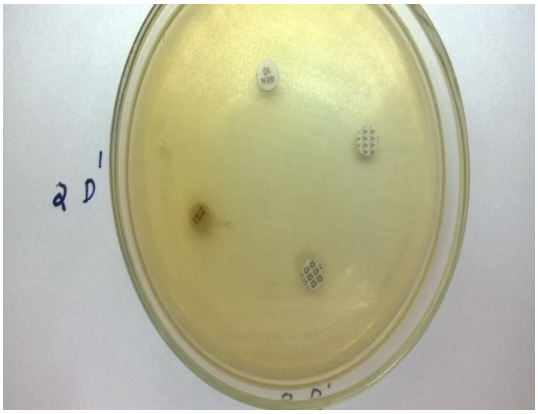

Fig9.0: Isolate 2D' against antibiotics

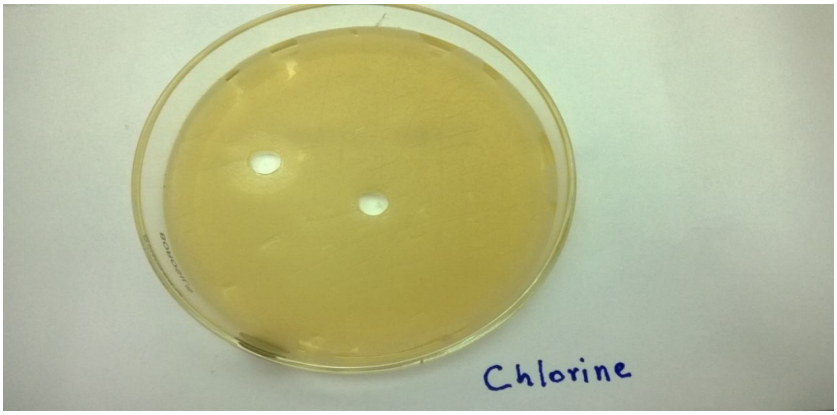

Fig10.0: Chlorine 3.43962 %(34396.2 ppm) shows anti Staphylococcal activity, vs. lower concentration.

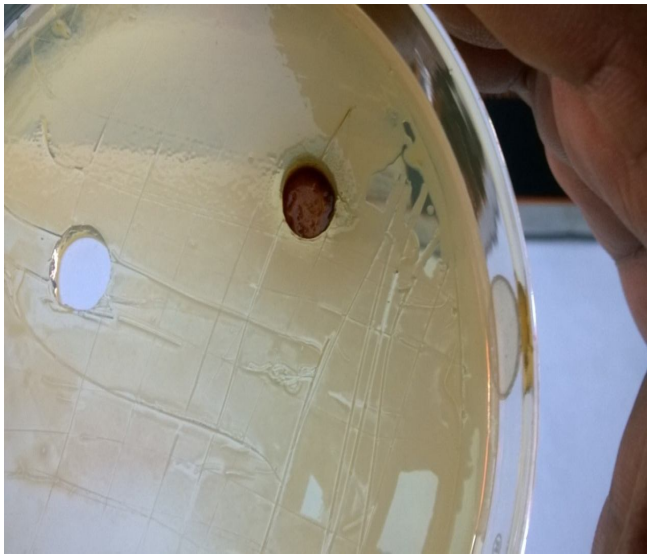

Fig11.0: Aqueous Turmeric extract shows anti Staphylococcal activity

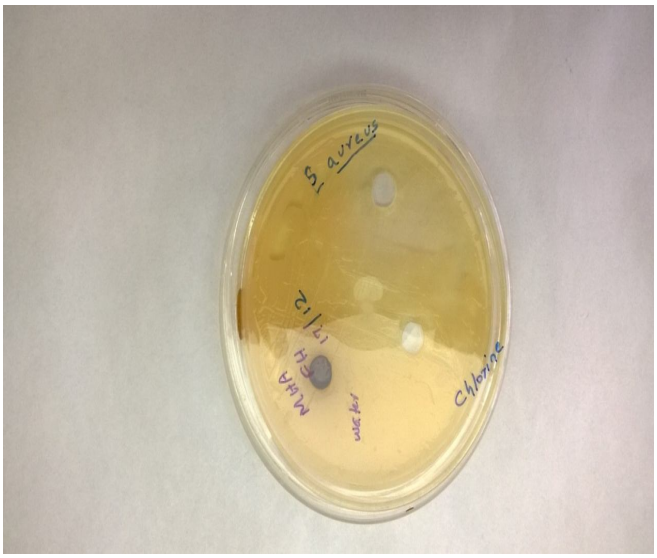

Fig 12.0: Antibacterial activity of Turmeric against *S. aureus* ATCC 6538

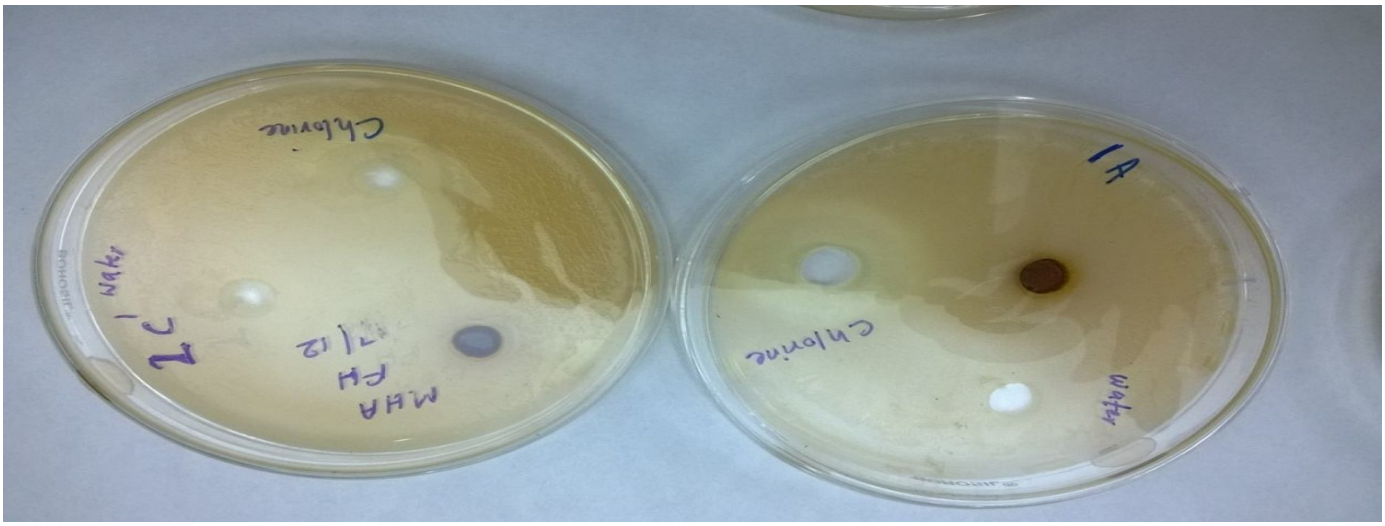

Fig13.0.:Antibacterial activity against Isolate 1A and 2C'

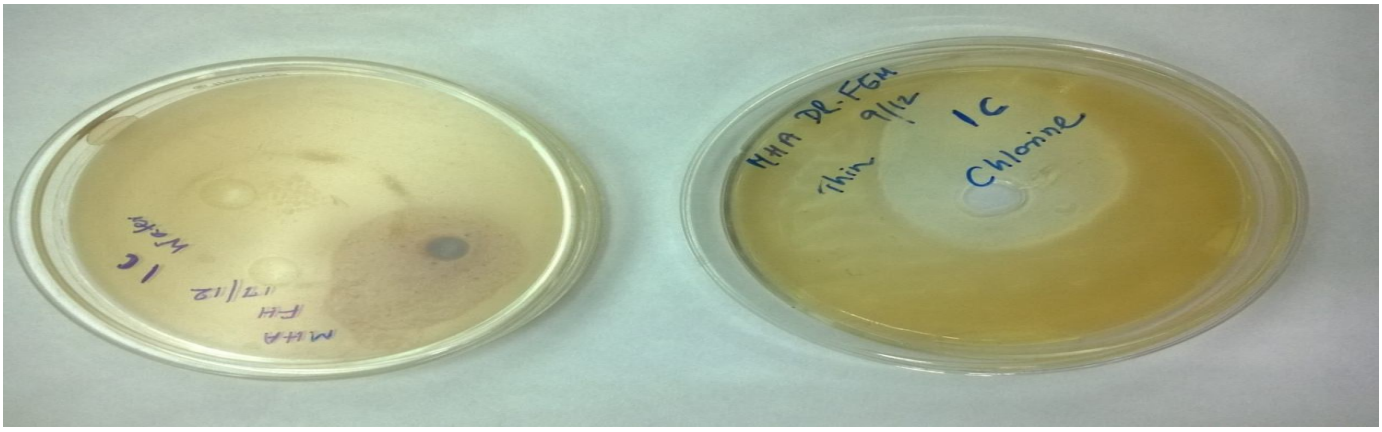

Fig 14.0: No antibacterial activity was seen against isolate 1C but Chlorine showed antibacterial activity against 1C

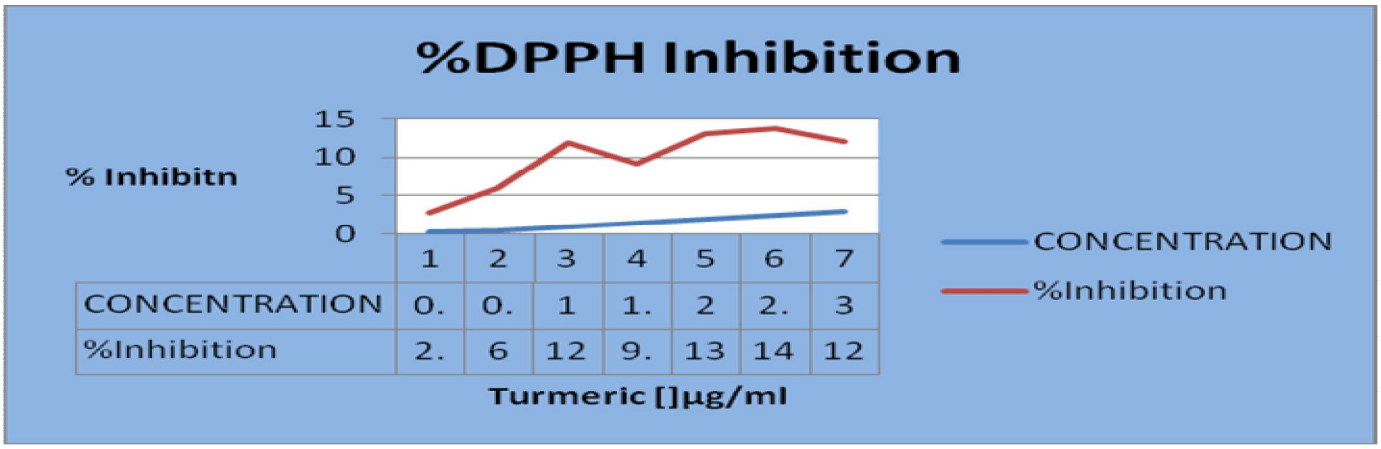

Fig 15.0: DPPH radical scavenging by different concentrations of turmeric

The molecular weight of protein bands of sarcoplasmic proteins of five varieties showed similarities and dissimilarities. (Table 2.0, Fig 2.0). The molecular weight of protein bands of myofibrillar proteins of the types showed positive (+) and negative (-).(Table 3.0, Fig. 3.0).

Cultural and Biochemical characteristics

The colonies showed different cultural characteristics. All the isolates were either gram negative rods or gram positive cocci in bunches (Table 4.0).

Biochemical characterisation showed gram negative rods resembling Enterobacteriaceae. (Table 5.0, Fig 5.0)

The organisms were identified as follows:

1A: CoNS (Cogulase Negative Staphylococcus)

2C': CoNS (Cogulase Negative Staphylococcus)

2E': CoNS (Cogulase Negative Staphylococcus)

1C: Bacillus species

2D: Candida krusei

TCBS: Enterococcus faecium

Antimicrobial activity

The selected isolates were shown to be sensitive and resistant to the commercially available antibacterial antibiotics namely Ampicillin 10 µg/disc, Tetracycline 30 µg/disc, Colistin 10 µg/disc and Gentamycin 10 µg/disc.(Table 6.0, Fig 6.0, 7.0, 8.0& 9.0). Seven bacterial isolates resistant to either of the antibiotics were tested for their sensitivity to turmeric-procured powder and raw rhizome extract.

Commercially available turmeric powder showed no activity against the selected isolates. (Table 7.0 i, Fig 10.0 & 11.0). Raw rhizome aqueous extract of Curcuma longa L showed activity against Staphylococcus aureus ATCC 6538, isolate 2C' and 2E' (Table 7.0 ii, Fig 11.0, 12.0 & 13.0) and no cidal activity against 1C, 1A, 2D' and TCBS isolates. The maximum zone diameter was observed against 2C' and S aureus ATCC 6538.

A commonly used sanitizer Chlorine (34396.2 ppm) showed antibacterial activity against 1C, 1A, 2D',2E' and TCBS isolates. (Table 7.0 ii , Fig 14.0). Maximum zone diameter was seen against 1C.

Antioxidant activity

The percentages of DPPH inhibition were 2.75%,5.95%, 11.85%, 9.21%, 13.03% and 13.7% at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 µg/ml of aqueous turmeric powder. There was a dose dependant increase in the value.(Table 8.0, Fig 15.0).

DISCUSSION

Undesirable microorganisms can cause illness. Toxic components may be formed when microorganisms grow in food before it is ingested resulting in the rapid onset of disease symptoms after consumption [11]. From a food safety perspective, intervention involves the addition of control measure into a process to reduce and ultimately prevent or eliminate food safety risks. In the present study, isolates from five varieties of prawns taxonomically endorsed to be different by SDS –PAGE studies were inhibited by the use of raw rhizome aqueous extract of turmeric. The supporting fact was that, the extract of C. longa inhibited the growth of S aureus, providing some scientific rationale that the local inhabitants used the extract as antimicrobial agent. Turmeric extract reported to be an antioxidant was investigated for the same. The aqueous extract of turmeric powder was found to scavenge the DPPH radicals generated in a dose dependant manner.

Incorporating turmeric rhizome extract into water can be categorized as a Post-harvest intervention in the Food Standard Agency's Food Code of Practice (2008)(FLCOP),[12]. Environment controls on the farm or in poultry growing houses can enhance cleaning procedures as intervention to reduce the incidence of pathogenic bacteria [13].

Ozonisation or application of sanitizers such as chlorinated compounds after the wash step, as an intervention to reduce the risk of pathogens is already one of the Post-harvest interventions for fruits and vegetables.

In the previous studies curcumin, demethoxycurcumin, bisdemethoxycurcumin, oleoresin and essential oils were isolated in C. longa. [14,15]. The potential role of essential oils as food preservatives has been recognized but the concentration necessary to inhibit food borne pathogens is high which leads to undesirable organoleptic effect.[16]

This study gave positive results for the use of freshly prepared aqueous extract from young tender rhizomes of C. longa. 200 µl of turmeric aqueous extract showed positive bactericidal activity against isolate 2C', 2E' and S. aureus ATCC 6538 is known to cause skin infections.

This study was taken up as there are scientific publications that support the use of some essential oils such as thyme and basil oils as effective against spoilage flora and food borne pathogens when used in washing water.[17,18] Previous studies indicated that some essential oils of Thai spices such as lemongrass, holy basil, turmeric and galangal oils had antibacterial properties[19,20,21]. This information prompted us to search for the active compounds of turmeric. However, an extensive study to identify the active components has only been reported on lemongrass oil, in which citral was identified as the active compound[19, 22].

REFERENCES

1. www.fsis.usdaigov/PDF/Reducing E coli Shedding in Cattle- 0510.pdf.
2. Akram M, Shahab-uddin, Afzal Ahmed, Khan Usmanghani, Abdul Hannan, Mohiuddin E, Asif M Burke. 1994, Curcumin dual defence against disease. Chemistry & Industry, n,8, p.289, 1994. Complemento.
3. Effect of Arecanut Extract on Oxidative Stress in- vitro and in-vivo. Amala Research Bulletin Vol.24 (2004). Pg 82-92.
4. Anti-inflammatory and anti-oxidant effect of Arecha catechu (2012). International Journal of Pharmaceutical Sciences and Research; Vol. 3(6): 1000-1006.
5. Jovanovic S.V, Steenton S, Tosic Mrjunovic M and Simic M.G (1994) Flavonoids as anti oxidants. J. Am. Chem. Soc. 116, 4846-4851.
6. Preethi KC, Kuttan G, Kuttan R. Antioxidant potential of Calendula offcinalis flower in vitro and in vivo. Pharmaceutical Biol. 2006; 44 (9): 691-697.
7. P.K. Surendran, Nirmala, T., Narayanan Nambiar, V., Lalitha, K.V., Laboratory Manual on Microbiological Examination of Seafood. 2nd Edn 2006 Central Institute of Fisheries Technology Publication.Cochin. 682029 Pg 9-14.
8. Prescott, L.M, Harley J.P, Klein D.A., Microbiology, 2nd Edn. Wm.C.Brown Publication pg 329.
9. Sharad Srivastava, Vineeta Singhal., Laboratory Methods in Microbiology 1st Edn 1994. Anmol Publications Pvt Ltd. New Delhi Pg 505-509.
10. Aquino R et al. Phenolic constituents and an oxidant activity of an extract of Anthrium versicolor leaves. J. of Natl. Products 2001; 64: 1019-1023.
11. Prescott, L.M, Harley J.P, Klein D.A., Microbiology, 2nd Edn. Wm.C.Brown Publication pg 867.
12. Allerdale Borough Council. Food safety Intervention Strategy United Kingdom www. Allerdale. Gov.uk/downloads/ intervention strategy. doc "Food safety Combria Food Liaisan group Food safety Intervention Strategy.
13. Cox, J.M and A. Pavic.2009. Advances in Enteropathogen Control in Poultry Production. J Appl. Microbiol 108:*745-755*.
14. Apisariyakul, A., Vanittanakom, N., Buddhasukh, D. Antifungal activity of turmeric oil extracted from Curcuma longa (Zingiberaceae). J Ethnopharmacol 49,163-169, 1995.
15. Song, E.K., Cho, H., Kim, N.Y., An, N.H., Kim, J.A., Lee, S.H., Kim Y.C. Diarylheptanoids with free radical scavenging and hepatoprotective activity in vitro from Curcuma longa. Planta Med 67, 876-877, 2001
16. Thoroski J, Blank G, Biliaderis C (1989) Eugenol induced inhibition of extracellular enzyme production by Bacillus cereus. J.Food Protect 52, 399-403.
17. Wan J, Wilcock A, Coventry MJ(1998) The effects of essential oils of basil on the growth of Aeromonas hydrophila and Pseudomonas fluorescens. J Appl Microbiol 84, 152-8.
18. Singh N, Singh RK, Bhunia AK, Stoshine RL(2002) Efficacy of chlorine dioxide, ozone and thyme essential oil or a sequential washing in killing Escherichia coli O157:H7 on lettuce and baby carrots. LWT Food Sci Tech 35, 720-9.
19. Orafidiya LO(1993). The effect of autooxidation of lemongrass oil on its antibacterial activity. Phytother Res 7,269-71.
20. Hammer K.A, Carson CF, Riley TV(1999). Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 86, 985-90.
21. Wannisorn B, Jarikasem S, Siriwangchai T, Thubthimthed S(2005). Antibacterial properties of essential oils from thai medicinal plants. Fitotherpia 76, 233-6.
22. Friedman M, Henika PR, Levin CE, Mandrell RE (2004). Antibacterial activities of plant essential oils and their components against Escherichia coli O157:H7 and Salmonella enteric in apple juice. J. Agr Food Chem 59, 6042-8.

