International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

STUDIES ON ANTIBACTERIAL ACTIVITY OF CRUDE ETHANOLIC EXTRACTS AND ESSENTIAL OILS OF SPICES AGAINST *SALMONELLA TYPHI* AND *E. COLI 0157:H7*

Dharmvir A.[1]*,
Chouhan B. [1], Kishor M. [1], Chandewar[1]

[1]Department of Microbiology, D.B. Science College, Gondia.

ABSTRACT

Based on reports from 1973 to 1997, cases of Salmonellosis other than typhoid have been reported almost each year. The objective of this study will be to assess the in vitro antibacterial activity of different extracts of commonly used spices like cloves and cinnamon against food borne Salmonella typhi and E.coli O157:H7. This study extended to determination of the minimum inhibitory concentration (MIC) against each bacterium. During this study food borne bacterial pathogens E.coli O157: H7 and Salmonella typhi isolated from choupati food samples, isolates were identified on the basis of Morphological, Biochemical and Cultural characteristics. The bacterial isolates were subjected to analysis for susceptibility or resistance towards different antibiotics. The four types of spices including Cinnamon, Clove, Coriander and Funnel were extracted using organic solvents like methanol, ethanol, ethyl acetate and steam distillation using water as a solvent. Well diffusion method was performed using standard procedure for Screening of Spices extract for their antibacterial potential. The dilution test was performed to determine minimum inhibitory concentration (MIC) using Standard procedure. This ethanolic clove extract have great activity against E.coli 0157:H7 as well Salmonella typhi isolates. But methanolic and ethyl acetate extracts were ineffective against S. typhi. Aqueous extract of all selected species were ineffective against E.coli 0157:H7 strain as well as S. typhi strains. Oils of clove were effective against all tested strains (Diameter of zone of inhibition (8 to 21). Oil of funnel was moderately effective against tested pathogens. The MIC value of ethanolic extract of clove against E.coli 0157:H7 strain was 6µg/ml; MIC for S. typhi isolates 5.0µg /ml, MIC value of methanolic extract against E.coli 0157:H7

Correspondence to Author

Dharmvir A.

Department of Microbiology, D.B. Science College, Gondia.

Email: dharmvir1703@yahoo.co.in

strain was 9 µg /ml MIC for S. typhi isolates 8µg/ml. The study indicate that clove bud extracts and clove bud oil can be used as antibacterial, antifungal and antiseptic agent so that they can be used as a food preservative hence, improve shelf life of foods.

INTRODUCTION

In recent years, food safety concerns have been focused on pathogens such as *Salmonella* which is recognized as one of the leading causes of food-borne bacterial diseases. The problem of human Salmonellosis following consumption of contaminated foods has increased worldwide. Based on reports from 1973 to 1997, cases of Salmonellosis other than typhoid have been reported almost each year. These outbreaks were epidemiologically linked to the consumption of several types of foods including chocolate, egg drink, cuttlefish, mayonnaise, fruit soup, fresh fruits and vegetables, dairy products, and fermented meat products.

Escherichia coli is one of the predominant species of facultative anaerobes in the human gut and usually harmless to the host; however, a group of pathogenic *E. coli* has emerged that cause's diarrheal disease in humans. Referred to as Diarrheagenic *E.coli*. The growing concern about safety of foods has recently led to the development of natural antimicrobials to control food-borne pathogens. Spices are some of the most commonly used natural antimicrobial agents in foods. Addition of spices in foods not only imparts flavor and pungent stimuli but also provides antimicrobial property. Natural antimicrobial compounds in spices were found to possess antimicrobial activity. Although some researchers have studied the antibacterial activity of spices against several species of bacteria, few serotypes of *Salmonella* have been tested, such as *S. typhimurium, S. enteritidis , S. infantis , and S. anatum.*

In developing countries, food materials sold by street vendors are widely consumed by millions of people

These foods provide a source of readily available and affordable source of nutrients to many sectors of the population, including the urban poor. Unpasteurized foods are preferred by the consumers because of the "fresh flavor" attributes and hence, in recent times, their demand has increased. The fruit juices are simply prepared by extracting, usually by mechanical means, the liquid and pulp of mature fruit and vegetables. The final product is an unfermented, clouded, untreated juice, ready for consumption. Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting. Contamination from raw materials and equipments, additional processing conditions, improper handling, prevalence of unhygienic conditions contributes substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables . The Geneva-based International Standards Organization (ISO) defines spices and condiments as: *Vegetable products or mixtures thereof, free from extraneous matter, used for flavoring, seasoning and imparting aroma in foods*.[1,2,3]

METHODOLOGY

Collection of Sample:

Five locations from Gondia city vending open restaurants, fresh fruits and vegetable juices were selected from every location were chosen on the basis of sell and during the rainy season. Based on the consumer demand to road side (Choupati) foods- Panipuri and Bhel, three types of juices namely sugarcane, lime and pineapple as well as meat pieces from mutton market were selected for microbial analysis and isolation of pathogenic strain of *S. typhi* and *E.coli 0157:H7*.

Sample Processing:

1:10 dilution of each sample was prepared in buffered peptone water. Mix the content of diluted tube thoroughly. These dilutions prepared then use for bacteriological analysis of food sample.

Enrichment and Isolation:

The approach recommended for qualitative determination of presence of pathogenic *E.coli 0157:H7* as well as salmonella typhi. For enrichment of *E.coli 0157:H7* aseptically dilute a food sample in brain heart infusion broth (I:10) Blend these suspension briefly incubate the homogenate for 10 min. at room temperature with periodic shaking then allow the sample to settle by gravity for 10 min. Decant medium carefully into a sterile conical flask and incubate for 3 Hrs at 35^0C to resuscitate injured cells. Transfer content to 225ml double strength tryptone phosphate broth in a sterile conical flask and incubate for 24 hrs at 37^0C. After incubation 0.2 ml enriched suspension was spread on Hicrome ECC selective agar plate and incubated 37^0C for 24 to 48 Hrs. After incubation observe for colony characteristics. The colonies which are appeared in pinkish red color indicating the *E.coli 0157:H7* on Hicrome ECC selective agar. *E.coli* giving blue color colonies with dark blue centre *E-coli 0157:H7* giving pinkish red color colonies. The colonies which are small and pink red color were selected as *E-coli (E-coli 0157:H7).* For enrichment and selective isolation of *S. typhi* initially 1:10 dilution of sample was prepared in sterile buffered peptone water, and mix thoroughly and homogenate for 10 min. at room temp. Then allow this suspension to settle by gravity for 10 min. Decant medium carefully into sterile conical flask and incubate for 3 Hrs at 37^0C to resituated injured cell. Transfer content to same volume of double strength tetra-thionote broth and incubate for 24 Hrs at 37^0C after incubation 0.2 ml enrich suspension spreaded and distributed on the surface of sterile BSA agar plate incubate this agar plate for 24 Hrs at 37^0C. After incubation plates were observe for colony characteristics. Black colored colonies indicating *S. typhi.* A single desired colony from each media plate selected and sub cultured on surface of sterile Nutrient agar slope and incubated at 37^0C for 24 Hrs.

Antibiotic Susceptibility testing of clinical isolates:

The *bacterial* isolates were subjected to analysis for susceptibility or resistance towards different antibiotics. The used were*, Penicillin, Ampicillin, Cotrimaxazole, Kanamycin, Streptomycin, Amoxicillin, Erythromycin Polymyxin-B, Nalidixic acid, Chloramphenicol, Gentamycin, Tobramycin, Imipenem and Ciprofloxacin.* The method used for determining antibiotic susceptibility of isolates was Kirby Bauer Disc diffusion method.

Muller Hinton agar medium was prepared using commercially available dehydrated media. According to manufactures instruction media was sterilized at 121^0 c for 20 minutes. After autoclaving media was cool to 45-50 ^{0}C.Freshly prepared sterilized molten cooled medium was poured into sterile Petri dishes to give uniform agar layers. Agar medium was allowed to solidity. A loop full pure culture from slant was selected and transfer into a tube containing 5ml of nutrient broth medium. Inoculated broth was incubated at 37^0C for 6- 8 hours.0.1 ml of broth culture of test bacterium (isolates) was inoculated on the surface of Muller Hinton agar. The broth culture was uniformly distributed on agar surface using sterile glass spreader. The lead of the plate was left for 3-5 min in ascetic zone. To allow for any excess surface moisture to be observed before applying the antibiotic disc.

In an ascetic zone surface of each inoculated is placed by different antibiotic disc at equidistance places. All plates were incubated at 37^0C for hours after incubated the plates were observed for diameter of zone of inhibition. The size of the zone of inhibition was interpreted by referring zone diameter interpretive standards of the NCCLS/M 100, S12 performance standard for antimicrobial susceptibility testing.

Multiple antibiotic resistance indexes*:*

MAR index of each isolates was calculated as recommended by Krumpreman

No of antibiotic to which test isolates

MAR index of isolates = displayed resistance / No of antibiotic used in studies

Collection of Spices:

4 types of spices including Cinnamon, Clove, Coriander and Funnel were purchased from local market of Gondia city. The spices were extracted using organic solvents like methanol, ethanol, ethyl acetate and steam distillation using water as a solvent.

Extraction of Spices

1. *Preparation of organic and aqueous extract:*

a. Organic extract: To obtain the solvent extracts, dried and finely powdered spices were weighed about 30 grams each and homogenized using 150ml of 70% organic solvent. They were added Soxhlet apparatus set up at boiling point of solvent. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solvent loses its color. The extract was then transferred to a sterile Petri dish and kept for evaporation of solvent at room temperature. Residues of extracts were collected and stored in the refrigerator.

b. Aqueous extract: To obtain the aqueous extracts, dried and finely powdered spices *were* weighed about 30 grams each and homogenized using 150ml of water. They were added to Soxhlet apparatus and the boiling point of water was set up at 100ºC. The water evaporates continuously and was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solution looses the color.

2. *Preparation of essential Oil:*

A small piece of spice material (30g) added in 100 ml of sterile distilled water. After steam distillation 100% pure essential oil was collected dispense into dark bottles and stored at 4^0C until used. Stock solution of organic extract and essential oils were used for well diffusion test and determination of MIC values.

Screening of Spices extract using well diffusion method:

Well diffusion method was performed using standard procedure. The inoculums suspension (06 Hrs broth culture) suspension of each bacterial strain was swabbed on entire surface of Muller Hinton agar using sterile 6mm gel borer equidistant wells were prepared on inoculated MH agar plate. These wells were labeled properly. Each well is then filled with 100 µl of each extract. Four wells were considered as negative control which was filled with 100µl of methanol, Ethanol, Ethyl acetate and sterile distilled water respectively. The plates are placed in freeze for 15 min to allow excess perfusion of extract. Then plates were incubated further at 37^0C for 24 Hrs. Diameter of inhibition zone were measured and activity index is calculated.

Antimicrobial potential of essential oils using well diffusion method:

Well diffusion method was performed using standard procedure. The inoculums suspension (06 Hrs broth culture) suspension of each bacterial strain was swabbed on entire surface of Muller Hinton agar using sterile 6mm gel borer equidistant wells were prepared on inoculated MH agar plate. These wells were labeled properly. Each well is then filled with 100 µl of oil of each spices.

Four wells were considered as negative control which was filled with 100µl of sterile distilled water. The plates are placed in freeze for 15 min to allow excess perfusion of extract. Then plates were incubated further at 37^0C for 24 Hrs. Diameter of inhibition zone were measured and activity index is calculated.

Determination of MIC of effective extract and oil using dilution test:

The dilution test was performed to determine minimum inhibitory concentration (MIC) using Standard procedure as described by *Jorgenson et.al. 1999*. 100 µl of 6 hours broth culture of test organisms (E5, E6 and E7) was added in each of 3 sets of 11 tubes each containing 10ml nutrient broth. These tubes were labeled as negative control, 2.0 µg/ml, 4µg/ml, 6 µg/ml, 8µg/ml-------20µg/ml. These labeled tubes then were added

with 0µl, 20µl, 40µl --------200µl of ethanolic extract of clove (conc. 1mg/ml) respectively in a tube. Mix the content thoroughly. These tubes were incubated at 37^0C for 2 hrs and turbidity was measured at 600 nm. The lowest concentration were inhibited visible growth of tested organism was recorded as MIC. Repeat the same procedure for clove methanolic extract, Cinnamon ethanolic extract and cinnamon methanolic extract. Same procedure was carried out for determination of MIC for *S. typhi* isolates (St1, St3 and St6).(MIC) of clove and funnel oil was determined using Standard procedure. 100 µl of 6 hours broth culture of test organisms (E5, E6 and E7) was added in each of 3 sets of 11 tubes each containing 10ml nutrient broth. These tubes were labeled as negative control, 2.0 µl/ml, 4µl/ml, 6 µl/ml, 8µl/ml-----20µl/ml. These labeled tubes then were added with 0µl, 20µl, 40µl -------200µl of clove oil respectively in a tube. Mix the content thoroughly. These tubes were incubated at 37^0C for 2 hrs and turbidity was measured at 600 nm. The lowest concentration were inhibited visible growth of tested organism was recorded as MIC. Repeat the same procedure for Funnel oil. Same procedure was carried out for determination of MIC for *S. typhi* isolates (St1, St3 and St6).

RESULTS AND DISCUSSION

In spite of the potential benefits offered by fruit juices and panipuri as well vitamins offered by mean pieces, concerns over there safety and quality have been raised. During preparation of fruit juices as well Choupati food material little or no process steps that reduce pathogens level were implemented which resulting in emergence of food born diseases in human being (Ref. Vicroria Government Department of human services 2005)

In the present investigation fresh juices of sugarcane, lime and pineapple, Choupati food like Panipuri and Bhel as well mead pieces were subjected to bacteriological analysis. These samples showed occurrence of pathogens, *E.coli* and *Salmonella typhi.*

All bacterial isolates were identified on the basis of morphological, cultural and biochemical characteristics.

Antibiotic resistance is a common phenomenon shows among food borne pathogens. All clinical isolates of *E.coli157:H7* were tested for their susceptibility to 9 different antibiotics which includes- Gentamycin (10 mcg), Streptomycin (10 mcg), Tobromycin (10mcg), Imipenem (10mcg), Ciprofloxacin (5mcg), Penicillin (100 mcg), Amoxicillin (10mcg) Erythromycin (15 mcg) and Co-trimaxazole. In present study different antibiotic selected against *Salmonella typhi* isolates were Penicillin (10mcg), Amoxicillin (10mcg), Ampicillin (10mcg), Co-trimaxozole (10mcg), Kanamycin (10mcg), Streptomycin (10mcg), Erythromycin (15mcg), Nalidixic acid (10mcg), Polymyxin-B(300units) and Chloramphenicol(10mcg).

In present study clinical isolates were reported to exhibit multiple drug resistance.

Figure 1 MAR indices of *E.coli O157:H7* isolates:

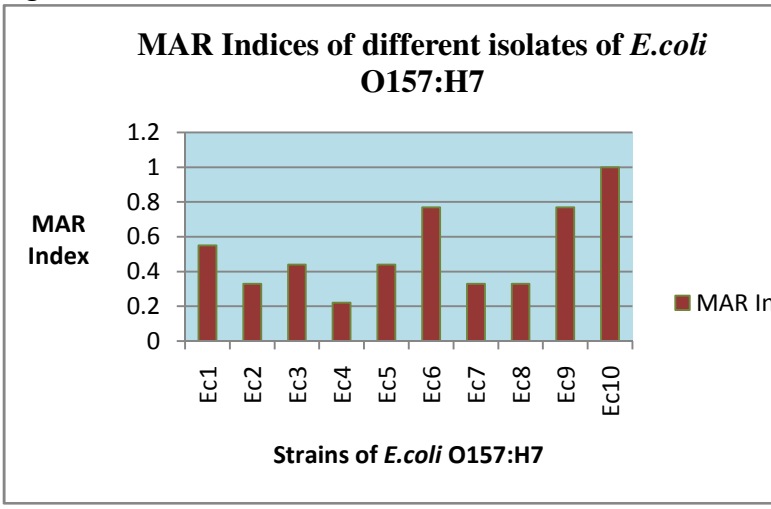
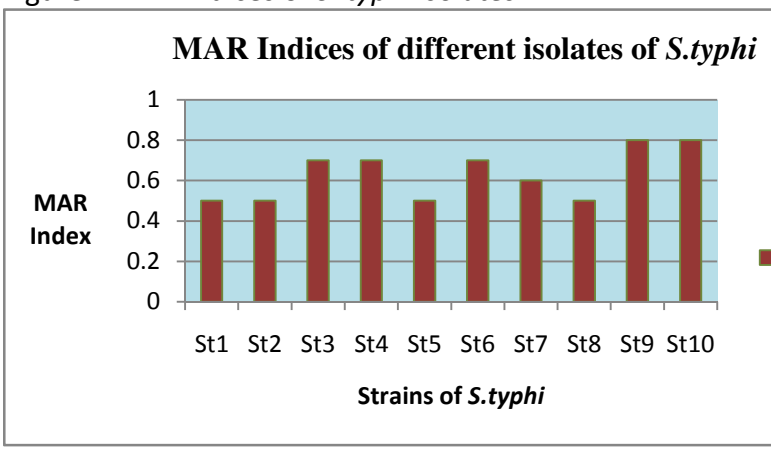


Figure 2 MAR indices of *S. typhi isolates*:



Generally pathogens like *E.coli 0157:H7* causing diarrhea in human beings. These isolates reported to exhibit multiple drug resistance. 50% isolates of *E.coli* found to be resistant to gentamycin 40% *E.coli* isolates found to be resistant to streptomycin and Tobromycin. While 80% isolates of *E.coli* were resistant to Imipenem and Erythromycin, 10% strains were resistant to Co-trimaxozole while 100% strain was resistant to Amoxicillin and Piperacillin. 10% clinical isolates of salmonella resistant to co-trimaxazole 30% isolates of *S. typhi* were resistant to Polymyxin –B 40% isolates were resistant to Chloramphenicol, 70% isolates resistance to Kanamycin, 90% isolates resistant to Amoxicillin and Nalidixic acid while 100% isolates of *S. typhi* showing resistant to penicillin ampicillin and Erythromycin while streptomycin and Cotrimaxazole were most effective antibiotics against all isolates of *Salmonella spp.*MAR indices of clinical isolates were calculated by dividing number of antibiotic to which test isolates displayed resistance divided by total number of antibiotics used in sensitivity test.

Multiple antibiotics resistance (MAR) Index is a tool that revels the spread of antibiotic resistance in given bacterial population .[15]

MAR index greater than 0.2 implies that strain of bacterial originate from environment where several antibiotics are used. MAR indices of different clinical isolates are given in *table 5.9 and 5.10.* These MAR indices of different clinical isolates were multi drug resistant and possibly pre-exposed to several antibiotics. *Farzana K. et al (2009)* giving the same result they reported E.coli isolated from food environment exhibiting 100% resistant to Ampicillin.

The growing concern about food safety has recently lead to the development of natural antibacterial to control food born pathogen spices are one of the most commonly used natural antimicrobial agents in food and have been used traditionally for preserving foods and as food additives to enhance aroma and flavor [21].

Antibacterial potential of different extracts of different species were tested against bacterial isolates of *E.coli 0157:H7* and *Salmonella typhi* by Kirby Bauer well diffusion method. The result of these well diffusion test indicated that different extracts of clove, funnel, coriander and cinnamon showed different degree of growth inhibition depending of bacterial strain.

Results were summarized in Table 1,2,3,4,5,6, and 7. The ethanolic extract of clove showed highest activity among all the tested species extracts. This ethanolic clove extract have great activity against *E.coli 0157:H7* as well *Salmonella typhi* isolates. The diameter of inhibition zone ranging from 27 to 45 mm for *E.coli 0157:H7* while 15mm to 30mm for *S. typhi* ethanolic extract of Coriander, Cinnamon and Funnel inhibited growth of all tested strains of *E.coli 0157:H7* with zone of inhibition from 7 to 12 mm. But methanolic and ethyl acetate extracts were ineffective against *S. typhi.*

Aqueous extract of all selected species were ineffective against *E.coli 0157:H7* strain as well as *S. typhi* strains. Oils of clove were effective against all tested strains (Diameter of zone of inhibition (8 to 21). Oil of funnel was moderately effective against tested pathogens. Oils of Cinnamon and Coriander were not extracted because of very small amount of oil in plant material. In general inhibitory activity of essential oil that of ethanolic extracts, In case of *E.coli 0157:H7* isolates while among the *S. typhi* isolates tested ethanolic extract as well as oil exhibited same degree of inhibition. Against *S. typhi* isolates methanolic extract of clove was also effective.

Table 1Antimicrobial activity of Clove against *E.coli O157:H7:*

Sr. No	Strain	Inhibition zone (mm)					Activity index			
		Ciprofloxacin	EE	ME	EaE	AE	EE	ME	EaE	AE
1	Ec1	22	25	27	26	00	1.13	1.22	1.18	00
2	Ec2	22	40	30	20	00	1.81	1.36	0.90	00

3	Ec3	25	28	27	20	00	1.12	1.08	0.80	00
4	Ec4	18	23	21	25	00	1.27	1.16	1.38	00
5	Ec5	22	32	32	20	00	1.45	1.45	0.90	00
6	Ec6	24	27	36	23	00	1.12	1.50	0.95	00
7	Ec7	32	38	33	35	00	1.18	1.03	1.09	00
8	Ec8	21	26	31	21	00	1.23	1.47	1.00	00
9	Ec9	26	45	28	24	00	1.73	1.07	0.92	00
10	Ec10	08	25	26	20	00	3.25	3.12	2.50	00

Table 2 Antimicrobial activity of Clove against *S. typhi*

Sr. No	Strain	Inhibition zone (mm)					Activity index			
		Cotrimaxazole	EE	ME	EaE	AE	EE	ME	EaE	AE
1	St1	23	21	24	18	00	0.91	1.04	0.78	00
2	St2	17	16	18	15	00	0.94	1.05	0.88	00
3	St3	18	27	22	24	00	1.20	1.50	1.33	00
4	St4	14	24	19	12	00	1.30	0.83	0.85	00
5	St5	20	21	13	24	00	0.65	0.54	1.20	00
6	St6	20	28	27	19	00	1.35	0.55	0.95	00
7	St7	20	23	24	20	00	1.20	0.00	1.00	00
8	St8	23	30	22	16	00	0.95	0.00	0.69	00
9	St9	17	15	18	30	00	1.05	0.80	1.76	00
10	St10	18	18	20	16	00	1.10	0.84	0.88	00

$$\text{Activity index} = \frac{\text{Inhibition zone by extract}}{\text{Inhibition zone by std. Antibiotic}}$$

Table 3Antimicrobial activity of *Coriandrum* against *E.coli O157:H7*

Sr. No	Strain	Inhibition zone (mm)					Activity index			
		Ciprofloxacin	EE	ME	EaE	AE	EE	ME	EaE	AE
1	Ec1	22	14	16	00	00	0.63	0.72	0.00	0.00
2	Ec2	22	15	16	00	00	0.68	0.72	0.00	0.00
3	Ec3	25	00	00	00	00	0.00	0.00	0.00	0.00
4	Ec4	18	24	20	22	00	1.33	1.11	1.20	0.00
5	Ec5	22	15	23	00	00	0.68	1.04	0.00	0.00
6	Ec6	24	00	15	17	00	0.00	0.62	0.70	0.00
7	Ec7	32	21	22	22	00	0.65	0.68	0.68	0.00
8	Ec8	21	15	19	20	00	0.71	0.90	0.95	0.00
9	Ec9	26	23	23	21	00	0.88	0.88	0.80	0.00
10	Ec10	08	25	27	15	00	0.00	0.00	0.00	0.00

Table 4 Antimicrobial activity of *Coriandrum* against *S. typhi*

Sr. No	Strain	Inhibition zone (mm)					Activity index			
		Cotrimaxazole	EE	ME	EaE	AE	EE	ME	EaE	AE
1	St1	23	30	30	26	00	1.30	1.30	1.13	0.00
2	St2	17	09	11	12	00	0.52	0.64	0.70	0.00
3	St3	18	20	10	11	00	1.11	0.55	0.60	0.00
4	St4	14	22	10	14	00	1.50	0.71	1.00	0.00
5	St5	20	10	09	12	00	0.50	0.45	0.60	0.00
6	St6	20	10	00	00	00	0.50	0.00	0.00	0.00
7	St7	20	11	13	14	00	0.55	0.65	0.70	0.00
8	St8	23	11	10	00	00	0.47	0.43	0.00	0.00
9	St9	17	00	10	09	00	0.00	0.58	0.52	0.00
10	St10	18	09	10	10	00	0.50	0.55	0.55	0.00

Activity index= Inhibition zone by extract / Inhibition zone by std. Antibiotic

Table 5 Antimicrobial activity of Cinnamom against *E.coli O157:H7:*

Sr. No	Strain	Inhibition zone (mm)					Activity index			
		Ciprofloxacin	EE	ME	EaE	AE	EE	ME	EaE	AE
1	Ec1	22	25	27	26	00	1.13	1.22	1.18	00
2	Ec2	22	40	30	20	00	1.81	1.36	0.90	00
3	Ec3	25	28	27	20	00	1.12	1.08	0.80	00
4	Ec4	18	23	21	25	00	1.27	1.16	1.38	00
5	Ec5	22	32	32	20	00	1.45	1.45	0.90	00
6	Ec6	24	27	36	23	00	1.12	1.50	0.95	00
7	Ec7	32	38	33	35	00	1.18	1.03	1.09	00
8	Ec8	21	26	31	21	00	1.23	1.47	1.00	00
9	Ec9	26	45	28	24	00	1.73	1.07	0.92	00
10	Ec10	08	25	26	20	00	3.25	3.12	2.50	00

Table 6 Antimicrobial activity of Cinnamom against *S. typhi*

Sr. No	Strain	Inhibition zone (mm)					Activity index			
		Cotrimaxazole	EE	ME	EaE	AE	EE	ME	EaE	AE
1	St1	23	21	24	18	00	0.91	1.04	0.78	00
2	St2	17	16	18	15	00	0.94	1.05	0.88	00
3	St3	18	27	22	24	00	1.20	1.50	1.33	00
4	St4	14	24	19	12	00	1.30	0.83	0.85	00
5	St5	20	21	13	24	00	0.65	0.54	1.20	00
6	St6	20	28	27	19	00	1.35	0.55	0.95	00
7	St7	20	23	24	20	00	1.20	0.00	1.00	00
8	St8	23	30	22	16	00	0.95	0.00	0.69	00
9	St9	17	15	18	30	00	1.05	0.80	1.76	00
10	St10	18	18	20	16	00	1.10	0.84	0.88	00

Table 7 Antimicrobial activity *of funnel* against *E.coli O157:H7:*

Sr. No	Strain	Inhibition zone (mm)					Activity index			
		Ciprofloxacin	EE	ME	EaE	AE	EE	ME	EaE	AE
1	Ec1	22	08	21	14	00	0.36	0.95	0.63	00
2	Ec2	22	09	21	00	00	0.40	0.95	0.00	00
3	Ec3	25	12	29	00	00	0.48	1.16	0.00	00
4	Ec4	18	09	10	00	00	0.50	0.50	0.00	00
5	Ec5	22	11	12	00	00	0.50	0.54	0.00	00
6	Ec6	24	11	20	23	00	0.45	0.83	0.95	00
7	Ec7	32	15	22	16	00	0.46	0.68	0.50	00
8	Ec8	21	13	13	14	00	0.61	0.61	0.66	00
9	Ec9	26	07	18	18	00	0.20	0.69	0.69	00
10	Ec10	08	15	12	12	00	0.00	0.00	0.00	00

Table 8 Antimicrobial activity *of funnel* against *S. typhi*

Sr. No	Strain	Inhibition zone (mm)					Activity index			
		Cotrimaxazole	EE	ME	EaE	AE	EE	ME	EaE	AE
1	St1	23	23	19	19	00	1.00	0.82	0.82	00
2	St2	17	13	11	14	00	0.76	0.64	0.82	00
3	St3	18	00	00	11	00	0.00	0.00	0.61	00
4	St4	14	17	08	13	00	1.20	0.57	0.76	00
5	St5	20	15	15	08	00	0.75	0.75	0.40	00
6	St6	20	09	13	15	00	0.45	0.65	0.75	00
7	St7	20	20	12	14	00	1.00	0.60	0.70	00
8	St8	23	00	20	17	00	0.00	0.86	0.73	00
9	St9	17	08	22	20	00	0.29	1.29	1.17	00
10	St10	18	08	13	08	00	0.41	0.72	0.40	00

Against *Salmonella typhi* as well as *E.coli 0157:H7* isolates with range of zone of inhibition 19mm to 24 mm and 21 mm to 36 mm for salmonella and *E.coli* respectively. Methanolic extract of other species are also moderately effective against *S. typhi* as well as *E.coli 0157:H7* isolates. Ethyl acetate extract of each species is also reported to show moderate activity against both classes of bacteria under study. The extract of Cinnamon, Coriander showing inhibitory activity against tested bacteria due to presence of potent antimicrobial compound. The major constituent are 1,8 cineole (20 to 60%) and α-terpinyle acetate (20 to 53%) in Cinnamon, linalol (74%) and other components (small amount of a-pinene, y-terpinie, geranyle-acetate camphor and geranole) in coriander[1]. Garlic extracts posse's antibacterial activity against *S.typhi* as well as *E.coli.* The major antibacterial compound in garlic Allicin [2,7,14.]

The major pangene component of Ginger is gingeron and gingerol which have strong inhibitory activity against pathogenic bacteria [12].The antibacterial activity of spices may differ between strains within the same species moreover, the antimicrobial properties of species may differ depending on the no. of form of species added, such as fresh dried, or extracted forms (*Nanasombat, S. 2005*) and also differ depending on the harvesting season [3].

However there is evidence that essential oils of species are more strongly antibacterial than is accounted for by the additive effect of their major antimicrobial component (*Postar N et al 1995 and Lattoui N. 1994).*Clove and Cinnamon have been used in food silver ancient time major antimicrobial

component in clove and cinnamon have been reported to be Eugenol and cinnamaldehyde respectively. *Bullarmon 1997,* which have been given special attention to find their antibacterial activity against food borne pathogens. Eugenol has been reported to inhibit growth of *E.coli 0157:H7.* [4] Cinnamaldehyde by has been reported to inhibit growth of *E.coli* and *S. typhi*[10].Aqueous extract were unable to inhibit growth of *E.coli 0157:H7*[18].

3 Strain of *E.coli 0157:H7* (E5, E6 and E7) and 3of *Salmonella typhi* (St 1, St3 and St6) was selected as test organism for MIC determination of spices extracts and oil. The MIC value of effective extract (ethanol and methanolic) of Clove and Cinnamon indicated that clove had highest antibacterial action against both bacterial strains tested (Table 5.15, Table 5.16, Table 5.17 Table 5.18, Table 5.19 and Table 5.20).

MIC values of oils of funnel as well as Clove also tested against both bacterial strains. Oils of clove strongly inhibit growth of both strains. The oil of clove had the lowest MIC (4.2 µg/ml) to inhibit growth of every strain tested. The MIC value of ethanolic extract of clove against *E.coli 0157:H7* strain was 6µg/ml; MIC for *S. typhi* isolates 5.0µg /ml, MIC value of methanolic extract against *E.coli 0157:H7* strain was 9µg /ml MIC for *S. typhi* isolates 8µg/ml. The oil of funnel had the lowest MIC (4.5 µg/ml) to inhibit growth of every strain tested. The MIC value of ethanolic extract of cinnamon against *E.coli 0157:H7* strain was 8µg/ml, MIC for *S. typhi* isolates 7.0µg /ml, MIC value of methanolic extract of cinnamon against *E.coli 0157:H7* strain was 11µg /ml MIC for *S. typhi* isolates 10 µg/ml. In this study different extract and essential oil of seven was screen for their antibacterial properties. The degree of antibacterial activity was considered from MIC values against the bacterial strain. The result of present study indicated that clove exhibited the strongest antibacterial activity in all forms of extract followed by Cinnamon Coriander and Funnel. Eugenol having wide spectra of antibacterial activity[14] similar finding was reported by *Suksringam B (1975)* and *Tarhad R.S. et al (1989)*.

CONCLUSION

Among food borne bacteria antibiotic resistance is a most common phenomenon. This leading to immergence of multiple antibiotic resistant bacteria due to horizontal spread of resistance. This problem if ignored leading to immergence of food borne diseases in human population habitual to eat ready to eat food material from local venders. So this study carries out for determining presence of pathogenic. Diarrheagenic *E. coli 1057:H7* strain as well strains of *S. typhi* in food sample from local venders. Choupati Food material showing occurrence of multiple antibiotic resistant strains of *E. coli 0157:H7* as well Salmonella typhi.

Traditionally various species like clove, coriander, cardamom, funnel, cinnamon, garlic, black pepper, lemon grass is used. These species having antibacterial properties. The result obtained in our study indicates that clove bud extract as well as oil most effective against both kind of pathogenic organism (*E.coli 0157:H7, S. typhi*). These results provide an effective mean of inhibiting the growth of tested pathogenic bacteria (*E.coli 0157:H7, S. typhi)*

The highest inhibitory effect of clove bud extract is found in case of ethanolic and methanolic extract. The study indicate that clove bud extracts and clove bud oil can be used as antibacterial, antifungal and antiseptic agent so that they can be used as a food preservative hence, improve shelf life of foods. The tested organism are considered as important food borne pathogens, raising the possibility of using clove bud oil to prevent food borne diseases. However the application of this oil in food industry will require safety and toxicity issues to be addressed.

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