

Antibacterial effect of kitchen herbs against pathogenic Multi Drug Resistant E. coli isolates from Calf Diarrhoea

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Abstract

Calf diarrhoea remains the biggest challenge both in the small and large farms. Infectious diarrhoea is associated with many pathogens, *E. coli* being one but, majority are systematically treated with antibiotics. Since antimicrobial resistance (AMR) is a growing menace, the need to find alternative prophylactic solutions using popular kitchen herbs such as *Trachyspermum ammi* (Carom seeds), *Curcuma Longa* (Turmeric) and Cinnamon (*Cinnamomum sp.*) extracts is been investigated against virulent form of *E. coli* isolated from calf diarrhoea. The virulence factors identified in these isolates were ST (32.5%), LT (20%), *eaeA* (15%), *stx1* (2.5%) and *stx2* (5%) with the occurrence of the most common serogroups as O18 (15%) followed by O111 (12.5%). Highest resistance was seen with β lactamase inhibitor (Amoxicillin/Clavulanic acid) followed by β lactams (Ampicillin, Cefuroxime and Cefepime). Antibacterial effect of cinnamon (methanol) and carom seed (ethanol) extracts was found to be highly sensitive at higher concentration of the extract (500 to 250 $\mu\text{g/ml}$) while, minimum inhibitory concentration (MIC) of turmeric was significantly higher ($P < 0.05$) followed by cinnamon. Turmeric, cinnamon and carom had the potency of inhibiting the pathogenic *E. coli* which maybe suggestive of its use in calf diets as prophylaxis against diarrhoea.

Introduction

Escherichia coli are Gram negative bacteria which easily colonize the gastrointestinal tract that even derive benefit from the host for decades (Foster and Smith, 2009). Many strains are harmless but, certain strains acquiring virulence genes can cause moderate to severe scour, sometimes even death (Wani et al. 2004). Some diarrheagenic *E. coli* pathotypes reportedly are Enterotoxigenic *E. coli* (ETEC) (Foster and Smith, 2009), attaching and effacing *E. coli* (AEEC), Shiga toxin producing *E. coli* (STEC) etc. ETEC produces ST and LT enterotoxins causing rapid onset of secretory diarrhea and dehydration (Kolenda et al. 2015) in young animals. Studies have indicated that these respective strains possessed *stx*, *stx*₂, *hlyA*, and *eaeA* gene determinants that have the potential to cause infections, diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) in humans (Farrokh et al. 2013). Antibiotics such as oxytetracycline, chlortetracycline, sulfamethazine, ampicillin, furazolidone, neomycin, and streptomycin have been used to treat calf diarrhoea (Constable, 2004) but the problem of resistance is of growing concern (WHO, 2014). We are running out of newer groups of antibiotics and use of herbal medicines is the only alternative. Though sufficient study on microbial resistance to herbal drugs are lacking, it is often stated that bacteria cannot develop resistance to herbal medicines (Singh, 2013). Herbs used in our kitchen are blessed with a diverse array of secondary metabolites such as phenols, terpenoids, flavonoids, tannins, lectins polypeptides etc. (Das, 2013). These biomolecules are not only known for its antimicrobial activity (Srivastava et al. 2010) but also possess good antioxidant property (Lee et al. 2010). When used as feed additives in animals, beneficial effect on feeding patterns as well as microorganisms in the rumen, secretion of various digestive enzymes, utilization of nutrients and overall performance have been reported (Bakshi et al. 2004). Carom seed (*Trachyspermum ammi*) is a traditional herb widely used for flatulence, atonic dyspepsia and diarrhea). The seeds have 50% thymol which has the ability to kill bacteria resistant to third generation antibiotics and MDR (Multi Drug Resistant) pathogens (Bentley and Trimen, 1999). The rhizome *Curcuma longa*, (Turmeric) has an important natural component called Curcumin which has been known to possess *in vitro* anti-microbial properties against several microorganisms (Lüer et al. 2012, Mun et al. 2013). Confirmed membrane leakage in Gram-negative and Gram-positive bacteria on exposure to curcumin I by membrane permeabilization assays have been reported (Tyagi et al. 2015). Among all the essential oil, cinnamon has been found possess the most effective antimicrobial properties (Aggarwal et al. 2000). Cinnamaldehyde in cinnamon (*Cinnamomum sp.*) are known to interfere with proteins and nucleic acids (Fadli et al. 2012) thereby, inhibiting cell division, ATPase, biofilm formation altering the lipid profile (Vasconcelos and Croda, 2018) and causing auto aggregation (Clemente et al. 2016).

Therefore, the study is made to evaluate the antibacterial effect of *Trachyspermum ammi* (Carom seeds), *Curcuma Longa* (Turmeric) and Cinnamon (*Cinnamomum sp.*) extract against virulent form of Multi Drug Resistant (MDR) *E. coli* isolates from calf diarrhoea.

Materials And Methods

Sample collection

The work was carried at Indian Council of Agricultural Research – National Dairy Research Institute, Eastern Regional Station, Kalyani, West Bengal, India. Diarrhoea samples were collected from neonatal calves (38 no.) per rectally using sterile gloves, stored in an ice box and delivered to the laboratory for immediate processing within 2 h. within one day to three months of age.

Isolation and identification of E coli

The sample was then cultured in MacConkey's Agar (Himedia Lab. Pvt. Ltd.) at 37°C for 24 hrs. The colonies were further streaked on EMB Agar (Himedia, Lab. Pvt. Ltd.) at 37°C for 24 hrs. Metallic sheen colonies were picked up for *E coli* identification using biochemical test (Indole, Citrate utilization, Methyl Red and Voges Proskauer, Grams staining) (Buchanan and Gibbon, 1994). Confirmation was done by PCR amplification using specific primers. Serotyping of the somatic antigen (O) for the *E coli* isolates was carried at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli (HP) – 173204, India.

Antibiotic susceptibility testing

The isolates were tested against 12 antimicrobial agents by the Kirby Bauer disc diffusion method and break even points as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2006). The antimicrobial agents tested were Cefuroxime (30 mcg, CXM), Ceftazidime (30 mcg, CAZ), Ceftazime + Clavulanic Acid (30/10 mcg, CAC), Cefepime (30 mcg, CPM), Gentamicin (10mcg, GEN), Amikacin (30 mcg, AK), Ciprofloxacin (5 mcg, CIP), Ofloxacin (5 mcg, OF), Co-Trimoxazole (25 mcg, COT), Amoxicillin/Clavulanic Acid (30 mcg, AMC) and Imipenem (10 mcg, IPM). Mueller Hilton Agar was poured in sterile petri plates and the inoculum was seeded at 0.5 McFarland. Then the antibiotic disc was placed on the surface of the agar plate and incubated at 37° for 18 hr after which the zone of inhibition was measured (mm). All interpretation was performed according to guidelines. Isolates with intermediate resistance was considered as resistant.

Detection of virulent genes in the E coli isolates

DNA was extracted by boiling method (Wani et al. 2004). Detection of virulent genes, *stx*₁, *stx*₂, *hlyA*, *eaeA*, *st* and *lt* was done by PCR. Primer sequence used to amplify specific product is given in Table 1.

Table1: Primer sequences and predicted lengths of PCR amplification products

No.	Primer		Sequence	Size	Ref.
1	E coli	F	TGACGTTACCCGCAGAAGAA	832	25
		R	CTCCAATCCGGACTACGAC		
2	ST	F	AGGAACGTACATCATTGCCC	521	26
		R	CAAAGCATGCTCCAGCACTA		
3	LT	F	GCACACGGAGCTCCTCAGTC	218	26
		R	TCCTTAATCCTTTCAATGGCTTT		
4	stx 1	F	AACTGGATGATCTCAGTGG	614	27
		R	CTGAATCCCCCTCCATTATG		
5	stx 2	F	CCATGACAACGGACAGCAGTT	779	27
		R	CCTGTCAACTGAGCAGCACTTTG		
6	EHEChly	F	ACGATGTGGTTTATTCTGGA	165	28
		R	CTTCACGTGACCATACATAT		
7	eaeA	F	GTGGCGAATACTGGCGAGACT	890	29
		R	CCCCATTCTTTTTCACCGTCG		

The PCR assay were carried out in 25µl reaction volume containing 1U Taq Polymerase, 200µM dNTP, 0.5 µM primer concentration, 2.5µl of 10xPCR buffer MgCl₂ concentration of 1.5mM. The positive control was taken from West Bengal University of Animal and Fishery Sciences, Veterinary Microbiology Division, Belgachia, Kolkata, India and sterile distilled water was used as a negative control. The PCR cycling condition was as follows: initial denaturation at 95 °C for 1 m followed by 30 cycles of denaturation at 95 °C for 15 s, annealing at 58 °C for 40 s, extension at 72 °C for 10 s, and final extension at 72 °C for 10 m. Amplified PCR products were resolved in 1.0% agarose gel containing ethidium bromide.

Collection of spices, drying and extraction

Spices such as turmeric carom seeds and cinnamon were dried and grounded to fine powder for extraction in distilled water, ethanol (60% v/v) and methanol (100%) at 1:10 ratio. The solvent was evaporated at 40°C to 45°C under reduced pressure. The dried extract was calculated by the following formula: R/S x100 (where R: weight of extracted plant residue and S: weight of plant raw sample). Plant extracts were dissolved in DMSO at a concentration of 100mg/mL and filtered through syringe filters before use. All extracts were assessed for its pH (Himedia®) (Nagappan, 2012).

Determination of Total Phenolic Content

Total phenolics of the extracts were determined using Folin and Ciocalteu reagent (Sigma®) as described by (Singleton and Rossi, 1965) with slight modifications mentioned (Chandra et al. 2014). Readings of the sample and standards were made using a UV Spectrophotometer (Labman®) at 765 nm wavelength. The phenolic content was measured as gallic acid equivalents (GAE/g) of dry plant material on the basis of standard curve of Gallic acid (5-500mg/L, Y=0.0027x-0.0055, R²=0.9999). All estimations were in duplicates.

Determination of Total Flavonoid Content

Aluminium chloride colorimetric method was used for total flavonoid content as described (Marinova *et al.*, 2005). The absorbance was measured at 420nm wavelength with the spectrophotometer. The concentration of the flavonoid content in

the sample was calculated from the calibration plots ($Y=0.0162x + 0.0044$, $R^2=0.999$) and expressed as mg quercetin equivalent/g of spice powder. All estimations were in duplicates.

Determination of antioxidant activity (2, 2- Diphenyl –l- picrylhydrazyl (DPPH) Assay)

The extracts were measured at a concentration of 500µg/mL as mentioned (by the method described by (Cheng et al. 2006). The absorbance was measured at 515nm. Gallic acid is used a positive control. Percentage of DPPH radical scavenging activity was calculated as follows:

$$\% \text{ Radical scavenging activity} = \left[1 - \frac{(\text{Sample} - \text{blank})}{(\text{Control} - \text{blank})} \right] \times 100$$

Antimicrobial and minimum inhibitory concentration (MIC) assay

Microorganisms used in the study are virulent *E coli* isolates from calf diarrhoea. Positive control is procured from West Bengal University of Animal and Fishery Sciences, Microbiology Department, Belgachia- Kolkata, WB, India. The antimicrobial activity of Cinnamon, Carom seeds and Turmeric were determined by agar well diffusion method at different concentrations. Microplate method is used to determine MIC of the herb extract against bacterial species with virulence factors causing calf diarrhoea. Bacterial cultures used for both the assay are grown overnight and adjusted to McFarland standard 0.5, equivalent to 10⁸cfu/ml. The extracts are dissolved in DMSO to a concentration of 10mg/ml and 100ul is added to the first well of the microtitre plate and serially diluted with water. Bacterial cultures of 100ul are added to each well. Gentamicin is used as a positive control and DMSO is solvent control. As an indicator growth, 40µl of 0.2mg/ml p-iodonitrotetrazolium violet (INT) (Sigma) is added to the microplate wells and incubated at 37° for 2 h. This dye is an electron acceptor and is reduced by the viable microorganism to a coloured product. Therefore the yellow tetrazolium dye is reduced by viable microorganisms to a pink/purple colour (Elisha et al. 2017).

Statistical Analysis

Statistical analysis was carried out by one way ANOVA using SPSS (Ver.20) and the significance was determined at p<0.05 level.

Results

Antimicrobial resistance by the disc diffusion method is presented in Table 2 against antimicrobials such as Ampicillin 10 mcg (AMP), Cefuroxime 30 mcg (CXM), Ceftazidine 30 mcg (CAZ), Ceftazine + Clavulinic Acid 30/10 mcg (CAC), Cefepime 30 mcg (CPM), Gentamicin 10mcg (GEN), Amikacin 30mcg (AK), Ciprofloxacin 5mcg (CIP), Ofloxacin 5mcg (OF), Co-Trimoxazole 25mcg (COT), Amoxicillin/Clauvalinic Acid 30mcg (AMC), Imipenemn 10mcg (IPM). Breakpoint was used using CLSI, 2006 guidelines. The samples from young calves had the highest incidence of resistance with B lactamase inhibitor (Amoxicillin/Clavalinic acid) followed by B lactams (Ampicillin, Cefuroxime and Cefepime). Least resistance have been encountered with Aminoglycosides (Gentamicin, Amikacin) and Ceftazine + Clavulinic Acid (Table 2). The young calves have been treated against diarrhea using either of these antibiotics such as Ciprofloxacin, Ampicillin, Cefuroxime and Ofloxacin; notably, resistance to other antibiotics have also been observed.

Table 2: The antimicrobial susceptibility pattern of E coli isolated from calf diarrhoea

Antimicrobial Group	Antimicrobial Agents	Resistant + Intermediate (No)	Sensitive (No)
B Lactams	Ampicillin 10 mcg (AMP)	24	16
	Cefuroxime 30 mcg (CXM)	20	20
	Ceftazidime 30 mcg(CAZ)	9	31
	Ceftazime + Clavulinic Acid 30/10 mcg (CAC)	1	38
	Cefepime 30 mcg (CPM)	21	19
Aminoglycosides	Gentamicin 10mcg (GEN)	0	40
	Amikacin 30mcg (AK)	3	37
Quinolones	Ciprofloxacin 5mcg (CIP)	18	22
	Ofloxacin 5mcg (OF)	17	23
Folate inhibitor	Co-Trimoxazole 25mcg (COT)	18	22
B Lactamase inhibitor	Amoxicillin/Clavulinic Acid 30mcg (AMC)	34	6
Non B Lactam	Imipenem 10mcg (IPM)	14	26

Incidence of Virulent Genes

Isolates resistant to these antimicrobials were tested for the presence of the most virulent genes through PCR. The virulence factors were ST (32.5%), LT (20%), eaeA (15%), stx1 (2.5%) and stx2 (5%), respectively. A total of 26 (65%) isolates were typable while 14 (35%) were untypable. The identified serogroups were O2, O18, O26, O126, O63, O111, O84, O7, O135, O118, O98, O128, O157 and Untypable. (Table 3, Table 4, Fig. 1).

Table 3: Number of Samples positive for virulent genes

<i>ST</i>	<i>LT</i>	<i>eaeA</i>	<i>stx1</i>	<i>stx2</i>	<i>ST+LT</i>	<i>ST+eaeA</i>	<i>ST+LT+ stx2</i>	<i>Total</i>
13	8	6	1	2	1	1	1	34

Table 4. Frequency of serogroups in the diarrhoea samples of neonatal calves

Serotypes	No (%)	Genes present	Multiple Drug Resistant
O18	6 (15%)	<i>aeA, st, lt</i>	AMC, CAZ, CAC, COT, CPM, IPM, CXM, AMP, CIP, OF
O63	1 (2.5%)	-	AMC, CAZ, CAC, COT, CPM, OF
O111	5 (12.5%)	<i>st, lt, stx2</i>	AMC, CAZ, CAC, COT, APM, IPM, CXM, AMP, CIP, OF
O84	1 (2.5%)	-	AMC, CAZ, COT, CPM, CIP, OF
O7	2 (5%)	-	COT, CPM, CXM
O135	1 (2.5%)	-	CPM, CXM, OF
O118	1 (2.5%)	-	AMC, COT, CPM, IPM, AMP
O98	1 (2.5%)	-	AMC, CAZ, COT, CPM, IPM, CXM, AMP, CIP, OF
O2	1 (2.5%)	-	AMC, CPM
O128	1 (2.5%)	<i>st</i>	COT
O157	2 (5%)	<i>stx1, aeA</i>	COT, IPM, CIP, OF
O26	1 (2.5%)	<i>lt</i>	AMC, COT, CPM, CXM, AMP, CIP
O126	1 (2.5%)	-	AMC, COT, CPM, CXM, AMP, CIP, OF
O149	2 (5%)	<i>st</i>	AMC, CAZ, COT, CPM, IPM, CXM, AMP, CIP, OF
Total typable	26 (65%)	-	AMC, CAZ, CAC, COT, CPM, IPM, CXM, AMP, CIP, OF
Total untypable	14 (35%)	<i>aeA, st, lt, stx2</i>	AMC, AK, CAZ, COT, CTM, IPM, CXM, AMP, CIP, OF

Extract Yield: Total extract percentage and pH of turmeric, carom seeds and cinnamon in different solvents ranged from 1.70±0.11 to 2.57±0.63 % (w/w) in Table 5. Higher extract percentage is seen in Ethanolic extraction of carom seeds. **Collection of spices, drying and extract preparation for total flavonoid, phenolics and DPPH scavenging activity**

Table 5. Extract yield in different solvents

(%) w/w	Aqueous (pH)	Ethanol (pH)	Methanol (pH)	P value
Cinnamon	2.16±0.10 (6)	1.93±0.05 (6.5)	1.94±0.04 (6)	0.106
Carom Seeds	1.96±0.34 (6.5)	2.06±0.05 (6)	2.57±0.63 (6.5)	0.501
Turmeric	1.95±0.16 (6)	1.70±0.11 (4.5)	1.98±0.03 (4)	0.152
P Value	0.84	0.007 (**)	0.270	
**significant p<0.001				

Total Phenolic content: The total phenolic content ranged from 0.155±0.05 to 0.221±0.014 mg/g tannic acid equivalent is presented in Fig.2. The aqueous extract of carom seeds showed the highest level of phenolics while methanolic extracts of turmeric showed the lowest ($P<0.05$).

Total Flavonoid Content: The total flavonoid content varied from 0.014±0.001 to 0.078±0.011 mg/g Quercetin Equivalent (Fig. 4). Cinnamon in aqueous extraction had the highest flavonoid content ($p<0.05$) while ethanolic extraction of turmeric was the lowest.

DPPH Scavenging Activity: All the extracts of turmeric, carom seeds and cinnamon revealed the DPPH scavenging activity between 70.81 ± 1.85 to 87.67 ± 1.81 (%) respectively. The maximum activity was observed in methanolic extraction of carom seeds ($p < 0.001$) and minimum in aqueous extraction of turmeric. The correlation between DPPH activity of herb extracts on total phenolic and flavonoid contents were positively correlated with linear correlation coefficient values of $r = 0.273$ and $r = 0.230$, respectively.

Antibacterial and MIC activity of the herb extract : The antibacterial activity of Cinnamon, Carom seeds and Turmeric extracts against *E. coli* is given in table 6 and Fig. 5. Use of the food herbs is tested on the pathogenic *E. coli* isolates (random samples positive for *stx1*, *stx2*, *ST*, *LT* and *eAea* genes) from calf diarrhoea. Antibacterial effect of methanolic extracts of Cinnamon and Ethanolic extracts of Carom seeds was found to be highly sensitive at higher concentration (500 µg/ml & 250 µg/ml) respectively.

Table 6: Antibacterial effect of food herbs using different extracts on selected pathogenic species of E coli isolates from calf diarrhoea

Conc. (w.w)	Diameter of ZOI								
	Cinnamon			Carom Seeds			Turmeric		
	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol
500 µg/ml	+	+++	+++	+	-	+++	+	+	+
250 µg/ml	-	+++	+	+	-	+++	-	+	+
125 µg/ml	-	++	+	-	-	++	-	+	+
62.5 µg/ml	-	++	-	-	-	-	-	+	+
32.6 µg/ml	-	-	-	-	-	-	-	+	-
Gentamycin (10mcg, mm)	24.66±0.66			27.66±1.45			28.00±1.15		

Average ZOI (mm): Non sensitive (<8mm, -), Sensitive (Between 9-14mm, +), Very sensitive (Between 15-19 mm, ++), Extremely sensitive (Larger than 19 mm, +++) (Watanya et al. 2016)

Table 7. MIC of the food herbs using different extracts of food herbs

MIC (mg/ml)	Aqueous	Ethanol	Methanol	P value
Cinnamon	0.07±0.01	0.05±0.01	0.05±0.01	0.317
Carom Seeds	0.17±0.12	0.02±0.00	0.01±0.00	0.135
Turmeric	0.36±0.19	0.02±0.01	0.03±0.01	0.043*
P Value	0.356	0.207	0.089	
Gentamicin	0.008			

*significant $P < 0.05$

The result showed good potency of turmeric ($P < 0.05$) followed by cinnamon. Ethanolic extraction of turmeric had better ($P < 0.05$) minimum inhibitory concentration (MIC) followed by cinnamon hence, seeming a good antibacterial quality.

Discussion

The incidence of calf diarrhoea is very common during the first few week of life. Care of the calves during the first few hours of life is very crucial, where sufficient colostrum is required to build up natural passive immunity failure of which may incite diarrhoea and sickness. *E. coli* isolates from calf diarrhoea were screened for antimicrobial resistance by the disc diffusion method against twelve antimicrobial. The young calves have been treated against diarrhea using either of these antibiotics such as Ciprofloxacin, Ampicillin, Cefuroxime and Ofloxacin; notably, resistance to other antibiotics have also been observed. High incidence of resistance with B lactamase inhibitor (Amoxicillin/Clavulanic acid) followed by B lactams (Ampicillin, Cefuroxime and Cefepime) is noted. This kind of multidrug resistance is alarming in young calves. The neonatal calves do not have fully functional intestinal microbiota (Boerlin et al. 2005) and therefore consumption of sub MIC level of antimicrobials result in the intestinal colonization of multi drug resistant or pathogenic bacteria (Andersson and Hughes, 2014). The farm itself serves as a best medium from where the resistant genes will spread to the new animal or environment though inverse relationship have been established between resistant *E. coli* and animal age (Artashes et al. 2004). Others like feeding milk to the neonates from cows treated with antimicrobials and shedding of resistant *E. coli* is a common phenomenon. Isolates resistant to these antimicrobials were tested for the presence of the virulent genes responsible for calf diarrhoea. But only few isolates carried the pathogenic genes, while the rest were non pathogenic. Even for the non pathogenic ones, they were the potential reservoir for resistance in farm animals as suggested (Brien, 2002). The incidence of heat stable (ST) enterotoxins was highest followed by heat labile (LT) enterotoxin. There are reports of significant association of diarrhoea to ST positive *E. coli* strains in cattle and buffaloes. Even LT positive *E. coli* was present in diseased, asymptomatic contact, and healthy groups (Mohammad et al. 1985) while Zhang et al. (2006) reported the occurrence of diarrhoea in piglets appreciably caused by LT and STb positive strains. EHEC/hly observed was negative in these isolates. Serotyping of *E. coli* strains help to identify the pathogenic groups essential for surveillance in case of any outbreaks (Scheutz et al. 2004). The most common serogroup was O18 (15%) followed by O111 (12.5%) and not all of them are carrying the virulent genes. Such fecal shedding of *E. coli* O157 producing Shiga toxins *stx1* and *stx2* are known to be highly pathogenic (Paton and Paton, 1998) thereby posing risk to the cattle owners, women and children. Manna et al. (2006), also reported significant occurrence of Shiga toxin producing multiple antibiotic-resistant pathogens from the calves. Among the six major diarrheagenic *E. coli* pathotypes (Croxen et al. 2013) ETEC was confirmed as the main causative agent precipitating diarrhoea in new born calves, lambs and piglets (DebRoy and Maddox, 2001) while STEC and EHEC often isolated from diarrheic and healthy calves role in calf disease remains controversial (Kolenda et al. 2015). But according to Ryu et al. (2020), there was no association between the virulence factors and diarrhoea in neonatal calves and diarrhoea incidence was age related.

Known for the potential antioxidant and antibacterial effect of kitchen herbs, carom seeds, cinnamom and turmeric was collected and processed to test against the important pathogenic forms of *E. coli* isolated from the calves. There are many variations in the extract yield reported by other workers. The extraction yield is not only affected by the technique followed but also by the type of solvent used, plant and bioactive compounds to be isolated etc. (Ajanal et al. 2012) therefore making it difficult to make recommendations on the solvent used. The pH of these herbs evaluated ranged from normal to acidic pH in different solvents. It maybe that the inhibitory effect of these herbs due to bioactive components maybe active at different pH as that of sulfide or thiol extracts from garlic, onion and shallot demonstrating the inhibitory activities at pH level 5 and 6 (Jarriyawattanachaikula et al. 2016). Phenolic compounds contribute to the antioxidant in many plants and herbs (Duthie and Crozier, 2000) where the aqueous extract of carom seed was 0.221 (mg/g tannic acid equivalent) as against 0.199±1.3 (aqueous extract) and 0.210±0.83 (methanolic extract) (mg/g tannic acid equivalent) reported by Goswami and Chatterjee, (2014) while cinnamon yielded 1.918±0.528 mmol/g (Dvorackova et al. 2015). Results related to research work of Kaur and Kapoor (2002), analyzed that the total phenolic content in turmeric varied from 1.72 to 7.46 g gallic acid equivalent/100g. The total flavonoid content in different solvents was lower than the ones reported by Tacouri et al. (2013) as 37.6±3.95 in carom seeds and 2.8±0.06 mg QE/g in distilled water. Different species of cinnamon differ significantly in their flavonoid content and antioxidant potential (Prasad et al. 2009) as well as the age of bark (Singh et al. 2007) and climatic growth conditions (Loizzo et al. 2013). The recovery of phenolic and flavonoid content from plant source depends on the solvents used, polarity, and preparation of samples, particle size and density of the extracts that can limit the accessibility of solvents used for extraction (Dvorackova et al. 2015). Studies have shown that the polyphenolic contents present in spices were

responsible in decreasing the oxidative stress (Khan *et al.*, 2010) and DPPH activity. The correlation between DPPH activity of herb extracts on total phenolic and flavonoid contents were positively correlated with linear correlation coefficient values of $r=0.273$ and $r=0.230$, respectively. Carom seeds have the ability to mitigate oxidative damage in the calf thymus DNA (Goswami and Chatterjee, 2014). Also, the antioxidants present in these spices have numerous beneficial activities on inflammation, infections, microbes, pain, wounds, immunity, digestion etc. (Rather et al. 2016 and Singh et al. 2017).

The antibacterial activity of Cinnamon, Carom seeds and Turmeric extracts against *E. coli* is tested on the pathogenic *E. coli* isolates (random samples positive for *stx1*, *stx2*, *ST*, *LT* and *eAea* genes) from calf diarrhoea. Use of the food herbs acquiring diverse array of biomolecules known for its antimicrobial activity is tested on the pathogenic *E. coli* isolates (random samples positive for *stx1*, *stx2*, *ST*, *LT* and *eAea* genes) from calf diarrhoea. The efficacy is tested through the determination of minimum inhibitory concentration (MIC) by microbroth dilution method using iodinitrotetrazolium chloride (INT). The sensitivity of these isolates to different extracts are represented by the mean MIC values ranging from The result showed good potency of turmeric ($P<0.05$) followed by cinnamon suggesting that it can be used to control and prevent the growth of pathogenic bacteria in the gut. Antibacterial effect of methanolic extracts of Cinnamon and Ethanolic extracts of Carom seeds was found to be highly sensitive at higher concentration (500 µg/ml -250 µg/ml) respectively. The variation in the MIC concentrations may arise from the extraction process of the various herbal extracts, chemical and volatile nature of the constituents. Several researchers have reported the efficiency of herbal extracts (phenols, terpenoids and alkaloids etc. to interact with the enzymes and disrupt the microbial cell membrane (Burt et al., 2004).

Increase attention has been given to the occurrence of AMR in livestock; therefore alternating to readily available kitchen herbs for their bioactive components and prophylactic use since "Prevention is Better than Cure". We have known from literatures the potential benefits of kitchen herbs and the problem of resistance is still unknown. Previous studies have reported that bacteria may not develop resistance to herbal medicines due to the presence of multiple components in their extract acting on different sites of the target organism thereby contributing to the overall activity of the extract. Likewise the residual organic solvents may add up to the aversion of incorporating in feed or milk. Therefore, kitchen herbs still finds scope in incorporating directly in powdered form in milk or grains to prevent organism causing calf diarrhoea. However, *in vivo* studies are still required to confirm it.

Declarations

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Ethics approval: Not Applicable

Consent to participate: Yes

Consent for publication: Yes

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Code availability: Not applicable

Authors contributions: Saroj Rai- conceived the idea and designing of experiment; M. Kumar- conducted the experiment; G.P. Mandal and S. Tripura: Herbal extractions and estimation assisted by them; I.Samanta: guided Microbiology works; M. Mondal and M. Rajendar helped the molecular works; S.K.Das did the draft evaluation and D.K. Mandal did the statistical analysis

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Figures

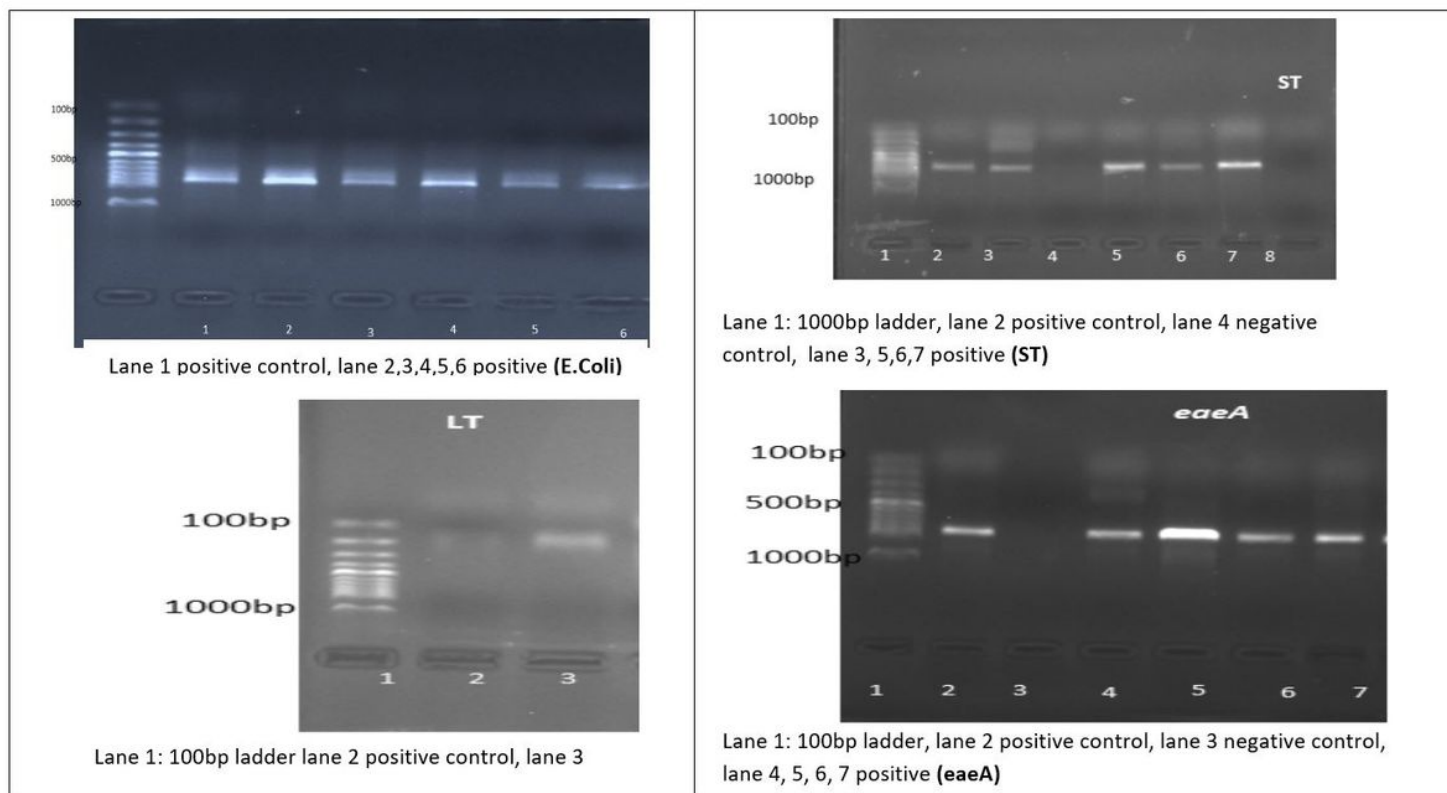


Figure 1

Prevalence of virulence factor in the isolates

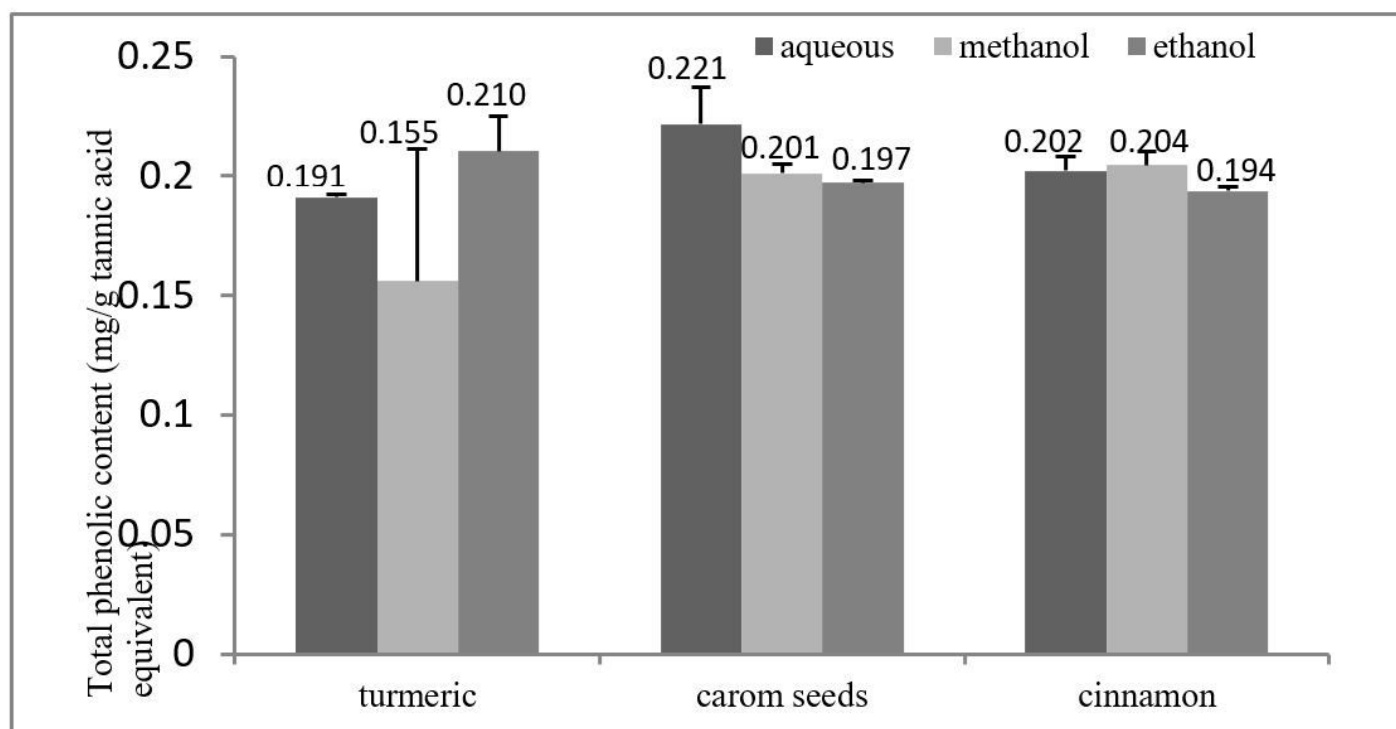
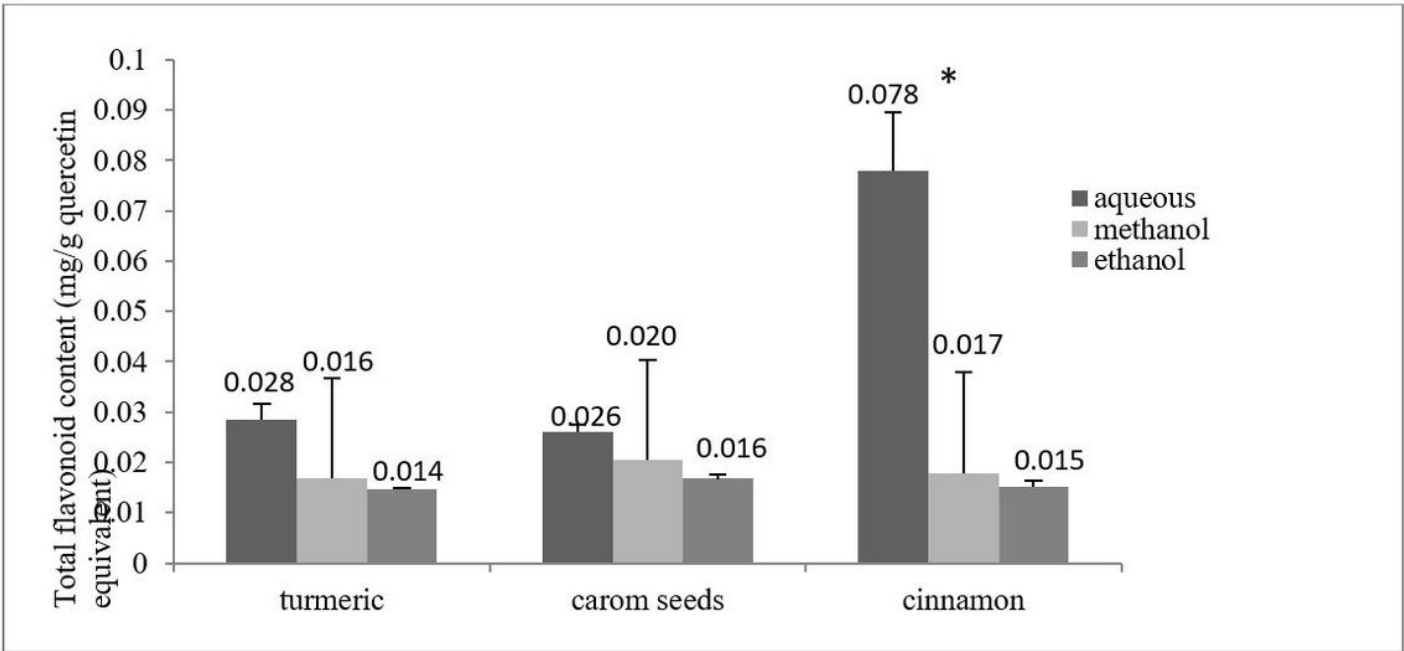


Figure 2

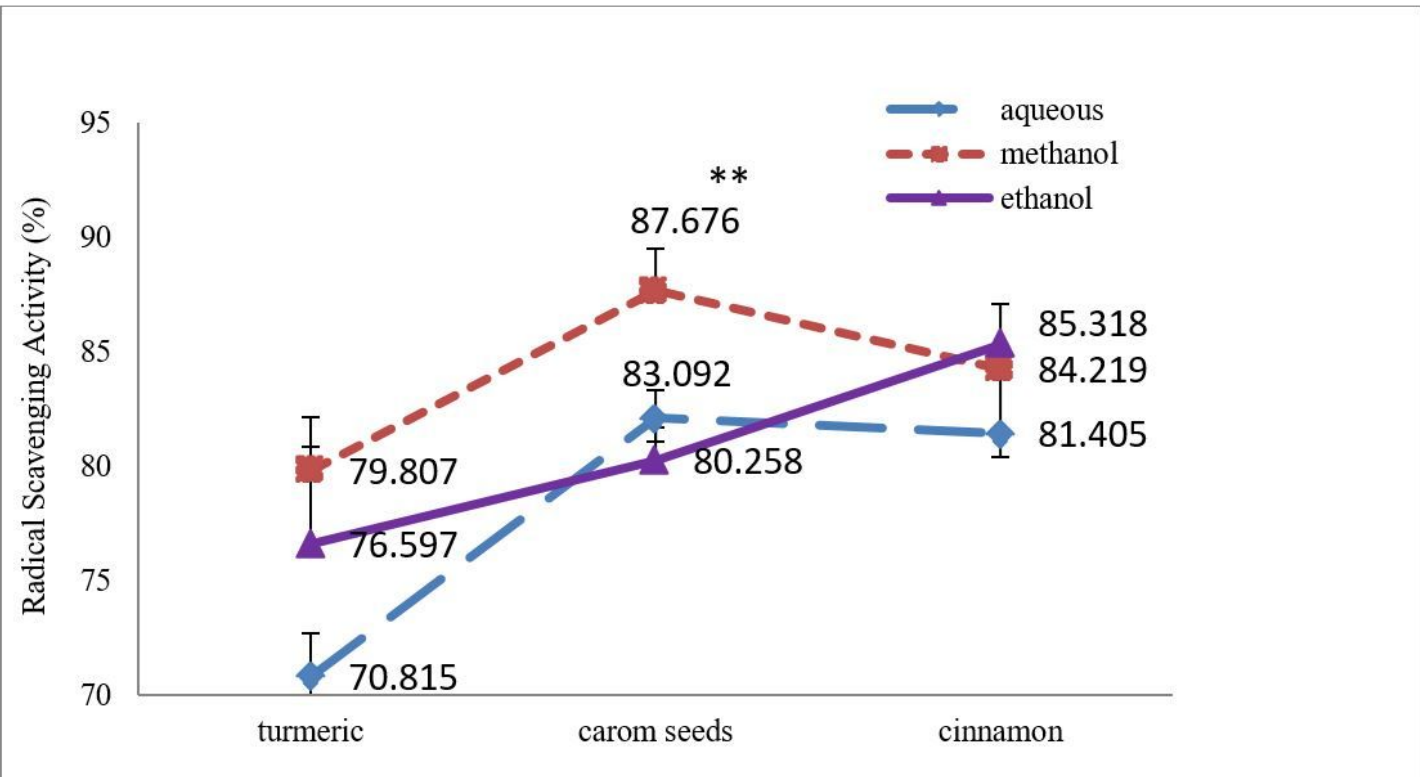
Total phenol content (mg /g dry wt) in herb extracts



*significant $p<0.05$

Figure 3

Total Flavonoid content (mg QE/g dry wt) in herb extracts



**significant $p<0.01$

Figure 4

Antioxidant activity of turmeric, carom seeds and cinnamon by DPPH assay



Figure 5

Antibacterial screening of kitchen herb extracts on E coli