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RESEARCH ARTICLE

Inhibitory effect of spices on beta lactamase enzyme of resistant bacteria isolated from milk of healthy cattle

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Abstract: Present study was undertaken to observe the effect of spices on the extended Spectrum beta lactamase enzyme of ESBL Producing Enterobacteriaceae Group of Bacteria isolated from healthy cattle milk. Out of 100 samples collected randomly from various dairy farms located at the different areas of Rewa 14 samples characterized by the phenotypic standard methods were found to be ESBL positive giving a prevalence rate of 14%. Inhibitory potential and per cent inhibition of beta lactamase enzyme were observed against dry powder of Methi seeds (*Trigonella foenum-graecum*), dry zinger, (*Zingiberofficinale*), Ajwain seeds (*Trachyspermum ammi)* Kalonji seeds (*Nigella sativa*), Black pepper (*Piper nigrum*), Clove bud (*Syzigium aromaticum*) (10mg/ml) and one test drug Tazobactum by colorimetric assay method performed on the individual spices and in their different combinations on the basis of absorbent value. The absorbance level of each spice was observed using NITROCEFIN as a chromogenic substrate at 405nm wavelength. Minimum absorbance value was observed by *Zingiber officinale* (0.48 ± 0.009) and the maximum absorbance value of *Syzgium aromaticum* (1.698 ± 0.069). The inhibitory effect of combination of the spices showed maximum absorbance value (0.46 ± 0.06) by *Piper nigrum* and *Zingiber officinale* No significant difference was seen between the absorbance value of *Z.officinalis*, *N.sativa* (0.61± 0.05) and *P.nigrum T.faenum graecum* (0.62±0.03). Study observed the *in vitro* inhibitory potential present in the spices and could be used in near future to combat antimicrobial resistance to some extent.

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Introduction

Extended spectrum beta lactamase are enzymes that hydrolyze most penicillins and cephalosporins, including oxyimino-beta lactam compounds (cefuroxime, third- and fourth-generation cephalosporins and aztreonam) but not cephamycins or carbapenems (Rahman et al. 2004) Antimicrobial therapies may hasten the emergence of antimicrobial resistance due to these enzyme producing organisms that would, otherwise, be delayed. Exchange of resistance genes between bacteria from different sources can also occur in the environment (Batabyal et al. 2018). Since 2000, the European Antimicrobial Resistance Surveillance Network has reported a steady increase in the rates of invasive *E. coli* and *Klebsiella pneumonia* isolates resistant to third-generation and fourth-generation cephalosporins (Tenover et al. 1999, Paterson and Bonomo,2005)

Milk is a major part of human food and plays a prominent role in the diet. The presence of pathogenic bacteria in milk is of considerable public health concern, especially for those individuals who still drink raw milk. *Escherichia coli* and *Klebsiella pneumoniae* are humans and animals opportunistic pathogens, responsible for a wide range of infections. Milk is an ideal medium for the rapid multiplication of bacteria, particularly under unhygienic production and storage.

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Spices include leaves (coriander, mint), flower (clove), bulbs (garlic, onion), fruits (red chilli, black pepper), stem (cinnamon), rhizomes (ginger, turmeric) and other plant parts. Apart from providing aroma and flavour spices have been recognized for their properties of preserving foods and medicinal values due to the presence of bioactive compounds (Dhiman et al. 2015, Faujdar et al. 2020). Resistance among pathogenic microbes against various antimicrobial drugs has been an increasingly important and most appalling problem, globally. Synthetic chemicals can be toxic in nature; hence, these spices containing phytochemical, which have both antimicrobial and antioxidant properties, must be taken to control this problem and

could be a better alternative with growing concern of antimicrobial resistance.

Material and Methods

Isolation of ESBL producing *Enterobacteriaceae* group of bacteria

Milk samples were collected from various dairy farms for isolation of ESBL producing *Enterobacteriaceae* group of bacteria. Milk sample (100 samples) enriched in Tryptose soya broth (10 ml/1 ml sample) fresh overnight broth culture was streaked in Tryptone bile glucuronic agar media. Broth and media both were supplemented with cefotaxime(2µgm/ml) and aztreonam(4µgm/ml) to isolate ESBL producing *Enterobacteriaceae* bacteria.

Phenotypic Characterization

Double Disc Synergy Test (DDST): Two cephalosporins (cefotaxime) disc with (amoxicillin-clavulanic acid disc in the centre were placed in the plates. Augmentation of the inhibition zones around any of the cephalosporin discs in the direction of the disc containing amoxicillin-clavulanic acid indicated the positive result. The distance between the discs was kept 20 mm centre-to-centre however it could be reduced or expanded for strains with very high or low levels of resistance(EUCAST, 2013, Castanheira et al.2021).

Ezy MIC strip test: Cefotaxime and Cefotaxime + clavulanic acid E strip were used and test was confirmed positive if MIC ratio e" 8 or deformed ellipse was present around Cefotaxime + clavulanic acid

Combination Disc Diffusion Test (CDDT): For each test, discs containing the cephalosporin alone (cefotaxime 30µgm) and in combination with clavulanic acid (30-10µgm) were applied. The inhibition zone diameter around the cephalosporin disc combined with clavulanic acid was 5mm larger to the zone around the disc with the cephalosporin alone indicated positive results (Garrec et al. 2011)

Inhibitory potential of on extended spectrum beta lactamase enzyme

Preparation of beta lactamase enzyme - Fresh overnight cultures of bacteria were inoculated into broth and grown for 2 h at 35°C in a rotary shaker. Inducer (penicillin-G 400 µg/ml) was added, and incubation was continued for an additional 4 h. The cell pellets were collected by centrifugation, resuspended, and washed with potassium phosphate buffer (0.05M, pH 7.0) at 4°C. The bacteria were recentrifuged and subsequently resuspended in the same buffer that is 10-fold concentrated. The bacteria were disrupted by sonic treatment for 5 minutes in an ice bath. Cellular debris was removed by centrifugation at 10000 rpm for 4 minutes at 4°C. The resulting supernatants containing beta lactamase enzyme were stored in portions at -20°C until required.

Preparation of spices

Spices were collected from local market and grinded. Powder was mixed with distill water to prepare concentration of 10 mg/ml which was further used for colorimetric assay.

Colorimetric assay – Beta lactamase inhibitory potential of seeds of dried powder of each spices *Trigonella foenum-graecum*,*Zingiber officinale*, *Trachyspermum ammi*, *Nigella sativa*, *Piper nigrum*,*Syzigium aromaticum* were analysed by the beta lactamase enzyme inhibitory assay using Chromogenic substrate Nitrocefin. Nitrocefin (98% pure) was dissolved in dimethyl sulfoxide at a final concentration of 0.4 mmol/L. Dried powder of each spices were taken respectively (10 mg/ml) along with the standard beta lactamase inhibitor Tazobactum (100 µM concentration). Briefly 8µl of enzyme was initially stabilized with the 72µl sodium phosphate buffer (100m Micro litre) with pH 7.0 for 10-15 minutes at 25°C. Later on standard beta lactamase inhibitors and powder of each spices were added into the respective wells and plate was again incubated for 20-25 minutes at 25°C.After 25 minutes substrate was added to the wells and again incubated for 20 minutes at 25°c.After desired incubation period plate was read using Lisa plus make Elisa reader – 96 well Micro titre plate. Colour development was analyzed at 405nm wavelength (Linscott and Brown 2005, Solanki and Selvanayagam 2013)

Minimum inhibitory Concentration detection of the Spices

MIC of the spices powder were also determined using serial tube dilution technique as per the method described by CLSI(2011). Spices powder aqueous solution were prepared and serially diluted in the range of 0.1mg/ml to 10 mg/ml. The tubes were inoculated with 100 µl of bacterial culture The density of selected bacteria was adjusted equal to that of the 0.5 McFarland standard (1.5 x 108 CFU/ml) by adding sterile distilled water. Tazobactum was used as the standard drug for comparison. The tubes were incubated at 37°C for 12-18 hours Broth along with inoculum without drug/spices were used as growth control. The growth of inoculum was decreased next tube was taken as MIC.

Statistical Analysis:

The data were analyzed using student's t test and ANOVA when appropriate. Results are presented as mean ± standard deviation. Values of pÂ0.05 were considered as statistically significant.

Results and Discussion

Isolation of ESBL producing Enterobacteriaceae group of bacteria

After initial screening of 100 samples 14 samples was found to be ESBL positive and 86 samples was negative giving a prevalence rate of only 14%. Present study included both pooled and mastitis milk samples. The prevalence rate in the mastitis milk was 0% as no samples turned out to be ESBL positive (Table 1).

Phenotypic Characterization

After initial screening of the 100 samples 14 positive samples were further confirmed by phenotypic methods. As per the CLSI and EUCAST three standard methods have been used for the phenotypic confirmation of ESBL producing isolates. Among the three methods CDDT method showed the maximum sensitivity than the Among the 14 samples all the samples were positive by CDDT method giving 100% sensitivity,10 samples were positive by DDST method giving71% of sensitivity and only 5 samples gave ellipsoidal shape in enzyme MIC strip method giving 35% sensitivity (Gutmann 1985, Taslima 2012).

Inhibitory potential of on extended spectrum beta lactamase enzyme

Inhibitory potential and per cent inhibition of beta lactamase enzyme were observed against dried powder of meethi seeds (*Trigonella foenum-graecum*), Dry Zinger (*Zingiber Officinale*), Ajwain seeds (*Trachyspermum ammi*), Kalonji seeds (*Nigella sativa*), Black pepper (*Piper nigrum*) , Clove buds (*Syzgium aromaticum*). Each of the spices was grinded and prepared and used in the final concentration of 10mg/ml by Colorimetric assay performed on the individual spices and in their different combinations. Inhibitory effect was observed on the basis of absorbent value. The absorbance level of each spices was observed using NITROCEFIN as a chromogenic substrate at 405nm wavelength. Minimum absorbance value was observed by Zingiber officinale (0.48 ± 0.009) and the maximum absorbance value of *Syzgium aromaticum* (1.698 ± 0.069). *T.ammi*, (1.172±0.125), *Piper nigrum* (1.047±0.103) *S.aromaticum* (1.698± 0.069) *Z.officinalis* (0.449± 0.009) showed significant difference

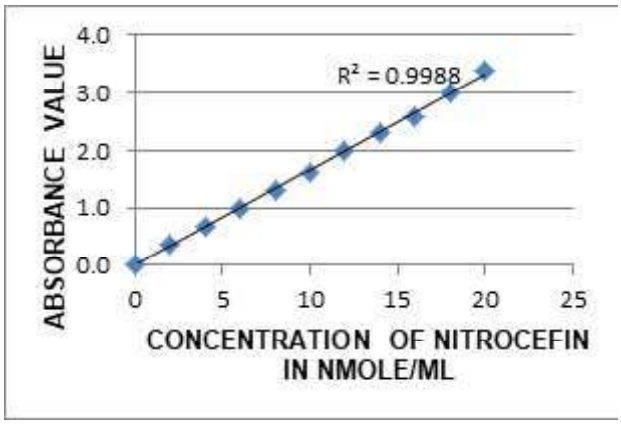

Fig. 1 Standard curve of Nitrocefin in Distill water

Table 1 Per cent Prevalence of ESBL isolates from Mastitis/Pooled milk

No. of Samples Pooled/Mastitis	Positive Samples	Negative Samples	Percent Prevalence
Pooled milk	14	79	14
Mastitis milk	0	7	0

Table 2: Inhibitory Effect of Individual Spices on ESBL enzyme by Colorimetric Method (Absorbance value)

Sample	*T .Ammi*		*P .Nigrum*		*S. Aromaticum*		*T. Fgraecum*		*Z.Officinale*		*N. Sativa*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	1.229	0.066	1.110	0.065	1.712	0.133	1.30	0.034	0.444	0.007	0.932	0.013
2	1.347	0.228	1.216	0.077	1.625	0.222	1.18	0.250	0.476	0.016	1.157	0.063
3	1.253	0.067	1.171	0.298	1.942	0.041	1.59	0.330	0.469	0.004	0.892	0.066
4	1.342	0.250	1.038	0.039	2.007	0.121	1.82	0.044	0.44	0.011	1.052	0.046
5	1.046	0.030	0.985	0.050	1.806	0.034	1.27	0.260	0.465	0.011	1.152	0.031
6	1.181	0.041	1.102	0.008	1.795	0.044	0.92	0.078	0.451	0.014	0.926	0.037
7	0.971	0.021	0.999	0.041	1.829	0.032	0.90	0.177	0.439	0.004	0.970	0.031
8	1.229	0.331	1.068	0.036	1.725	0.025	1.49	0.210	0.447	0.003	1.000	0.019
9	1.001	0.173	0.853	0.135	1.676	0.032	1.03	0.022	0.450	0.014	0.996	0.021
10	1.131	0.050	1.193	0.077	1.627	0.040	1.33	0.061	0.438	0.008	0.993	0.024
11	1.217	0.124	0.917	0.189	1.579	0.048	1.23	0.117	0.441	0.013	0.990	0.026
12	1.233	0.142	1.132	0.038	1.530	0.056	1.21	0.097	0.444	0.010	0.987	0.029
13	1.159	0.166	1.037	0.099	1.481	0.064	1.21	0.064	0.437	0.004	0.983	0.032
14	1.073	0.068	0.840	0.298	1.432	0.072	1.16	0.078	0.437	0.009	0.980	0.036
Mean ± SE	1.172^{c}	0.125	1.047^{b}	0.103	1.698^{d}	0.069	1.26^{c}	0.130	0.449^{a}	0.009	1.001^{b}	0.034

Mean with different superscript differ significantly ($p < 0.05$)

in their absorbance value ($p<0.05$). Whereas *N.sativa* (1.001±0.034) and *Piper nigrum* (1.047±0.103); *T.ammi* (1.172±0.125), and *T.faenum graecum* (1.26±0.130) showed no significant difference in the absorbance value and inhibitory potential (Table 2 & Fig. 1).

The inhibitory effect of combination of the spices by colorimetric method showed maximum absorbance value (0.46 ± 0.06) by *Piper nigrum* and *Zingiber officinale*. Significant difference in the absorbance value was observed between *Z.officinalis*, T.faenum graecum (0.240±0.03); *Z.officinalis*, *Nigella sativa* (0.61±0.05), *Piper nigrum Z.officinalis*. (0.46±0.06). ($p<0.05$) .No significant difference was seen between the absorbance value of *Z.officinalis, N.sativa* (0.61± 0.05) and *P.nigrum T.faenum graecum* (0.62±0.03). Combination of *T.ammi Z.officinalis* (0.77±0.03); *N.sativa, T.faenum graecum* (0.93±0.04) and *T.ammi, T.faenum graecum* (0.99±0.03) showed significant difference in their absorbance value.($p<0.05$). Significant difference was also observed between P.nigrum, N.sativa (1.23±0.12); *S.aromaticum T.faenumgraecum* (1.38±0.07); *S.aromaticum, Z.officinalis* (1.54±0.02); *T.ammi, S.aromaticum* (2.00±0.14), *P.nigrum, S.aromaticum* (2.23±0.10); *S.aromaticum N.sativa* (2.34±0.03). In each of the above work conducted on the positive isolates Tazobactum was taken as the standard drug in the concentration of 8 µgm/ml. Tazobactum showed lowest absorbance value (0.213± 0.025) giving almost 98% of per cent inhibition against enzyme obtained from each isolate (Table 3-4 & Fig. 2-4).

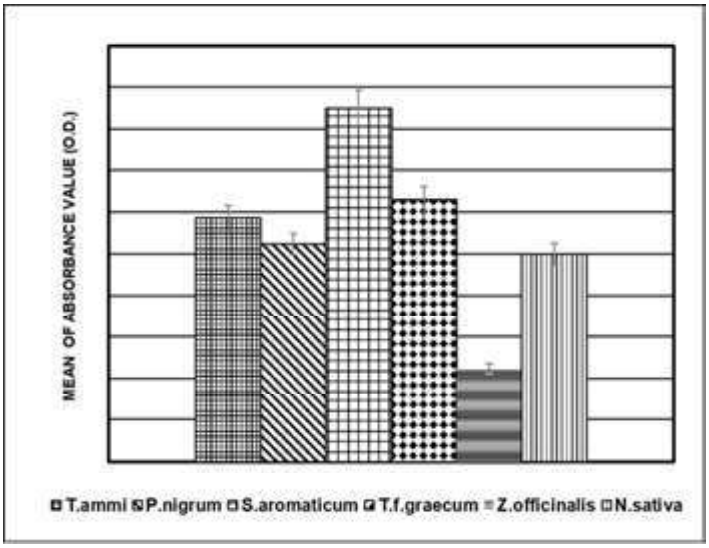

Fig. 2 Inhibitory Effect of Spices on ESBL enzyme by Colorimetric method

Extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae have become a matter of great concern in human and veterinary medicine as these pathogens pose a major challenge for the treatment of general infections and cause a problem with the extensive use of second- or third-generation cephalosporins for the treatment of bacterial infections (Serrano et al. 2009). Out of100 samples 14 samples were found to be ESBL producing Enterobacteriaceae group of bacteria in the initial screening giving a prevalence rate of 14%. Corelating with the study conducted by Batabyal *et al* (2018) in West Bengal where prevalence rate was 12% in healthy animals and another study conducted on healthy broilers of Jabalpur and its adjoining areas confirmed prevalence of 38% of ESBL *E.coli* (Shrivastav et al. 2016)

The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oil, and flavonoids (Keite et al. 2012). Spices, herbs, and their constituents are generally recognised as safe (GRAS) and approved by several regulatory agencies such as US Food and Drug Act, theEuropean Union standards, Codex Alimentarius, and Food Safety and

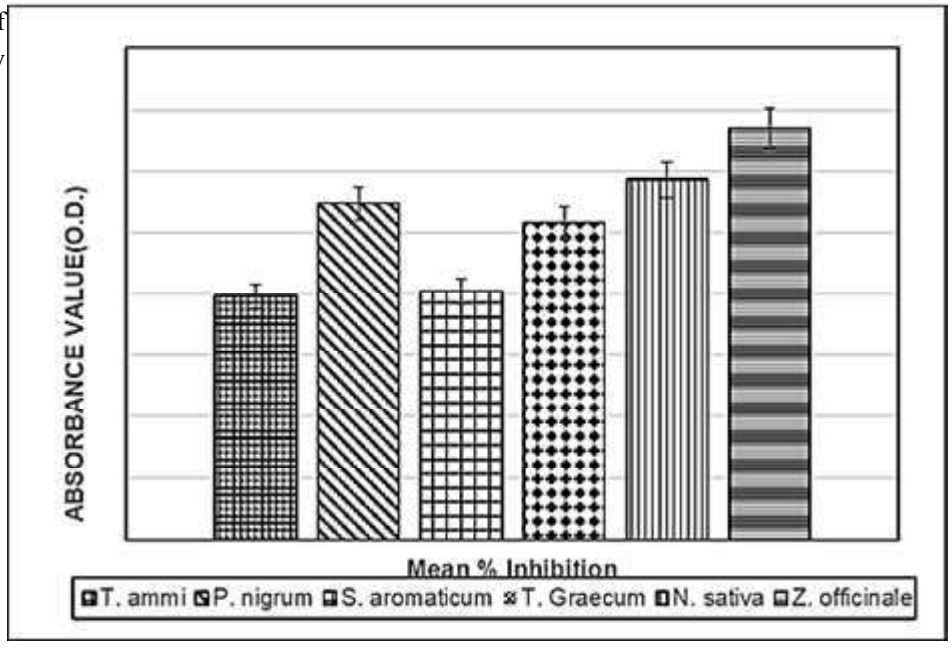

Fig. 3 Inhibitory Effect of Combination of two Spices on ESBL enzyme by Colorimetric method

Table3: Inhibitory Effect of Combination of Spices on ESBL enzyme by Colorimetric method (Absorbance value)

	ZT		***ZN***		***PZ***		***PT***		***TZ***		***NT***		***TT***	
Sample	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	0.30	0.06	0.61	0.02	0.47	0.04	0.63	0.03	0.77	0.02	0.94	0.03	1.09	0.00
2	0.24	0.02	0.58	0.02	0.46	0.06	0.66	0.05	0.81	0.03	0.89	0.02	1.00	0.02
3	0.21	0.04	0.58	0.03	0.45	0.05	0.63	0.03	0.77	0.04	0.84	0.03	0.93	0.07
4	0.26	0.08	0.55	0.08	0.44	0.06	0.59	0.02	0.79	0.02	0.95	0.02	1.01	0.04
5	0.33	0.04	0.57	0.03	0.45	0.08	0.62	0.01	0.80	0.01	0.91	0.04	0.96	0.04
6	0.27	0.03	0.55	0.06	0.44	0.07	0.62	0.01	0.78	0.02	0.95	0.02	0.99	0.03
7	0.27	0.03	0.54	0.07	0.42	0.09	0.61	0.01	0.76	0.01	0.97	0.04	1.03	0.03
8	0.17	0.03	0.61	0.08	0.47	0.05	0.63	0.04	0.79	0.02	0.97	0.04	1.02	0.04
9	0.22	0.04	0.60	0.04	0.44	0.08	0.60	0.03	0.80	0.03	0.95	0.03	0.92	0.03
10	0.22	0.01	0.59	0.05	0.45	0.07	0.61	0.04	0.74	0.02	0.97	0.05	0.97	0.04
11	0.25	0.02	0.58	0.06	0.47	0.06	0.60	0.05	0.79	0.00	0.86	0.07	0.96	0.03
12	0.20	0.03	0.59	0.05	0.46	0.07	0.64	0.02	0.69	0.05	0.92	0.04	0.96	0.01
13	0.20	0.03	0.60	0.05	0.49	0.04	0.64	0.02	0.74	0.02	0.94	0.06	0.99	0.05
14	0.24	0.02	0.62	0.05	0.48	0.04	0.61	0.04	0.73	0.06	0.92	0.08	0.97	0.05
Mean±SE	0.240^{a}	0.03	0.61^{c}	0.05	0.46^{b}	0.06	0.62^{c}	0.03	0.77^{d}	0.03	0.93^{e}	0.04	0.99^{f}	0.03

Mean with different superscript differ significantly ($p<0.05$).
ZT: Zingiber + T ammi; **ZN**- zingiber + Nigella sativa **PZ** – Piper nigrum + Zingiber **PT**-Piper nigrum+ T ammi; **TZ** T foenum-graecum+ Zingiber **NT**- Nigella sativa+ T ammi ; **TT**: T foenum-graecum+ T ammi

Table 4: Inhibitory Effect of Combination of Spices on ESBL enzyme by Colorimetric method (Absorbance value)

Sample	*PN*		*ST*		*TS*		*SZ*		*TS*		*PS*		*SN*		*TN*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	1.07	0.03	1.27	0.03	1.41	0.02	1.56	0.01	2.18	0.03	2.34	0.01	2.42	0.02	2.43	0.05
2	1.07	0.03	1.23	0.01	1.44	0.02	1.56	0.02	2.05	0.04	2.28	0.10	2.38	0.02	2.40	0.11
3	1.36	0.27	1.29	0.04	1.43	0.04	1.57	0.01	2.06	0.11	2.34	0.07	2.36	0.03	2.34	0.08
4	1.33	0.24	1.38	0.05	1.44	0.03	1.53	0.03	2.15	0.05	2.28	0.10	2.33	0.02	2.33	0.12
5	1.09	0.00	1.33	0.07	1.48	0.01	1.55	0.01	2.04	0.04	2.21	0.10	2.34	0.05	2.42	0.06
6	1.05	0.03	1.36	0.06	1.40	0.08	1.55	0.03	1.89	0.15	2.18	0.06	2.35	0.03	2.28	0.14
7	1.39	0.29	1.46	0.02	1.47	0.02	1.55	0.02	1.99	0.07	2.15	0.07	2.34	0.01	2.54	0.16
8	1.13	0.03	1.40	0.08	1.47	0.01	1.50	0.01	2.09	0.21	2.02	0.01	2.36	0.03	2.40	0.03
9	1.23	0.10	1.33	0.10	1.47	0.02	1.55	0.03	2.07	0.23	2.09	0.10	2.37	0.05	2.36	0.05
10	1.17	0.03	1.36	0.03	1.48	0.01	1.58	0.00	1.96	0.19	2.28	0.13	2.26	0.06	2.39	0.08
11	1.03	0.03	1.46	0.15	1.46	0.01	1.49	0.03	1.90	0.19	2.11	0.12	2.23	0.07	2.30	0.04
12	1.12	0.03	1.41	0.08	1.46	0.03	1.57	0.01	1.88	0.22	2.35	0.27	2.34	0.05	2.33	0.07
13	1.69	0.29	1.50	0.09	1.47	0.03	1.53	0.04	1.88	0.25	2.28	0.12	2.32	0.01	2.34	0.09
14	1.50	0.22	1.50	0.11	1.46	0.04	1.53	0.04	1.80	0.24	2.33	0.15	2.30	0.02	2.36	0.13
Mean± S.E.	1.23^{g}	0.12	1.38^{h}	0.07	1.45^{h}	0.03	1.54^{j}	0.02	2.00^{k}	0.14	2.231	0.10	2.34^{m}	0.03	2.37^{m}	0.09

Mean with different superscript differ significantly ($p<0.05$).
PN- Piper nigrum+Nigella sativa ; ST Syzigium aromaticum + T ammi; TS T fenugraecum + S aromaticum; SZ Syzigium aromaticum+ Zingiber PS – Piper nigrum+ Sygizium aromaticum SN- S. aromaticum+ Nigella sativa; TN - T fenugraecum + Nigella sativa

Standards Authority of India (Dhiman et al. 2015). Colorimetric assay performed with Nitrocefin as the chromogenic substrate against beta lactamase enzyme for all the spices both individually as well as in combination. Nitrocefin, which has been used previously as a competing substrate to monitor enzyme-inhibitor interactions (Gutmann et al.1985; Hedges et al.1975; James 1983) was used in to calculate a relative substrate affinity index based on a 5-min reaction between enzyme, inhibitor, and nitrocefin.

Absorbance value/OD value determined inhibitory potential of spices against the ESBL enzyme. Highest inhibitory potential was observed by *Zingiber officinalis* with OD values of mean of triplicates (0.449±0.009). Lowest inhibitory potential was observed by *Syzygium aromaticum* (1.698± 0.069) correlating with the data reported earlier. No significant difference ($p>0.05$) was observed in the inhibitory effect of *T.ammi* and *T. foenum-graecum* (1.172±0.125; 1.26±0.130) and *Piper nigrum* and *Nigella sativa* (1.047± 0.103; 1.001±0.034) against ESBL enzyme. Inhibitory

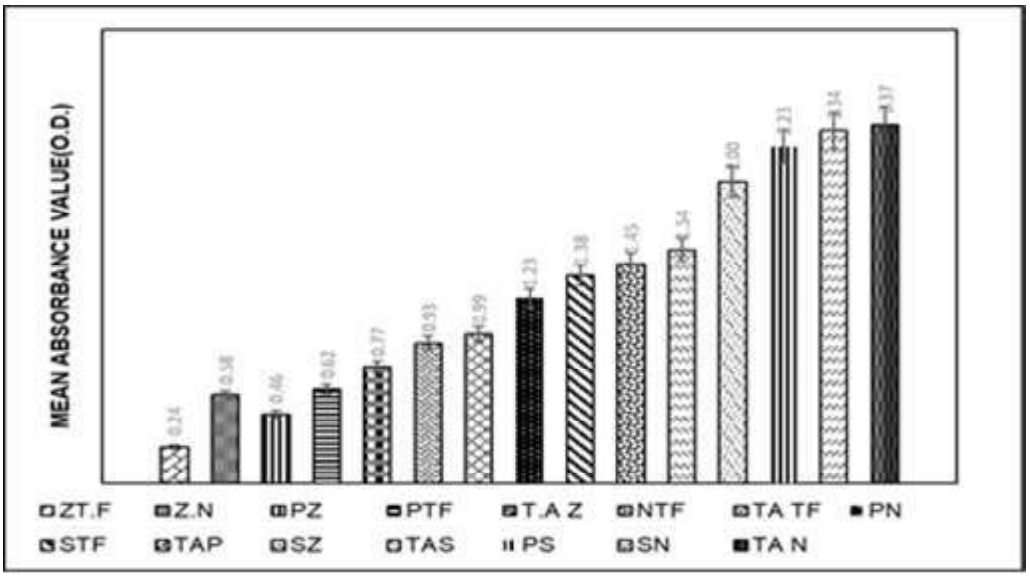

Fig. 4 Per cent Inhibition (Inhibitory Potential) of Spices by Colorimetric method

potential of *T.ammi* and *Piper nigrum* showed significant difference ($p<0.05$) with mean values (1.172±0.125, 1.047±0.103) corelating with the results obtained by Iodometric method (Yang et al.2004) for *Piper nigrum*, *T.ammi* ($p<0.05$). When two spices in equal concentration mixed together inhibitory potential was little different. Maximum inhibitory potential was shown by *Piper nigrum* and *Zingiber officinale* (0.46± 0.06). No significant difference ($p>0.05$) was observed with the combination of *Zingiber officinale* and *Nigella sativa* and *Piper nigrum* and *T.foenum-graecum* (0.61±0.05; 0.62±0.03). Significant difference in the mean values was observed with *Zingiber officinale T.foenum-graecum*; *Zingiber officinale T.ammi*; *Nigella sativa T.foenum-graecum; T.ammi T.foenum-graecum; Nigella sativa T.ammi; Zingiber officinale Piper nigrum Zingiber officinale T.ammi; Zingiber officinale T.foenum-graecum* .Tazobactum taken as standard control gave inhibitory effect as absorbance value of 0.213±0.025 showing almost 95% of inhibition of beta lactamase activity. MIC values for the aqueous solution of the spices were observed by tube dilution method. The MIC of *Zingiber Officinale* was found to be 0.7mg/ml, *Nigella sativa* showed minimum inhibitory concentration at 0.8mg/ml, MIC value of *T.foenum-graecum P.nigrum* and *T.ammi* seed powder in water was observed as 0.9mg/ml,1mg/ml and 0.9mg/ml and highest MIC value was observed for *S.aromaticum* (2mg/ml). Correlating with the data reported earlier with the nitrocefin competition assay. The antimicrobial activity of *C. longa* and *Z. officinalis* was also observed in water and other solvents against food borne pathogens. The solvent extracts of *C. longa* and *Zingiber officinalis* displayed antibacterial and anti-yeast activity. (Sunilson et al. 2009). Similar findings of *Zingiber officinalis* were observed by Lakshmi *et al* (2015) against ESBL isolates. Perusal of their data revealed commonly used herbs and spices such as garlic, black cumin, cloves, cinnamon, thyme, all spices, bay leaves, mustard, and rosemary, possess antimicrobial properties that, in some cases, can be used therapeutically (Kaveri 2019; Lai and Roy, 2004). β-lactamase inhibitors screened from the extracts of traditional Chinese medicines and concluded that the solution of the extracts is often brown or yellow and hinders the reasonable judgment of screening experiments. Tazobactum used as the standard drug showed minimum inhibitory concentration of 8µg/ml. Higher values in many species indicate only a very limited antibacterial efficacy. The drug-resistant ESBL gene is significantly present in approximately 14% of the bacterial strains isolated from pooled milk samples which may be of great health concern for human beings. This drug resistance can easily be transferred between closely related pathogens *in vivo* which may result in risky and fatal health hazards due to unsuccessful treatment with common antimicrobials.

Conclusion

The results of the present study warn the need for stricter preventive measures. For this, regular sterilization of dairy equipment, washing of utensils, milker's hands, udders, eradication of diseased animals, pasteurization/boiling of milk is required before collection and distribution for consumption and product making. The inhibitory effect of spices on the activity of beta lactamase enzyme further states the utility of herbs in the dairy management and could help in limiting the use of antimicrobial agents.

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