2011 2nd International Conference on Biotechnology and Food Science
IPCBEE vol.7 (2011) © (2011) IACSIT Press, Singapore

Effect of turmeric extract, fermented vinegar and their mixture on *Salmonella* Typhimurium Reduction *in vitro*

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Abstract— **This study was conducted to determine the efficacy of turmeric extract, vinegar and their mixture in reduction of *Salmonella* Typhimurium (ST). Two extraction methods consisting of cold solvent extraction (maceration method) and hydro-distillation extraction were compared for preparation of stock crude turmeric. By GC-MS analysis of major components in crude turmeric extract shows that the total turmerone (82.88%) in the former extract was higher than the latter extract (52.20%). Moreover, the cold solvent extraction method also provided more β-tumerone (21.40%), AR-tumerone (41.97%) and α-tumerone (19.51%) than the hydro-distillation extraction. Therefore, the crude turmeric from cold solvent extraction is used in subsequent study. The survival patterns of ST were determined in Trypicase Soy Broth (TSB). Results showed that after 10 min in TSB containing turmeric extract 0.05 mg/mL, the ST was decreased from 8.18 log CFU/ mL to 7.59 log CFU/mL (71.15% inhibition) and completely inhibited within 15 min. When using vinegar containing 1.7% (v/v) with pH 3.86, the ST was inhibited from initial population of 8.12 log CFU/mL to 7.94 log CFU/mL (34.09%) within 10 min and completely inhibited within 25 min. The combination effect of the mixture of turmeric extract (0.05 mg/mL) in vinegar (1.7%v/v) with pH 3.8 showed the significant inhibition of ST than individual use of The inhibition of bacteria growth may have been due to the interaction between vinegar and turmeric extract.**

Keywords- turmeric extract, fermented vinegar, Salmonella Typhimurium, inhibition, in vitro

I. INTRODUCTION

In recent years, *Salmonella* spp. has been increasingly concerned in food safety as the leading causes of foodborne bacterial diseases, causing human infections in particular by *S.* Typhimurium (ST). The ST commonly results in severe gastroenteritis and is non-typhoid. It also leads to degeneration into systemic invasive septicemia chronic condition [4]. Cross contamination with ST may still occur during the production cycle and originate from soil, water, equipment, animals and humans [7]. Therefore, the efficacy of sanitizing agents to control and reduce population of ST is studied depending on treatment conditions such as concentration and contact time [8]. However, higher concentrations of chemical sanitizers may cause residue and result in potential health risks. This is an important issue to search for novel antimicrobial compounds from natural sources instead of chemical substances. Many studies have focused on turmeric (*Curcuma longa* Linn). It is recognized as a rich source of essential oil, especially from rhizome and leaves. It contains antibacterial activity. The minimum concentration (MICs) of 15.62 and 125 µl/ml was found in rhizome and leaves, respectively [3]. In addition, the antibacterial potential of turmeric rhizome extracts had evaluated and found that it had a potential against pathogenic strains of Gram-positive (*Staphylococcus aureus, Staph. epidermidis*) and Gram negative (*E. coli, Pseudomonas aeruginosa, Salmonella* Typhimurium) bacteria [20]. Moreover, a quantity of 0.3 mL. of turmeric juice could against *E. coli, S.* Paratyphi, *S.* Typhimurium and *Staph. aureus* [21]. However, turmeric extract is limited in its application because of coloring compound of curcuminoids. Turmeric contains a major yellow pigment which amounts to about 3-6%. When it is dissolved in a base solution, it changes to a brownish-red color, while it has a light yellow color when it reacts with acid [15]. Normally, fermented vinegar can be applied for adjusting the pH condition in the acid solution. Also several studies have reported that it could inhibit the growth of microorganisms in various products such as fungi on dried yellow strip trivially fish [11], *Salmonella* Anatum of fresh pork loin [12], *S.* Enteritidis on surface of eggshell [23]. In addition, fermented vinegar was recommended to extend shelf life of fresh chicken [14] as well as fresh strawberries [8]. According to the report [18] had explained the reduction of *S.* Typhimurium on carrot was 0.79-3.95 log cfu/g by using lemon juice–vinegar mixture (1:1). Additionally, the efficacy cleaning method in reducing bacterial contamination of fresh produce such as apples, tomatoes and lettuce by using 5% vinegar solution as well as 13% lemon solution followed by rinsing the fruit and vegetables under tap water were reported [10]. Consequently, the purpose of this study was to investigate the reduction of *S.* Typhimurium in vitro by using turmeric extract, fermented vinegar and their mixture onward to a natural sanitizer instead of a chemical substance.

II. MATERIALS AND METHODS

A. Preparation of turmeric extract

Turmeric (*Curcuma longa* Linn.) was provided by the Applied Thai Traditional Medicine Center, Faculty of Medicine, Thammasat University, Patumthani. The moisture content of dried turmeric was investigated [16]. Dried turmeric was kept in a plastic zip bag at room temperature

before extraction. The two extraction methods of turmeric were used as follows:

- Cold solvent extraction method or maceration method [22]. Firstly, 100g of dried turmeric were soaked in 200 mL of 95% ethanol for 72 h at ambient temperature (30± 2°C). After maceration, the mixtures were then filtered by vacuum and, then, the filtrates were evaporated by a vacuum rotary evaporator at 60°C and 175 mbar. Finally, 2g of crude extract was diluted with 8 mL of 10% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 0.2 mg/mL which was collected for stock solution and kept in a dark bottle at 4°C until use.
- The elemental hydro-distillation method [2], 100g dried turmeric and 500 ml distilled water were placed in a 1,000 mL round bottomed flask. Distillation was conducted continuously for 60 h. After turning on the valve to remove the distilled water, the essential oil was collected as stock solutions and kept into a dark bottle and stored at 4°C until use.

B. *Analysis of major components of turmeric extract by GC-MS method*

Turmeric extracts were analyzed by the GC-MS using 6890n GC (Agilent, USA) equipped with a 5973-GC mass selective detector. At this stage, the turmeric extracts from both cold solvent extraction and hydro-distillation methods were determined for their major components by the optimal condition recommended [2] as shown in Table 1.

TABLE 1. GC/MS OPERATING CONDITIONS FOR ANALYSIS OF COMPONENTS AND CONSTITUENTS OF TURMERIC EXTRACTS.

Equipments/Operating conditions	Details
Column	Capillary column DB-5
Dimension of film thickness	30 m x 250 µm i.d. x 0.25 µm
Carrier Gas	Helium
Column Flow rate (mL/min)	1
Injector Temp. • Initial Temperature (°C) • Final Temperature (°C)	 50 300
Injection mode	Splitless mode
Injection flow rate (µL)	1

As a result of the analysis, the extract from an appropriate extraction method was used in subsequent study of the inhibition effect on *S.* Typhimurium.

C. *Preparation of fermented vinegar*

Fermented vinegar, My garden brand (AGRONEGAR Co, Ltd., Thailand), was used in this study. The vinegar stock solutions were prepared at a various acetic acid concentration of 1%-3% from the original vinegar containing 10% acetic acid concentration and, then, kept in a dark bottle and stored at 37°C.

D. *Microorganism preparation*

The *S.* Typhimurium (ST) ATCC 13311 was provided by the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nontaburi, Thailand. The elemental analysis [17], one loop full of ST was transferred to 20 mL of trypticase soy broth (TSB) and incubated at 37ºC for 18-20 h. Subsequently, the suspension was diluted with 80 mL of 0.1% peptone solution in order to achieve the ratio of suspension 1:5. The density was adjusted to 0.5 McFarland Standard to achieve concentration of cell number 10^7cfu/mL

E. *In vitro inhibition test of ST by various concentration of turmeric extract (TE)*

The TE obtained from an appropriate method [22] after analysis by GC/MS was used to prepare a concentration of 0.1, 0.05, 0.01, 0.005 and 0.001(mg/mL) in TSB by calculating volume from stock solution with a final volume of 10 mL in each tube. The 1 mL of ST suspension containing 10^7 cfu/mL was inoculated into each tube. After incubating for 0, 5, 10, 15, 20, 25 and 30 min, the survival ST in each tube was enumerated using a spread plate technique on TSA and XLD. The three replicates were conducted.

F. *In vitro inhibition test of ST by various concentration of fermented vinegar (FV)*

The in vitro inhibition test of ST by FV was conducted by modified method [12]. The vinegar solutions containing acetic acid concentration of 1%-3% with 0.1% interval were prepared in TSB by calculating based on the original vinegar containing 10% acetic acid concentration with a final volume of 10 mL in each tube. Then, the 1 mL of ST suspension containing 10^7 cfu/mL was inoculated into each tube. One tube was to measure pH. The other tubes were incubated for 0, 5, 10, 15, 20, 25 and 30 min at 37ºC. The survival ST in each tube was enumerated using a spread plate technique on TSA and XLD. The three replicates were conducted.

G. *In vitro inhibition test of ST by the mixture of turmeric extract (TE) and fermented vinegar (FV)*

The appropriate concentrations of TE and FV from previous experiments were used in this study. Based on modified method [18], the mixture was prepared to a suitable concentration (w/v) in TSB by calculating with a final volume of 10 mL in each tube. The 1 mL of ST suspension containing 10^7 cfu/mL was inoculated into each tube. After incubating for 0, 5, 10, 15, 20, 25 and 30 min, the survival ST in each tube was enumerated using a spread plate technique on TSA and XLD. The three replicates were conducted.

H. *Statistical analysis*

The experiments were 7x5 and 7x10 factorial in completely randomized design for turmeric extract and fermented vinegar, respectively. Data were interpreted by using SPSS Version11.5. Analysis of variance (ANOVA) was determined. Additionally, Duncan's new multiple range test (DMRT) at $P \leqslant 0.05$ significance level was used to determine the differences between treatments.

III. RESULTS AND DISCUSSION

A. Major components of turmeric extract (TE)

Sliced dehydrated turmeric contained an average moisture content of 7.19±0.02% (Moisture, % by weight). Two methods consisting of cold solvent extraction and hydro-distillation extraction were used to extract components of turmeric. The TE was subsequently identified for their major components by GC-MS method. Results in Table 2 that indicated that β-tumerone, AR-tumerone, α-turmerone and total turmerone in the former extract was higher than the latter extract. Moreover, hydro-distillation method provided more α-terpinolene and phellandrene in the extract. As shown in the report [19], the turmeric oil consisting of ketone group such as Ar-turmerone, β-tumerone, α-tumerone and AR-curcumyl alcohol in crude extract provided an effective bacterial inhibition. Consequently, the extract of cold solvent extraction method provided more important components than the extract of hydro-distillation method, as shown in Table2. Then, the TE from cold solvent extraction method was used in subsequent studies for inhibition of ST.

TABLE 2. COMPARISON OF COMPONENTS OF TURMERIC EXTRACT FROM COLD SOLVENT EXTRACTION METHOD AND HYDRO-DISTILLATION METHOD BY GC-MS ANALYSIS.

Extraction method	Components (%Area)*						
	Phellandrene	Limonene	α-Terpinolene	β-Turmerone	AR-Turmerone	α-Turmerone	Total Turmerone
Cold solvent extraction	-	-	-	21.4	41.97	19.51	82.88
Hydro-distillation	1.21	-	4.67	16.9	20.3	15	52.2

*Analyzed by GC-MS model Agilent 6890n GC with5973GC/MSD, Agilent USA

B. In vitro inhibition test of ST by various concentration of turmeric extract (TE)

Text Survival of ST in TSB by various concentration of TE concentration at 0.1, 0.05, 0.01, 0.005 and 0.001 mg/mL (w/v) in vitro was shown in Table 3. In no turmeric extract treatment, the initial population of ST was 8.14 log CFU/mL and, then, increased to 8.17 log CFU/mL after 30 min of incubation time. At 0.01 mg/mL concentration of TE, the ST was completely inhibited within 30 min. It could be noticed that more concentration of TE was used, the decrease of ST was resulted. In the study [13] showed that *Salmonella* spp. was inhibited by 0.016 mg/mL concentration of TE which extracted by 95% ethanol. Our result at 0.01mg/mL concentration of TE provided the similar result as mentioned [13]. Although the complete inhibition of ST was found at 0.01 mg/mL concentration of TE, but it needs longer period of TE treatment, a 30 min. However, the TE at higher concentration such as 0.05 mg/mL provided exponential reduction of ST when compared with 0.01 mg/mL. Consequently, it was used for subsequently study due to the extraction cost and suitable contact time.

TABLE 3. SURVIVAL OF ST IN TSB AND PERCENTAGE OF REDUCTION OF ST AFTER TREATMENT WITH VARIOUS OF TURMERIC EXTRACT (TE) CONCENTRATION AT 31±10C FOR 30 MIN.

TE(mg/ml)	Surviving population of ST *in Vitro* (log CFU/ml)* during contact time for 30 min.							Percentage of Reduction of ST					
	0	5	10	15	20	25	30	5	10	15	20	25	30
Control***	8.14 ^{aa} ± 0.00	8.15 ^{Ba} ± 0.01	8.15 ^{Ca} ± 0.01	8.15 ^{Ba} ± 0.01	8.14 ^{Ea} ± 0.00	8.15 ^{Fa} ± 0.02	8.16 ^{Ga} ± 0.02	-	-	1.70	6.81	-	-
0.001	8.14 ^{Aa} ± 0.01	8.13 ^{Ba} ± 0.01	8.10 ^{Cb} ± 0.02	8.10 ^{Db} ± 0.02	8.05 ^{Eb} ± 0.01	8.01 ^{Fb} ± 0.02	8.03 ^{Gb} ± 0.02	1.90	8.33	9.76	19.52	25.48	23.57
0.005	8.13 ^{Ac} ± 0.04	8.12 ^{Bb} ± 0.02	8.08 ^{Cc} ± 0.03	8.04 ^{Dc} ± 0.03	7.97 ^{Ec} ± 0.02	8.03 ^{Fc} ± 0.05	7.88 ^{Gc} ± 0.03	2.67	11.89	19.66	32.04	41.50	42.48
0.01	8.13 ^{Ad} ± 0.01	8.08 ^{Bd} ± 0.02	8.02 ^{Cd} ± 0.01	7.97 ^{Dd} ± 0.02	7.89 ^{Ed} ± 0.00	7.63 ^{Fd} ± 0.12	0.00 ± 0.00	10.24	21.71	30.24	42.20	68.29	**100.00**
0.05	8.13 ^{Ae} ± 0.01	7.91 ^{Be} ± 0.05	7.59 ^{Ce} ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	39.61	71.15	**100.00**	**100.00**	**100.00**	**100.00**
0.1	8.13 ^{Af} ± 0.03	7.50 ^{Bf} ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	77.05	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Surviving population of ST enumerated on XLD. Means of three replication with standard deviations (SD).

** Different letters in the same row means no significant difference in survival population of ST of contact time for 30 min by DMRT. ($p<0.05$).

Different letters in the same column means no significant difference in survival population of ST of turmeric concentration by DMRT. ($p<0.05$).

***Samples indicate no exposure of Turmeric solution.

C. In vitro inhibition test of ST by various concentration of fermented vinegar (FV)

The results in Tables 4 and 5 showed the survival of ST in TSB treated with FV containing 1-3% acetic acid concentration. It was found that the initial population of ST in no treatment of FV with pH 7.48 was 8.14 log CFU/ml and increased to 8.17 log CFU/ml after 30 min of incubation. The 1.7% acetic acid concentration of FV caused complete inhibition of ST after treatment in TSB for 25 min. Simultaneously, it is commonly noticed that increase of acetic acid concentration of FV caused the drop of pH as shown in Fig.1. But it was related to the gradual decrease of ST growth in TSB. However, more acetic acid concentration of FV increased, more effective inhibition of ST was observed. This result belonged to the mode of action of acetic acid attributed to direct pH reduction, depression of the internal pH of ST cells by ionization of the un-dissociated acid molecule, or disruption of substrate transport by cell membrane permeability as affected to inhibition mechanisms of the pathogens, ST [5].

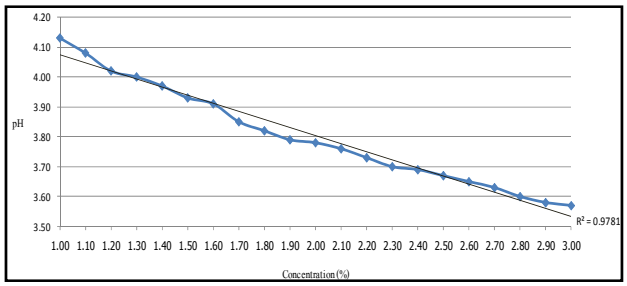

Figure 1. The pH of TSB at various acetic concentration in fermented vinegar at 31±10C.

TABLE 4. SURVIVAL OF ST IN TSB AND PERCENTAGE OF REDUCTION OF ST AFTER TREATMENT WITH 1-2% ACETIC CONCENTRATION OF FERMENTED VINEGAR (FV) AT 31±10C FOR 30 MIN.

FV (V/V)	Surviving population of ST *in Vitro* (log CFU/ml)* during contact time for 30 min							Percentage of Reduction of ST					
	0	5	10	15	20	25	30	5	10	15	20	25	30
Control***	8.13 ^{Ae**} ± 0.01	8.16 ^{Ab} ± 0.01	8.14 ^{Ab} ± 0.02	8.12 ^{Ad} ± 0.01	8.10 ^{Ae} ± 0.01	8.15 ^{Ag} ± 0.02	8.16 ^{Cf} ± 0.02	-	-	1.70	6.81	-	-
1.0	8.13 ^{Be} ± 0.01	8.10 ^{Bb} ± 0.01	8.10 ^{Bc} ± 0.01	8.01 ^{Cd} ± 0.02	8.05 ^{Be} ± 0.01	8.00 ^{Bg} ± 0.01	8.02 ^{Dh} ± 0.03	5.62	7.58	23.23	17.36	25.92	22.74
1.1	8.14 ^{Ce} ± 0.03	8.11 ^{Ab} ± 0.03	8.12 ^{Bc} ± 0.03	8.06 ^{Bd} ± 0.05	8.06 ^{Bf} ± 0.03	8.00 ^{Bg} ± 0.03	7.98 ^{Ah} ± 0.01	5.78	3.13	16.63	15.66	26.51	30.12
1.2	8.12 ^{De} ± 0.04	8.01 ^{Bb} ± 0.04	7.98 ^{Cc} ± 0.01	7.94 ^{Cd} ± 0.01	7.93 ^{Cf} ± 0.02	7.90 ^{Cg} ± 0.05	7.89 ^{Bh} ± 0.03	22.94	26.93	33.67	35.41	39.90	40.90
1.3	8.11 ^{Ee} ± 0.05	8.00 ^{Cb} ± 0.01	7.97 ^{Dc} ± 0.02	7.93 ^{Dd} ± 0.02	7.91 ^{Df} ± 0.02	7.91 ^{Bg} ± 0.04	7.87 ^{Ch} ± 0.03	22.05	27.18	34.36	37.31	36.54	41.92
1.4	8.11 ^{Ee} ± 0.03	8.00 ^{Cb} ± 0.04	7.96 ^{Dc} ± 0.05	7.92 ^{Dd} ± 0.02	7.88 ^{Ef} ± 0.02	7.84 ^{Dg} ± 0.06	7.69 ^{Ch} ± 0.05	21.99	29.92	36.06	41.18	46.04	61.89
1.5	8.14 ^{Ce} ± 0.02	7.99 ^{Db} ± 0.02	7.95 ^{Ec} ± 0.02	7.89 ^{Ed} ± 0.02	7.90 ^{Ef} ± 0.04	7.81 ^{Eg} ± 0.03	7.52 ^{Dh} ± 0.06	28.52	35.02	42.72	42.48	53.07	75.69
1.6	8.12 ^{De} ± 0.02	7.92 ^{Eb} ± 0.06	7.95 ^{Ec} ± 0.02	7.86 ^{Fd} ± 0.03	7.88 ^{Ef} ± 0.03	7.87 ^{Eg} ± 0.03	7.47 ^{Ch} ± 0.12	37.31	33.33	45.52	43.28	43.53	77.86
1.7	8.12 ^{Be} ± 0.01	7.92 ^{Eb} ± 0.03	7.94 ^{Ec} ± 0.02	7.65 ^{Gd} ± 0.02	7.52 ^{Ge} ± 0.04	0.00 ± 0.00	0.00 ± 0.00	37.11	34.09	65.79	74.84	**100.00**	**100.00**
1.8	8.11 ^{Ce} ± 0.02	7.89 ^{Fb} ± 0.03	7.87 ^{Fc} ± 0.03	7.69 ^{Hd} ± 0.05	7.43 ^{He} ± 0.03	0.00 ± 0.00	0.00 ± 0.00	38.92	42.01	61.73	80.41	**100.00**	**100.00**
1.9	8.12 ^{De} ± 0.03	7.87 ^{Fb} ± 0.03	7.56 ^{Gc} ± 0.04	**7.31** ^{Id} ± 0.02	**0.00** ± 0.00	0.00 ± 0.00	**0.00** ± 0.00	43.72	72.11	80.40	**100.00**	**100.00**	**100.00**
2.0	8.11 ^{De} ± 0.02	7.78 ^{Gb} ± 0.07	**7.47** ^{Hc} ± 0.02	**0.00** ± 0.00	**0.00** ± 0.00	0.00 ± 0.00	**0.00** ± 0.00	[illegible]	77.61	**100.00**	**100.00**	**100.00**	**100.00**

Surviving population of ST enumerated on XLD. Means of three replication with standard deviations (SD).
** Different letters in the same row means no significant difference in survival population of ST of contact time for 30 min by DMRT. ($p<0.05$).
Different letters in the same column means no significant difference in survival population of ST of turmeric concentration by DMRT. ($p<0.05$).
***Samples indicate no exposure of Turmeric solution.

TABLE 5. SURVIVAL OF ST IN TSB AND PERCENTAGE OF REDUCTION OF ST AFTER TREATMENT WITH 2.1-3% ACETIC CONCENTRATION OF FERMENTED VINEGAR (FV) AT 31±10C FOR 30 MIN.

FV 2-3% (V/V)	Surviving population of ST *in Vitro* (log CFU/ml)* during contact time for 30 min.							Percentage of reduction of ST					
	0	5	10	15	20	25	30	5	10	15	20	25	30
Control***	8.13 ^{Ae**} ± 0.01	8.16 ^{Ab} ± 0.01	8.14 ^{Ab} ± 0.02	8.12 ^{Ad} ± 0.01	8.10 ^{Ae} ± 0.01	8.10 ^{Ag} ± 0.01	8.15 ^{Ag} ± 0.02	-	-	1.70	6.81	-	-
2.1	8.13 ^{Ae} ± 0.03	7.66 ^{Bb} ± 0.05	7.02 ^{Cc} ± 0.15	0.00 ^{Dd} ± 0.06	6.42 ^{Ce} ± 0.32	6.42 ^{Cg} ± 0.32	0.00 ± 0.00	65.85	94.03	**100.00**	**100.00**	**100.00**	**100.00**
2.2	8.11 ^{Ae} ± 0.04	7.78 ^{Bb} ± 0.07	6.90 ^{Cc} ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	52.96	100.00	**100.00**	**100.00**	**100.00**	**100.00**
2.3	8.08 ^{Ae} ± 0.02	7.44 ^{Bb} ± 0.07	0.00 ^{Cc} ± 0.32	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	77.41	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
2.4	8.09 ^{Ae} ± 0.04	7.40 ^{Bf} ± 0.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	79.52	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
2.5	8.11 ^{Ae} ± 0.03	0.00 ^{Bb} ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
2.6	8.09 ^{Ae} ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
2.7	8.10 ^{Ae} ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
2.8	8.12 ^{Ae} ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
2.9	8.11 ^{Ae} ± 0.01	0.00 ± 0.00	0.00 ± 0.00	**0.00** ± 0.00	**0.00** ± 0.00	0.00 ± 0.00	**0.00** ± 0.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
3.0	8.10 ^{Ae} ± 0.02	0.00 ± 0.00	**0.00** ± 0.00	**0.00** ± 0.00	**0.00** ± 0.00	0.00 ± 0.00	**0.00** ± 0.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Surviving population of ST enumerated on XLD. Means of three replication with standard deviations (SD).
** Different letters in the same row means no significant difference in survival population of ST of contact time for 30 min by DMRT. ($p<0.05$).
Different letters in the same column means no significant difference in survival population of ST of turmeric concentration by DMRT. ($p<0.05$).
***Samples indicate no exposure of fermented vinegar solution.

D. In vitro inhibition test of ST by the mixture of turmeric extract (TE) and fermented vinegar (FV)

The mixed solution of 0.05% turmeric extract (TE) and 1.7% acetic acid concentration of fermented vinegar (FV) called "TEFV" was prepared for this study. The original pH of TEFV was 3.86.

TABLE 6. SURVIVAL OF ST IN TSB AND PERCENTAGE OF REDUCTION OF ST AFTER TREATMENT WITH 0.05 MG/ML TURMERIC EXTRACT (TE), 1.7% ACETIC ACID CONCENTRATION OF FERMENTED VINEGAR (FV) AND MIXTURE OF TURMERIC EXTRACT AND FERMENTED VINEGAR (TEFV) AT 31±10C FOR 30 MIN.

Treatment solution	Surviving population of ST in Vitro (log CFU/ml)* during contact time for 30 min.							Percentage of ST of reduction					
	0	5	10	15	20	25	30	5	10	15	20	25	30
TE 0.05 mg/ml	8.13 ± 0.01	7.91 ± 0.05	7.59 ± 0.09	**0.00 ± 0.00**	**0.00 ± 0.00**	0.00 ± 0.00	**0.00 ± 0.00**	39.61	71.15	**100.00**	**100.00**	**100.00**	**100.00**
FV 1.7% (V/V)	8.12 ± 0.01	7.92 ± 0.03	7.94 ± 0.02	7.65 ± 0.02	7.52 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	37.11	34.09	65.79	74.84	**100.00**	**100.00**
TEFV	8.10 ± 0.01	7.52 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	73.28	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

*
Surviving population of ST enumerated on XLD. Means of three replication with standard deviations (SD).

Results shown in Table 6 revealed that the 73.28% reduction of ST, from initial 8.10 log CFU/mL to 7.52 log CFU/mL, after 5 min of treatment of TEFV in TSB. Additionally, the complete inhibition of ST by TEFV was noticed at 10 min of treatment. Moreover, the ST was completely inhibited by individual TE and FV within 15 and 25 min ($p<0.05$), respectively. These results indicated that the TEFV was more effective inhibition activity than the individual TE as well as FV at its recommended concentration.

IV. CONCLUSIONS

The crude turmeric extract from cold solvent extraction method provided more effective inhibition activity on growth of S. Typhimurium (ST) than that from hydro-distillation method. As a result of cold solvent extraction, it consisted high major components such as β-turmerone, AR-turmerone, α-turmerone and total turmerone which provides the bacterial inhibition activity.

The combination of turmeric extract (TE) and fermented vinegar (FV) provided more effective inhibition activity on ST in vitro than individual use of TE or FV ($p<0.05$). The 0.05 mg/mL TE and 1.7% (v/v) acetic acid concentration of FV were recommended for preparation of TEFV mixture. The effective inhibition activity of TEFV on ST belongs to the integration of anti-bacterial property of both acetic acid in FV and major components of TE. In case of acetic acid, it is attributed to direct pH reduction by ionization of the un-dissociated acid molecule, or disruption of substrate transport into metabolism of ST cell membrane [9]. Furthermore, the turmerone, an anti-bacterial agent, from TE causes effective damage of bacterial cells in both inner and outer cell membrane. All substances in cytoplasm of bacterial cells are continually released externally and, finally, caused the death of bacterial cells. [1]. Therefore, as a result of this study, mixture of TEFV is recommended as a suitable natural sanitizer against the risk of chemical residue from chemical sanitizer for increasing safety for food consumers.

ACKNOWLEDGMENT

We would like to thank for technical support of extraction methodology and raw material from Associate Professor Dr Arunporn Itharat of Applied Thai Traditional Medicine Center, Faculty of Medicine, Thammasat University, Patumthani, Thailand. We are very appreciated to National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nontaburi, Thailand for providing S. Typhimurium culture sample.

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