Evaluation of Antimicrobial Activity and Synergistic Effect of Spices against Few Selected Pathogens

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ABSTRACT

Objectives: The main objective of this study was to evaluate antimicrobial activity of ethanolic extract of spices along with determination of its synergistic effect against few selected pathogens.

Methods: In this study, ethanolic extract of 5 different spices; *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), *Curcuma longa* (Turmeric), *Capsicum annum* (Chili) and *Allium cepa* (Onion) were obtained by using Soxhlet apparatus. The ethanolic extract was concentrated by evaporation and different concentrations of extract were prepared in Dimethy Sulphoxide (DMSO) solvent. Test organisms included mainly pathogens i.e. *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae*. The antimicrobial activities of the extracts were determined by well diffusion technique both individually and in combination. On the other hand, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was determined by serial dilution technique. The result were interpreted on the basis of the fact that the growth occurs in positive control and other tubes with inadequate amount of extract whereas the lowest concentration of agent that inhibits growth of organism, detected by lack of visible turbidity by inhibition of 99% is designed as the MIC. The MBC is identified by determining the lowest concentration of extract solution that reduces the viability of the initial bacterial inoculum by a predetermined reduction such as ≥99.9%. Likewise, for determination of Fractional Inhibitory Concentration Index (FICI), two extracts were combined along with standardized inoculum of bacterial strain. Tubes without visible turbidity were streaked on agar plate and observed for 99.9% killing.

Results: All the tested extract of spices were found effective against *S. aureus* and *K. pneumoniae* only. The highest zone of inhibition (ZOI) was found in chili extract (ZOI=26 mm) against *S. aureus* whereas lowest zone of inhibition was found in garlic extract against *K. pneumoniae* (ZOI=12mm). Similarly, highest ZOI was produced by combined extract of both Turmeric and Ginger (ZOI= 26 mm). Turmeric extract was found to be effective against *S. aureus* (MIC value = 62.5 mg /ml and MBC value = 31.25 mg/ml) and *K. pneumoniae* (MIC value 125 mg/ml and MBC value = 62.5 mg/ml). The Fractional Inhibitory Concentration (FIC) values of combined extract suggested synergistic and additive effect (0.5<FIC<1). Chili and ginger were effective with FIC value of 0.25.

Conclusion: To recapitulate, the extract of spices can be used to prevent the pathogenic organism.

Key words: Dimethyl Sulphoxide, Minimun Inhibitory Concentration, Minimum Bactericidal Concentration, Fractional Inhibitory Concentration Index

INTRODUCTION

Spices are indispensable components of Nepalese cuisines since ancient times and are considered medicinal purposes for several centuries due its extensive antimicrobial and antioxidant property. The activity of herbs and spices are not only limited to boosting flavor, but also recognized for their preservative and medicinal value (Panpatil et al. 2013). Food-borne illness caused by consuming food and

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beverages contaminated with bacteria, fungi can cause symptoms that range from upset stomach to more serious symptoms such as diarrhea, fever, vomiting, abdominal cramp, dehydration. Fifty years of increasing uncontrolled use of chemical antimicrobials have created a situation leading to an ecological imbalance and enrichment of multiple multi-resistant pathogen microorganisms (Aktug and Karapinar 1986). The burgeoning concern about safety of foods has recently led to the development of natural antimicrobials to control food borne pathogens. Addition of spices in foods imparts flavor and pungent stimuli and more importantly its natural products and naturally derived components shows antimicrobial property.

Ginger, a member of family Zingerberaceae, is an erect perennial plant growing from one to three feet in height; its stem is surrounded by sheathing bases of the two ranked leaves. Fresh ginger has been used for cold induced diseases, nausea, asthma, cough, colic heart palpation, dyspepsia, loss of appetite and rheumatism (Tyler 2002). Garlic (*Allium sativum*), belongs to Alliaceace, comprise numerous discrete cloves whereas leaves and stems are sometimes eaten, particularly whole immature and tender. It is claimed to help prevent heart disease including antherosclerosis, high cholesterol, high blood pressure and to improve immune system as well as protection against cancer (Maryland 2006). The onion (*Allium cepa*) belongs to Liliaceae family consisting of herbaceous plant part and edible bulb part; rich in proteins, carbohydrate, sodium, potassium and phosphorus (Cox et al. 2000). They are effective against common cold, heart disease, diabetes, osteoporosis, coughs and sore throat (Juven et al. 1994). Turmeric (*Curcuma longa*) is a member of the ginger family Zingiberceace. Various sesquiterpenes and curcuminoids have been isolated from the rhizome of *C. longa*, attributing a wide array of biological activities, anti-inflammatory, wound healing, anticancer and antibacterial (Sandur et al. 2007). The chili pepper (*Capsicum annum*), a member of night shade family, Solanaceae, is a diploid, facultative self-pollinating and closely related to potato, tomato, eggplant (UN FAOSTAT 2014). Capsaicin, a well-studied chemical component of *Capsicum* species has already demonstrated a high degree of biological activity affecting nervous, cardiovascular, digestive system (Britto et al. 2009). Chemical analysis has demonstrated that capsicum fruits contain relatively high concentration of several essential nutrient including vitamin C up to 6 times the concentration of orange (Sagdic et al. 2003)

Therefore, the investigation of antimicrobial properties of spices used as food additives to control the growth of food-borne pathogens may give useful results. But the main obstacle for using spices and medicinal plants as food preservatives is that their high concentration is required in food to inhibit the microbial growth as well as oxidation. This high concentration of spices and herbs causes negative organoleptic effects i.e. alter the taste, color, odor and texture of food and limit their use in food preservatives system as well as to develop safe and potent antimicrobial and antioxidant food preservatives from them (Burt 2004).

Several researchers have investigated the individual effects of essential oils of spices and medicinal plants on antimicrobial activity against food borne bacteria as well as antioxidant activity. Study performed by Maharjan et al. (2012) compares the sensitivity of some human pathogenic bacteria to various spice extract viz. essentials oils, acetone and methanol extracts by agar well diffusion method. Of the different spices tested clove, ajowan and cinnamon were found to possess relatively higher antimicrobial activities. The MBC value ranged from 0.39 to 25mg/ml. The lowest MBC value was given by essential oil of cinnamon against *E. coli, S. aureus and S.*Typhi. In contrast to these studies, this research mainly focuses on the spices that are used in food items in regular basis and focuses on synergistic interaction of selected spices against selected pathogens as their combination effects on both antimicrobial and antioxidant activities seem to be dubious. This approach may increase the antimicrobial and antioxidant efficacy at sufficiently low concentration by taking their advantages of possible synergistic interaction which may reduce their adverse side effects as well as negative organoleptic effect in food and facilitate their use in food preservation system (Foster 2004) .Therefore, the main aim of the study is to shed some light on the antimicrobial potency of spices used in Nepal with main regard to synergistic activity of spices.

MATERIALS AND METHODS

Collection of spices: Five different fresh spices viz., ginger, garlic, turmeric, chili, and onion free from disease were collected from various places of Lalitpur. The study was carried out in Microbiology laboratory of Pinnacle College, Lagankhel, Kathmandu.

Soxhlet extraction with 96% ethanol: Thoroughly washed dried leaves of 5 selected spices were dried under shade at room temperature for four days. The dried samples were cut into pieces by means of plant cutter and subjected to grinding. 25 gm of obtained dried powder was subjected to soxhlet extractor along with 150ml of 96% ethanol followed by filtration using Whatmann filter (No 1). For removal of ethanol from the extract, it was placed on evaporating dish placed over Bunsen burner. Finally, dense extract was diluted in 10% Dimethyl Sulphoxide (DMSO) to obtain standard working solution (Rajendhran 2008).

Preparation and standardization of inoculum: The Gram positive (*S. aureus*) and Gram negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa*) precultured in nutrient broth and incubated for about 2 hours. The turbidity of inoculum was adjusted by using Mc farland standard as a reference. The tubes were compared with turbidity of 0.5 Mc farland solution (1-2* 10^8 cfu/ml) (Tandukar et al. 2017).

Phytochemical screening: Phytochemical screening was carried out on ethanolic extract of spices for detecting its chemical composition especially for tannins (5% $FeCl_3$), flavonoids (1% NH_3)**,** terpenoids (0.5 chloroform) and alkaloids (dil HCl + Mayer's reagent) were employed during the study (Byadgi 2018).

Determination of antimicrobial activity: Antimicrobial activity of spices against the selected organism was determined by Agar well diffusion technique under aseptic condition. 20ml of sterilized molten Muller Hinton Agar was poured into sterile petri plates; after solidification, fleshly prepared inoculum was swabbed on respective plates with the aid of sterile cotton swab. By using sterile cork borer no 6, wells (diameter-7mm) were made in inoculated media plates which was finally filled with 50µl of working solution of different spices. The diameter of zone of inhibition was measured after incubation at 37^0 C for 24 hours (Dingle et al. 2009).

Determination of MIC and MBC: The crude extract which showed antimicrobial and DMSO solution were subjected to two-fold serial dilution method by Finegold and Baron (2014) to determine MIC and MBC. For each bacterium, a set of dry screw capped test tubes were taken and labeled as 1,2,3,4,5,6,7,8,9,10,11 – Tube no .1 taken as positive control (2ml plant extract) and Tube no. 11 as negative control (Nutrient broth). By mixing nutrient broth and plant extract followed by process of homogenization and dilution, the concentration of tube no 4, 5, 6, 7, 8, 9 and 10 becomes 125, 62.5, 31.25, 15.63, 7.81 and 1.92 mg/ml which is further added with 50µl of 4hrs culture of microorganism. All the tubes were incubated at 37^0 C for 24 hrs and observed turbidity by comparing with positive negative control. For MBC calculation, the tubes were sub cultured on nutrient agar plate and incubated at 37^0 C for 24 hrs. Then they were examined for the growth of bacteria.

Determination of synergistic activity: For detection of synergistic activity, combination of extract – Ginger & Garlic, Ginger & Chili, Turmeric & Ginger, Tumeric & Chili and Garlic & Onion – was prepared by mixing 2ml of each extract. Same procedure was performed as done for detection of antimicrobial activity of spices individually (Al-Mahmood 2009).

Determination of fractional Inhibitory concentration index (FICI): FIC index was determined by Multiple combinations bacterial testing (MCBT); two extracts were combined in test tube along with standardized inoculum of the bacterial cultured. Wells without visible turbidity was sampled by streaking a 10µl aliquot on agar plate, incubating for a day and observing 99.9% killing (Singh 2015).

RESULTS

A total of 6 different spices (Ginger, Gralic, Turmeric, Onion and Chili) were included in this study, collected from local houses of Lalitpur area. The crude extracts of spices were tested against altogether four pathogens.

Under phytochemical screening, tannins were present in all spices except garlic whereas flavinoids were present only in turmeric and onion. Furthermore, terpenoids were present in garlic, turmeric and onion only whereas the alkaloids were present in all spices except turmeric.

Table 1: Phytochemical screening of crude ethanolic extract of spices

Phytochemical test	Reagent used	Spices	Observation
Tannins	5% FeCl3	Ginger	+
		Garlic	-
		Turmeric	+
		Chili	+
		Onion	+

Phytochemical test	Reagent used	Spices	Observation
Flavonoids	1% NH_3	Ginger	-
		Garlic	-
		Turmeric	+
		Chili	-
		Onion	+
Terpenoids	0.5ml	Ginger	-
	Chloroform	Garlic	+
	& 1ml conc	Turmeric	+
	H_2SO_4	Chili	-
		Onion	+
Alkaloids	Mayer's	Ginger	+
	Reagent	Garlic	+
		Turmeric	-
		Chili	+
		Onion	+

All the four selected pathogens were tested with specific antibiotics by using Kirby-Brauer disk diffusion method. According to Clinical and Laboratory Standard Institute (CSLI 2014), six different sets of antibiotics were used to determine antibiotic susceptibility pattern of selected pathogens. Among them *E. coli* was seen resistant to most of antibiotic except Gentamicin (ZOI = 18mm). *S. aureus* was found highly sensitive to ceftriaxone with ZOI of 22mm but resistant to Amoxyclav and Bacitracin only. Similarly, *K. pneumoniae* was highly sensitive to Gentamicin (ZOI = 23mm) and resistant to Ceftriaxone whereas Ciprofloxacin was found most effective against *P. aeruginosa* (ZOI = 35mm).

Table 2: Antibiotic susceptibility pattern of selected test organisms

Test organisms	Antibiotic disc	Symbol	Concentration	Diameter of ZOI(mm)	Inference
S. aureus	Chloramphenicol	C	10mcg	21mm	Sensitive
	Gentamicin	GEN	10mcg	18mm	Sensitive
	Amoxiclav	AC	30mcg	12mm	Resistant
	Ceftriaxone	CTR	30mcg	21mm	Sensitive
	Ciprofloxacin	CIP	5mcg	22mm	Sensitive
	Bacitracin	B	8mcg	-	Resistant
E. coli	Chloramphenicol	C	10mcg	-	Resistant
	Gentamicin	GEN	10mcg	18mm	Sensitive
	Nitrofurantoin	NIT	100mcg	-	Resistant
	Ceftriaxone	CTR	30mcg	-	Resistant
	Nalidixic acid	NA	30mcg	-	Resistant
	Erythromycin	E	10mcg	-	Resistant
K. pneumoniae	Chloramphenicol	C	10mcg	13mm	Intermediate
	Gentamicin	GEN	10mcg	23mm	Sensitive
	Nitrofurantoin	NIT	100mcg	18mm	Sensitive
	Ceftriaxone	CTR	30mcg	-	Resistant
	Nalidixic acid	NA	30mcg	22mm	Sensitive
	Amoxyclav	AC	30mcg	8mm	Resistant
P.aeruginosa	Chloramphenicol	C	10mcg	-	Resistant
	Gentamicin	GEN	10mcg	32mm	Sensitive
	Amoxyclav	AC	30mcg	-	Resistant
	Ciprofloxacin	CIP	5mcg	35mm	Sensitive
	Nalidixic acid	NA	30mcg	-	Resistant

Antimicrobial activity was evaluated by two ways viz measuring zone of inhibition and quantitative determination of spices extract for MIC and MBC. Among four pathogens, spices extracts were found most effective against *S. aureus* and *K. pneumoniae* only. Chili extract proved to be most effective against *S. aureus* with zone of inhibition of 26mm. However, garlic and onion extract were found least effective.

Table 3: Antimicrobial activity of selected crude extract of spices against microorganisms

Spice extract	Diameter of zone of inhibition (mm) against microorganisms			
	S. aureus	*E. coli*	*K. pneumonia*	*P. aeruginosa*
Ginger	16mm	-	12mm	-
Garlic	15mm	-	14mm	-
Turmeric	16mm	-	14mm	-
Chili	26mm	-	14mm	-
Onion	15mm	-	14mm	-
DW	-	-	-	-
DMSO (10%)	-	-	-	-

The minimal inhibitory concentrations and minimum bactericidal concentrations for the spices extracts against examined bacterial strains are presented in table no (4). The lowest MIC and MBC which could inhibit microbial which could inhibit microbial growth was recorded for Turmeric. From the microbial sensitivity side of view, *K. pneumoniae* was the most sensitive bacteria to the examined spices with MIC of 31.25mg/ml and MBC of 62.5mg/ml.

Table 4: Minimum inhibitory concentration (mg/ml) and minimum bactericidal concentration of various spice extracts

spices extract	Minimum Inhibitory Concentration		Minimum Bactericidal Concentration	
	S. aureus	*K. pneumoniae*	*S. aureus*	*K. pneumoniae*
Ginger	500mg/ml	500mg/ml	1000mg/ml	1000mg/ml
Garlic	500mg/ml	500mg/ml	1000mg/ml	1000mg/ml
Turmeric	62.5mg/ml	31.25mg/ml	125mg/ml	62.5mg/ml
Chili	500mg/ml	250mg/ml	1000mg/ml	500mg/ml
Onion	500mg/ml	500mg/ml	1000mg/ml	1000mg/ml

Regarding the combined effect of spices, mixture of ginger and garlic showed highest effectiveness among all mixture with zone of inhibition of 26mm against *S. aureus*. Similarly, combined mixture of ginger and garlic was found least effective with zone of inhibition of 12mm against *K. pneumoniae*. Garlic and onion was found ineffective against both organisms.

Table 5: Antimicrobial activity of their combination against microorganism

Mixture of spices	Diameter of zone of inhibition(mm) against organisms			
	S. aureus	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
Gi+Ga	26mm	-	12mm	-
Tu+Ch	21mm	-	16mm	-
Tu+Gi	24mm	-	14mm	-
Ch+Gi	24mm	-	14mm	-
Ga+On	-	-	-	-

Note: Gi+Ga=Ginger and Garlic, Tu+Ch= Turmeric and chili,Tu+Gi= Turmeric and Ginger, Ch+Gi= Chili+Ginger, Ga+On= Garlic and Onion

Combined extract of Turmeric and Chili, Turmeric and Ginger, Chili and Ginger were found to have synergistic effect against *S. aureus* with FIC value of 0.28, 0.28,0.25 respectively whereas the extract of ginger and garlic was found to have additive effect (FIC value = 1). Similarly, these all combined extract were found to have additive effect against *K. pneumoniae*. Combined extract of Garlic and Onion was found to have antagonistic effect against both isolates.

Table 6: Minimum inhibitory concentration (mg/ml) of various spice extracts

Combined extract	*S. aureus*			*K. pneumonia*		
	MIC Value	FIC Value	Inference	MIC Value	FIC Value	Inference
Gi+Ga	250mg/ml	1	Add	250mg/ml	1	Add
Tu+Ch	15.63mg/ml	0.28	Syn	15.63mg/ml	0.56	Add
Tu+Gi	15.63mg/ml	0.28	Syn	13.63mg/ml	0.53	Add
Ch+Gi	62.5mg/ml	0.25	Syn	125mg/ml	0.75	Add
Ga+On	500mg/ml	2	Anta	500mg/ml	2	Anta

Keys: Add: Additive effect, Syn: Synergistic effect, Anta: Antagonistic

DISCUSSION

During study, it was found that most of extract was affective against *S. aureus* and *K. pneumoniae* whereas, *E. coli* and *P. aeruginosa* were resistant against extract. In case of *S. aureus*, the ginger extract produces ZOI of 16mm, whereas in case of *K. pneumoniae*, it produced ZOI of 12mm. The main constituents of ginger are sesquiterpenoids with zingiberene. Other compounds include β-sesquiphellandrene, bisabolene and farnesene, which are sesquiterpenoids and trace monoterpenoid fraction (Malu et al. 2008). In similar fashion ethanolic extract of turmeric produced ZOI of 16mm in case of *S. aureus* whereas only 14mm ZOI against *K. pneumoniae*. The antimicrobial activity of turmeric is reported to be due to the presence of essential oil, curcumins, curcuminoids, turmeric oil, turmerol and valeric acid (Gul et al. 2015). Ethanolic extract of chili produced highest zone of inhibition against *S. aureus* (ZOI = 26mm). Capsaicin, a well-studied chemical component of the capsicum species and one of the pungent capsainoids found in chili peppers, has already demonstrated a high degree of biological activity affecting nervous, cardiovascular and digestive system. The ethanolic extract of garlic and onion extract produced least ZOI of 15mm. In previous studies, it has been demonstrated that allicin is the main component of garlic that exhibits antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein synthesis are also partially inhibited (Yadav et al. 2015).

In this study, ethanol was used as a solvent although it itself has antimicrobial properties. The study is justified as the ethanol was evaporated when heated 40°C for 24 hrs. On other hand, it is also classified as polar solvent. This means the solvent is miscible in water and it will extract mostly ionic compounds from spices. It has better dissolving capabilities compared to water because it has a slightly low dipole and is dielectric (Ramli et al. 2017). Moreover, according to Marriott (2010), ethanol, ethyl acetate and acetone are the solvents permitted for use in the preparation of food ingredients.

Overall the effectiveness of ethanolic extract of spices is higher in Gram positive than Gram negative. Grace et al. (2017) found similar result i.e. ethanolic extract of ginger is best effective against *S. aureus* when compared to other Gram negative. Generally, in Gram negative bacteria, their outer membrane serves as permeability barrier which allows only small hydrophilic molecules to pass through into all, restricting their route of penetration for certain antimicrobial compounds and excluding larger molecules. Besides these, they also possess multidrug resistant pumps which exclude some of antibacterial compounds across barrier (Marriott 2010).

Similarly, effect of combination of spices extracts have proven to be feature of antimicrobial and antioxidant treatment due to number of important considerations viz (i) they increase activity through use of compounds with synergistic or additive activity, (ii) they thwart drug resistance (ii) they decrease required doses, reducing both cost and adverse/toxic side effects and (iv) they increase the spectrum of activity (Baljeet et al. 2015).

From the foregoing findings, combination of selected spices produced zone of inhibition larger than individual use. The combined extract of ginger and garlic showed highest ZOI of 26mm against *S. aureus* whereas turmeric and chili mixture produced highest ZOI of 16 mm against *K. pneumoniae.* Both the isolates were found resistant to the combined garlic and onion extract. But Aliyu et al. (2015) reported that ethanolic extract of garlic produced zone of inhibition ranging from 3.59-15.80mm against *S. aureus* whereas the ginger extract produced zone of inhibition ranging from 13-28mm. Their combined extract of garlic and ginger extract produced zone of inhibition of 33.60mm.

MIC value was calculated for only two organisms i.e. *S. aureus* and *K. pneumoniae* as these two organism were found to be most sensitive to ethanolic extracts whereas other two isolates were found to be insensitive to the extract as there was no any visible zone of inhibition. Although various spices extracts showed different inhibitory effect against tested microorganisms, similar value of MIC ranging from 500-1000µg/ml was observed in case of each individual spices extract against both isolates except for turmeric extract. This showed that the same concentration of various individual extract was effective to different extents in inhibiting the growth of tested microorganism. MIC value for turmeric was found to be 62.5mg/ml while MBC was found to be 125mg/ml. similarly, in case of *K. pneumoniae*, MIC value for turmeric was found to be 31.25mg/ml and MBC was found to be 62.5mg/ml.

The MIC of combined extracts however fluctuated from 15.63mg/ml to 500mg/ml and the most sensitive microbial species in relation to the MIC of combined extract was *S. aureus.* The MIC of most of combined extract was reduced to 25% of the MIC of individual extracts. The FIC values of combined extracts showed that the combination of ethanolic extract of chili and ginger (FIC index=0.25), turmeric and chili (FIC index= 0.28) & turmeric and ginger (FIC index = 0.28) displayed synergistic effect (FIC index < 0.5) against *S. aureus* while combined extract of ginger and garlic showed additive effect (FIC index = 1). On other hand all the combined extracts showed additive effect against *K. pneumoniae* except garlic and onion.

All the bacteria employed for the study were also subjected to antibiotic susceptibility test. The resistivity and sensitivity of antibiotics against the organism was determined based on CSLI (2014) guideline. The highest ZOI of inhibition was given by Ciprofloxacin against *P. aeruginosa*. Most of antibiotic was found resistant against *E. coli*.

Hence the synergistic and additive effect of these spices against tested spices against test microorganisms supports the use of these spices in combinations. The results of study revealed that combined ethanolic extracts of spices were more effective as antimicrobials, as antimicrobial properties of spices depend not only on chemical composition but also on the lipophilic properties and water solubilities. Combination of various compounds may have contributed to the observed synergistic and additive effects. The multiple mode of action may include degradation of cell wall, disruption of cytoplasmic membrane, leakage of cellular components, alteration of fatty acid and phospholipids constituents, changes in synthesis of DNA and RNA and destruction of protein translocation (Baljeet et al. 2015). Hence it is possible that combining spice extracts could lead to synergistic or additive inhibitory potential against both food spoilage and pathogenic microorganisms. Most studies attributed additive and synergistic effects to phenolic and alcoholic compounds.

Although the research is conducted by systematic protocol, the variables used in research are too small to draw strong and specific conclusion. The study only focuses on pathogenic organism by using four bacterial isolates and limited number of spices. From beginning to the end of research, tests were performed using crude extract without conforming its purity.

CONCLUSION

Spices extract seems more effective against *Staphylococcus aureus* than *Klebsiella pneumoniae* with MIC value of 62.5µg/ml and 31.25µg/ml and MBC value of 125µg/ml 62.5µg/ml respectively The MIC of most of combined extract was reduced 25% of the MIC of individual extracts. The FIC values of combined extracts showed that the combination of ethanolic extract of chili and ginger (FIC index=0.25), turmeric and chili (FIC index= 0.28) & turmeric and ginger (FIC index = 0.28) displayed synergistic effect (FIC index < 0.5) against *Staphylococcus aureus* while combined extract of ginger and garlic showed additive effect (FIC index = 1). On other hand all the combined extracts showed additive effect against *Klebsiella pneumoniae* except garlic and onion. Therefore, combinations of extracts can provide additive as well as synergistic effects making them more effective antimicrobial agents. Consequently, the selection of spices, for use in preventing food-borne bacterial infection, is both interesting and worthwhile for food safety.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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