ANTIBACTERIAL ACTIVITY OF COMMON SPICE EXTRACTS ON BACTERIAL ISOLATES FOUND IN KACHHILA

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ABSTRACT

Kachhila, one of the most popular snack items belonging to the Newari community, is made of raw meat of water buffalo, chicken or lamb. They get highly contaminated during processing, cutting with microorganisms among which may be pathogenic as well. The main aim of this study is to screen antibacterial activity of garlic, ginger, turmeric and their combination against test bacteria. All together 6 samples were collected. All these samples were collected from different meat shops. The samples were transported in ice box to microbiology laboratory and processed using bacteriological culture technique. Four different concentrations (100%,75%,50% and 25%) of extracts were used and their zone of inhibition was measured in millimeter (mm). Antibacterial activities extracts were carried out using agar well diffusion method against bacteria isolated from meat. The bacterial load was found to be decreased after the addition of spices in meat sample. *Escherichia coli* was the most dominant bacteria (40%). Result indicates that turmeric showed high activity against *Salmonella* spp, *Escherichia coli*, *Pseudomonas* spp and *Staphylococcus aureus* at high concentration and low activity at low concentration. Our study revealed that ginger and garlic extract could not inhibit the growth of bacteria. The antibacterial activity of combined spice extracts showed high effectiveness against bacteria which includes synergistic and additive effect. Therefore, the study revealed that spices used in kachhila have antibacterial property and enhance the shelf life of kachhila.

Keywords: Ginger, Turmeric, Garlic, Antibacterial property, Kachhila

INTRODUCTION

Kachhila, one of the most popular snack items belonging to the Newari community, is made of raw meat of water buffalo, chicken or lamb. Kachhila is one among the two most popular twin-sounding meat snacks in the Newari menu, Choila and Kachhila. The Newari community is considered one of the richest communities in terms of culinary experience. It is made by mixing minced meat with various spices such as garlic, coriander leaves, red chili, salt and chili powder (Sah et al 2020).

The term meat refers to the flesh, skeletal muscle and any attached connective tissue or fat excluding bone and bone marrow (Williams 2007). Meat is a good source of protein, essential fatty acids, minerals and vitamins but easily perishable because it provides the suitable medium for the growth of various microorganisms (Komba et al. 2012). The most important food borne bacterial pathogens associated with meat are *Salmonella spp , Staphylococcus aureus, Escherichia coli, Campylobacter jejuni, Listeria monocytogenes, Clostridium perfringens, Yersinia enterocolitica, and Aeromonas hydrophila* (Zhao et al 2001, Bhandare et al 2007).

Spices have been used for many centuries by various cultures to enhance flavor and aroma of our foods as our ancestors have recognized the usage of spices in food preservation and in treatment of clinical ailments and there are several reports on development of antibiotic resistance in diverse bacterial pathogens(Joe et al 2009). Spices are indispensable components of Nepalese cuisines since ancient times (Maharjan et al 2019). 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).The burgeoning concern about safety of foods has recently led to the development of natural antimicrobials to control food borne pathogens. (Maharjan et al 2019).

Spices have been defined as any aromatic plant substance in the whole, broken or ground form used to flavor food. Basically, spices are used for flavoring, masking the bad flavor of some foods, ayurvedic medicines and even in cosmetics and perfumes. The composition of spices includes proteins, lipids, vitamins, carbohydrate, and various mineral components. Spices have been widely used as flavoring and coloring agents since ancient times (Gottardi et al 2016). The meat is a nutrient-dense medium ideal for many pathogens and spoilage microbes to colonize because of its high in moisture, rich in nitrogenous foods of various complexity, plentifully supplied with minerals and accessory

growth factor, usually has some fermentable carbohydrate (glycogen), and is at favorable pH (Frazier and Westhoff 2009).

MATERIALS AND METHODS

The study method were quantitative and primary data were collected from February to March 2022. The study variables were microbial contamination, meat and the study was based on cross- sectional research design. The sample were collected from different meat shop inside Gokarneshwor municipality of Kathmandu district. These site are highly populated and has continuous buyers. A total number of 6 meat samples were collected convenientaly from different retail stores, which includes 3 meat samples that of chicken and 3 meat samples that of buff. The sample for the study were selected randomly. The meat samples were collected, transported to the laboratory within 1hour of the sample collection.

Sample processing

About 25g of buffalo and chicken meat sample was collectednin a clean, dry and sterile polythene bag from the local shops and transported to the laboratory and immediately processed for isolation of bacteria within one hour after purchase. 1 gram of the sample was first crushed and ground in a sterile mortar with the help of a sterile pestle and the 9 ml of sterile distilled water was added and further proceeded to serial dilution. The bacterial isolates were identified by studying their cultural, morphological and biochemical characteristics. Biochemical tests were used to confirm the identity of each isolate.

Preparation of spices extracts

Fresh garlic was purchased from local market. Garlic bulbs and ginger were peeled, weighed 100 g, and cleaned. Garlic and ginger were crushed in sterile mortar and pestle and then the mixture was filtered through a sterile cheese cloth. This filtrate was considered 100% fresh garlic extract, was stored at −20°C, and was thawed before use and different concentration were made using distilled water (Yadav et al 2015).

Enumeration of microorganisms

The total viable count was carried out by pour plate technique. For this purpose, serial dilution was used. 0.1ml sample was taken from diluted mixture, which was placed on the petri plate followed by agar medium. Sample and media was mixed by rotating the plate in 8 direction, allowed to solidify and incubated for 24 hours at 37ºC. Now the plates were screened for the presence of discrete colonies and the actual colonies were estimated by cfu/ml.

$$\textit{Colony forming unit/ml} = \frac{\textit{No. of colonies} \times \textit{dilution factor}}{\textit{Volume of sample plated}}$$

RESULT

Among 6 meat samples, a total of 30 bacterial isolates were isolated out of which 57.90 was Gram negative and 42.10 was Gram positive bacteria.

Total count of bacteria before adding spices

The homogenized diluted meat sample were inoculated in Plate count agar and incubated for 24 hours. The cfu/ml of colonies isolated on PCA plate were calculated and the highest cfu/ml isolated was 2.73×10^6 and that of lowest cfu/ml was 1.45×10^4. After the calculation of cfu/ml, the isolated colonies were sub cultured

Table 1 **:** Total count of bacteria before adding spices

Sample	Mean (Cfu/ml)
1	TMTC
2	2.73×10^6
3	TMTC
4	TMTC
5	1.54×10^4
6	1.45×10^4

Total count of bacteria after adding spices

The bacterial load of fresh meat was found to be 2.73×10^6 cfu/ml , 1.54×10^4 cfu/ml and 1.45×10^4 cfu/ml respectively. Again bacterial load was determined from kachhila after adding spices and found to be 3.87×10^3 cfu/ml, 3.75×10^3 cfu/ml, 3.61×10^3 cfu/ml, 3.0×10^3 cfu/ml, 2.5×10^3 cfu/ml and 2.37×10^3 cfu/ml respectively. This showed bacterial load in kachhila was decreased due to antibacterial activity of spice used.

Table 4**:** Total count of bacteria after adding spices

Sample	Mean (Cfu/ml)
1	3.87×10^3
2	3.75×10^3
3	3.61×10^3
4	3.0×10^3
5	2.5×10^3
6	2.37×10^3

Growth pattern of bacteria

All meat samples (inoculated into plate count agar) showed bacterial growth after 24hr of incubation. Based on Gram staining, morphological features, cultural characteristics, and biochemical test, the bacterial isolates were assigned to four bacterial species in which 95% was Gram negative and 5% was Gram positive bacteria. *E. coli* was the most common isolated bacteria which was 40%, followed by *Salmonella* (26.45), *Pseudomonas* spp (23.55).

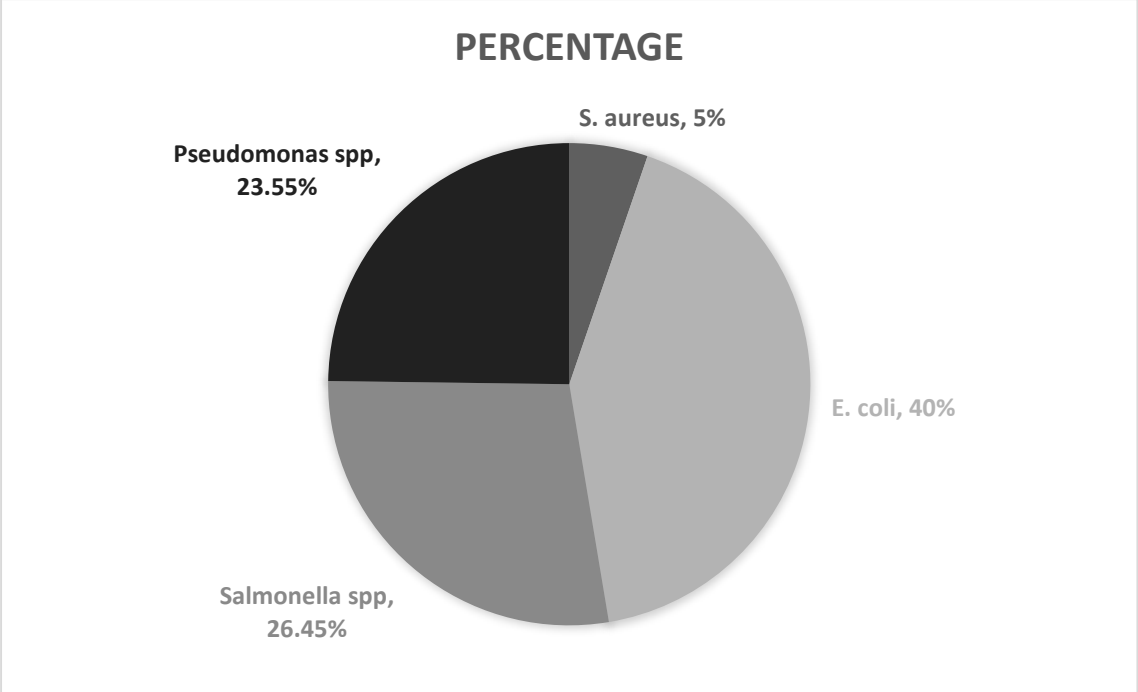

Figure 1**:** Growth pattern of bacteria on PCA

Antibacterial activity testing using agar well method

Overall, it was observed that among three spices, aqueous extracts of Turmeric had inhibitory effect on both gram-positive and gram-negative bacteria. The different concentrations (100%, 75%, 50% and 25%) of aqueous extract of turmeric showed least inhibition values (15 mm, 14 mm, 13 mm and 11 mm) against *S. aureus* and highest inhibition values (19 mm, 15 mm, 12 mm and 11 mm) against *Salmonella* spp. Similarly, the different concentrations (100%, 75%, 50% and 25%) of aqueous extract of turmeric showed zone of inhibition values such as (17 mm, 16 mm, 15 mm and 13 mm) against *E. coli*. and (17 mm, 16 mm, 14 mm and 12 mm) against *Pseudomonas* spp. respectively.

Table 3**:** Antibacterial activity of spices extract in different percentage against test organism

Spice extracts	**Concentration (%)**	**E.coli (mm)**	**Salmonella (mm)**	**S. aureus (mm)**	**Pseudomonas (mm)**
Ginger	100	–	–	–	–
	75	–	–	–	–
	50	–	–	–	–
	25	–	–	–	–
Garlic	100	–	–	–	–
	75	–	–	–	–
	50	–	–	–	–
	25	–	–	–	–
Local turmeric	100	17	19	15	17
	75	16	15	14	16
	50	15	12	13	14
	25	13	11	11	13

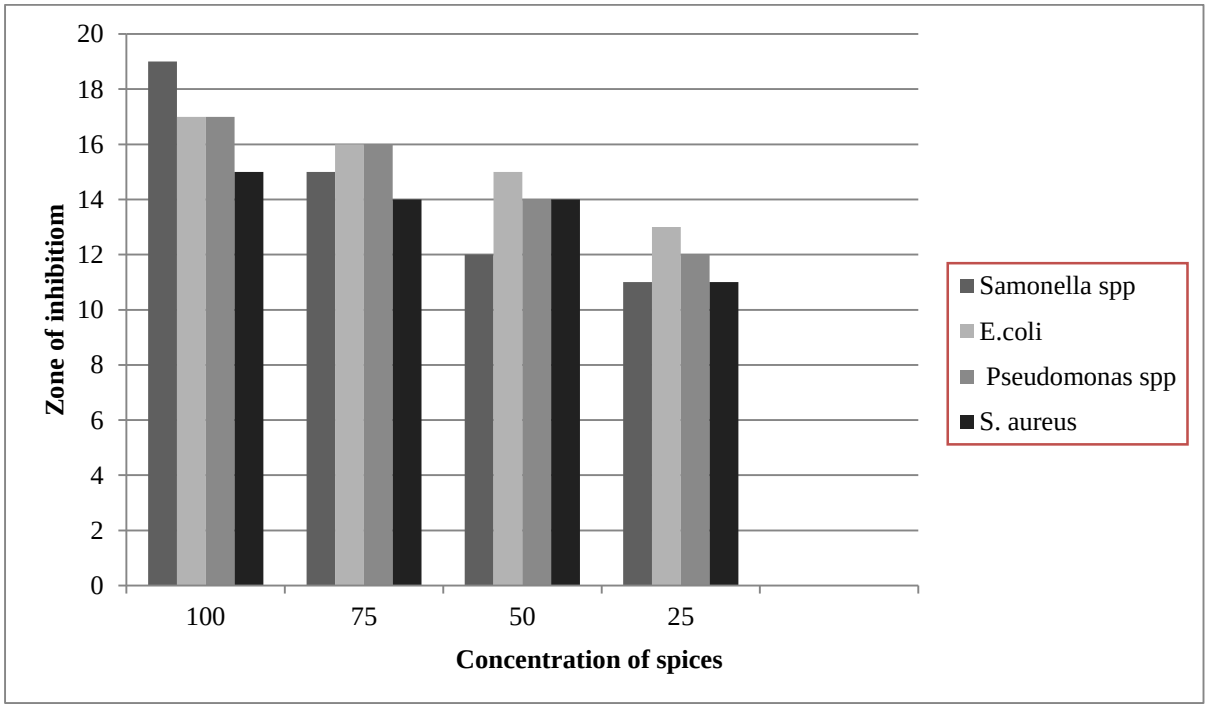

Figure 2**:** Antibacterial activity of Curcuma longa

Combine effect of garlic, ginger and turmeric

Garlic+ ginger, ginger+ turmeric, garlic+ turmeric and garlic+ ginger+ turmeric were combined together in different concentration and tested against gram positive and gram negative bacteria.

Regarding the combined effect of spices, different concentrations (100%, 75% 50% and 25%) mixture of ginger and garlic and turmeric showed highest effectiveness among all mixture with zone of inhibition of (19mm, 14mm, 12mm and 11m) against *E. coli* and least effective (15mm, 13mm, 10mm and 9mm) against *S. aureus*. Combined mixture of garlic and turmeric showed highest effectiveness with zone of inhibition of (16mm, 14mm, 13mm and 11mm) against Pseudomonas spp. And least effective (14mm, 12mm, 10mm and 8mm) against *S. aureus*.

Similarly, combined mixture of ginger and turmeric showed highest effectiveness with zone of inhibition of (18mm,16mm, 14mm and 11mm) against *Pseudomonas* spp. and least effective (13mm, 11mm, 10mm and 8mm) against *S. aureus*. Garlic and ginger was found ineffective against test organism.

Table 4**: Combine effect of garlic, ginger and turmeric**

Combine extracts	**Concentration (in %)**	**E.coli (mm)**	**Salmonella (mm)**	**S. aureus (mm)**	**Pseudomonas (mm)**
Gi+ Ga	100%	–	–	–	–
	75%	–	–	–	–
	50%	–	–	–	–
	25%	–	–	–	–
Ga + Tu	100%	15	14	14	16
	75%	13	12	12	14
	50%	11	11	10	13
	25%	10	9	8	11
Gi + Tu	100%	16	13	13	18
	75%	15	11	11	16
	50%	13	10	10	13
	25%	12	9	8	11
Gi+ Ga+ Tu	100%	19	15	15	17
	75%	14	12	13	13
	50%	12	11	10	10
	25%	11	10	9	8

Note: Gi+Ga=Ginger and Garlic, Tu+Ga= Turmeric and Garlic, Tu+Gi= Turmeric and Ginger, Gi+Ga+Tu= Ginger and Garlic and Turmeric.

DISCUSSION

Meat is regarded as one of the most indispensable and versatile food item, obtained from different cattle like goat, buffalo, sheep, poultry and the fishes. Meat and meat products plays a vital role in the maintenance of human health providing all the essential nutrients such as proteins, vitamin and minerals. It is pertinent to mention that about 30% of zinc for nutrients to human comes from meat and meat products (Pal Ma et al 2018). Due to abundance in nutrients, meat makes ideal condition suitable for microorganisms to grow and acts as important vehicles for many microbes, which can cause spoilage of meat and the food poisoning.

The result showed that bacterial load in prepared kachhila decreased in time. It was basically due to antibacterial property of spices used in kachhila but at the same time, some spices could not show antibacterial property. From these results, bacterial growth in kachhila was found to be resultant of growth promoting factors (Meat nutrients, moisture temperature etc) and growth retarding factors (spices extract). According to Sah et al 2020, addition of spices helped to decrease the load of bacteria in the meat. As spices have antibacterial property, they can be used for other various food items for better taste and preservation (Sah et al 2020). According to Venugopal et al (2018), addition of herbs and spices to the food preparations helps to keep a check on the concentration *Escherichia coli* in the body.

Spices are one of the most commonly used natural antibacterial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavor (Pundir and Jain 2010). Many of the spices and herbs used today have been valued for their antibacterial effects and medicinal powers in addition to their flavor and fragrance qualities (Bin et al 2007). A wide range of technologies is available for the extraction of active components and essential oils from medicinal and aromatic plants. The choice depends on the economic feasibility and suitability of the process to the particular situation. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Duhan et al 2013).

The mechanism of antibacterial action of spices and derivatives is not yet clear. Proposed hypothesis are : hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, followed by partition in the lipid bilayer; perturbation of membrane permeability consequent to its expansion and increased fluidity causing the inhibition of membrane embedded enzymes; membrane disruption; destruction of electrons transport systems and cell wall perturbation (Pundir and Jain 2010).

In this study, the representative organism isolate from 6 meat samples were *E.coli*, *S. aureus*, *Pseudomonas* spp and *Salmonella* spp which were cultured in anaerobic condition. A similar study was conducted in Dharan, Nepal which showed that 68% of samples were contaminated with *S. aureus*, 54% samples with *E. coli*, 34% samples with *Salmonella* spp., 40% of samples with *Pseudomonas* spp. and 6% samples with *Vibrio* spp. and *Shigella* spp (Bantawa et al 2018).

This study was performed to evaluate the antibacterial activity of aqueous extracts of some common spices against common gram positive and gram negative bacteria found in buff and chicken meat used in preparation of Kachhila. This study showed aqueous extracts of turmeric had a strong effect against all bacteria. The aqueous extracts of turmeric showed high activity for *Salmonella* spp, *E. coli*, *Pseudomonas* spp *and Staphylococcus aureus* at high concentration and low activity at low concentration. Chandrana et al (2005) and Kim et al (2005) reported that turmeric extract was effective against *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* which may be due to the presence of curcuminoid, a phenolic compound. The antimicrobial activity of turmeric is reported to be due to the presence of essential oil, curcumins, curcuminoids, turmeric oil, turmerol and veleric acid (Cikricki et al 2008; Rai et al 2008; Basniwal et al 2011). However, the Ginger and garlic extracts could show no zone of inhibition against any test bacteria.

Similarly, mixture of ginger+ garlic+ turmeric had strong effect against *E. coli* and *Pseudomonas* spp. Hence the synergistic and additive effect of these 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 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.

Based on this finding, Spices possess good natural antimicrobial agents against both gram-positive and gram-negative bacteria. The extracts of spices should be further analyzed to isolate the specific antibacterial component in them. Various experiments should be conducted to prove spices as food preservatives. Clinical trials should be carried out to explore the potential of the extracts in the treatment of the infectious diseases (Sah et al 2020).

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