



Antimicrobial activity of Ginger and Garlic against *salmonella* species isolated from Food products

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ABSTRACT

In the globe the most common reason for foodborne problems is Salmonella spp. The main serovar of Salmonella enterica connected with the human salmonellosis are Enteritidis and Typhimurium. The major cause of infection of these serovars is due to contamination of product of meat. According to food standard legislation salmonella spp in food product make the food unable to be consumed by human. Meat, poultry, egg, dairy products, and fruits and vegetables are primary transmission vehicles. The main transmitting vehicles of salmonella spp are poultry product, fishes, beef, vegetables and fruits. Garlic and Ginger are known to have medicinal importance. Along with other application ginger and garlic have antimicrobial property. In the present study the salmonella spp was isolated from different food products like poultry meat, beef, fish and vegetables. Salmonella spp was identified by cultural, morphological and biochemical tests. Agar diffusion assay was used to find out the antimicrobial activity of the ginger and garlic extract against salmonella spp isolates. 17% prevalence of salmonella spp in different food product was observed in our study as a whole. Out of which salmonella spp from poultry meat was 24% (6/25), beef 16% (4/25) and from fish it was 20% (5/25). In vegetables 8% (2/25) of Salmonella spp prevalence was observed. Sample. Our study observed that n-hexane and methanol extracts of garlic shows more potency than ginger extract against salmonella spp showing maximum inhibition zone of 23 mm at 40 mg/ml and 25 mm at 80 mg/ml while n-hexane and methanol extracts of ginger shows less potency than garlic extract showing maximum inhibition zone of 14 mm at 40 mg/ml and 16 mm at 80 mg/ml. our result direct that poultry meat, fish, beef and vegetables can be contaminated with Salmonella spp. The people of Pakistan are at great risk due to these contaminated foods. Against all isolates of salmonella spp Garlic extract shows excellent antimicrobial activity as compared to ginger extract. Our study encourage to use the medicinal value plants and spices by keeping in mind the various social and economic factors of Pakistan principally the poor people, and poor hygienic condition. This will help to decrease the high cost, side effects and will also help to reduce the emerging drug resistance of many pathogens against currently available antibiotics

Key words: *Salmonella spp; Ginger extract, garlic extract; antimicrobial activity, drug resistance*

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INTRODUCTION

In the globe the most common reason for foodborne problems is *Salmonella spp* [1]. Annually there is estimate of 94 million cases of gastroenteritis caused by *salmonella spp* in the whole world that lead to every year death in 155,000 patients [2]. Salmonellosis epidemics has been often implicated due to poultry meat [3,4]. Salmonellosis is one of the key health issue. Topographical divergences in reported degrees of *Salmonella spp* contamination in poultry probably imitate contrasts in systematic conditions instead of indigenous raw meat contamination. Non-typhoidal salmonellosis causes high rates of diseases worldwide because of food contamination in people which is related with food contaminated having animal origin [5]. Food-borne diseases and epidemics due to *salmonella spp* leads in many states in the world [6]. The main causative agent of food-borne diseases in the United States is *Salmonella enterica* [7]. In the Europe and United States the main causative agent for human salmonellosis epidemic is mainly considered due to *Salmonella enterica* serovar *Enteritidis* [8, 9]. This is principally associated by consuming poultry product that are contaminated with *salmonella spp* [10, 11]. According to food standard legislation *salmonella spp* in food product make the food unable to be consumed by human [12, 13]. In less developed countries, due to the absence of standard of hygiene and sanitation, typhoid fever is major health issue. Typhoid is considered to be the severe salmonellosis caused by *salmonella spp* [14, 15]. Food products and water is considered as the major transmitting vehicles of this disease and annually it cause problems in about 90 million people in the world and this disease shows variability in the morbidity and mortality in the world [16]. *Salmonella spp* could come into the food chain from crop level, poultry and livestock farm, during processing and manufacturing of food products and even retailing point can be used by *salmonella spp* to enter the food chain. In cattle asymptomatic infection due to *salmonella spp* can occur. Contamination of beef occur during slaughter of animal or contents of gastrointestinal origin processing and the milk is contaminated during milking [17]. Attention have gained as a vehicles of transmission of *salmonella spp* in fresh foodstuffs like fruits and vegetables which can enter the food processing chain by using many steps [18]. Alteration in eating habits and alteration in agricultural practices along with the multibillion dollar business of fresh foodstuffs between the countries are the major factor that are responsible for the salmonellosis in human caused by *salmonella spp* [19].

Contamination with *Salmonella spp* contamination in fresh foodstuffs is mainly from horticultural products. The usage of animal sources as an organic fertilizers and use of waste water for the irrigation purposes are the major factors that contribute as transmission vehicles in fresh foodstuffs [20, 21]. Fruits and vegetables can also be contaminated by the environmental *salmonella spp* as *salmonella spp* is present in the environment and it has ability of long period survival in the environment.

Many antimicrobial drugs are used commercially for controlling the pathogenic and infectious diseases agents since many years. Multiple drug resistance (MDR) have been developed by many microbial pathogens against the available antimicrobial agents due to excessive use of these available antimicrobial agents. The major limitation in treatment and control of disease is the increased drug resistance in the pathogens against current available antibiotics [22]. Correspondingly, the use of sulfites, nitrates, nitrites and antibiotics for preservation are unsafe for the health of human and it causes many side effects like headache, nausea, weakness, mental retardation, seizures, cancer and anorexia [23]. The major factors that power the development of new antimicrobial agents are the consumer interest of safe foodstuffs and the increased drug resistance developed in the microbes [24]. The treatment of the infectious disease are commonly done by extract of the higher plants in many parts of the world [25]. The medicines derived from plants can be used in numerous forms. It can be used in form of powder, liquid, raw or boiled mixtures and it can be used as liniments, ointments and incisions [26].

Ginger and garlic is used in many food preparation, in many countries including Pakistan. Neem, tulsi, ginger, garlic etc. are the medicinal plants that are recognized to have antimicrobial activity. Garlic (*Allium sativum*) that belong to Alliaceae family are used for cooking purposes but it has also important value in medicine along with cooking application. Mostly it is used in Asian countries including Pakistan. Whole garlic, garlic powder, garlic ketchup form are used in Pakistan. Application of garlic for their anti-tumor activity and uses for cardiovascular disorder and liver disorders are reported previously. Garlic use shows beneficial effects in controlling blood pressure, blood sugar and cholesterol [27]. Along with all these application garlic have potential to be used as antimicrobial agent. Ginger (*Zingiber officinale*) belonging to the family of Zingiberaceae have many medicinal application. It is reported that ginger use is important for many problems including arthritis, cramps, sprain, constipation vomiting, hypertension and fever. It is also important for their [28]. Keeping in mind all these this study was conducted with objective that was basically aimed on determination of antimicrobial activity of ginger and garlic against different isolates of *salmonella spp* from many food products in Pakistan.

MATERIAL AND METHODS

A total of 100 food samples were collected from different markets in Lahore. The distribution of samples are given in Table 1. The distribution of vegetables samples are given in table 2.

Table 1: Samples distribution of various food products

Type of sample	Number of samples
Poultry meat samples	25
Beef samples	25
Fish samples	25
Vegetables samples	25
Total samples	100

Table 2: Samples distribution of various vegetables

Type of vegetable sample	Number of samples
Cauliflower	5
Cabbages	5
spinach	5
Onion	5
Potato	5
Total sample	25

All the Samples were cut and placed in sterile bags in aseptic condition. Then the sample were placed in ice box and then for processing on the same day they were transported to the laboratory. *Salmonella species* was isolated from different food samples as follow.

For selective enrichment of *Salmonella species* Tetrathionate broth was used. Sterile distilled water was used for the samples washing firstly then for surface sterilization 3 % bleach was used. The samples were then chopped into small pieces by using sterile blades and then it was inoculated into the tetrathionate broth at the ratio of 1: 9.24 hour incubation time was given at 37 °C. Bismuth sulphite agar was then used for the plating of the samples from the enrichment broth. 48 hour incubation time was given at 37 °C. Composition of bismuth sulphite agar is given in the table 3

Table 3: Composition of bismuth sulphite agar

Serial Number	Ingredients	Gm/lit
1.	Peptone	10.000
2.	HM Peptone	5.000
3.	Dextrose (Glucose)	5.000
4.	Disodium phosphate	4.000
5.	Ferrous sulphate	0.300
6.	Bismuth sulphite indicator	8.000
7.	Brilliant green	0.025
8.	Agar	20.000

Isolates from different food samples having black color colonies on bismuth sulphite agar were suspected as positive for *Salmonella species* while isolates having colonies of other color were considered as negative. Gram staining was used for morphological identification of *Salmonella species*. Biochemical tests were performed for final identification. Oxidase, Catalase, TSI, Motility, Indole, Citrate and Urease tests were used for biochemical identification of *Salmonella species*. For the suspension preparation of the microorganisms McFarland standards was used as turbidity standard.

The plants materials were collected from vegetable market in Lahore. They were recognized as *Zingiber officinale* and *Allium sativum* correspondingly. Distilled water was used for the washing of ginger plant to remove the sand particles and it was then dried in air at room temperature for a period of six weeks. The garlic bulbs were detached into segments. The different segments were then peeled and it was then chopped. Then it was dried in air at room temperature for about six week. Homogenous sample of the dried materials was obtained by grinding it with sterile electric blender. Cold maceration method defined by [35] was used and from each one 120g powder were extracted by using 750ml of methanol and n-hexane. After extraction of plant material, rotatory evaporator at 40C⁰ was used for the concentration of the plant extract. To remove the water content from the plant extract, they were freeze dried. For further use they were placed in sterile bottles and then for preservation they were refrigerated at 4C⁰. For

determination of antimicrobial activity of the plant extract agar well diffusion method was used according to Clinical and Laboratory Standards Institute (CLSI) guidelines [38]. By using sterile cotton swabs the surface of the nutrient agar was inoculated with the standardized suspension. 8 mm diameter wells were bored using sterile core borers in the solidified agar. Sterile borer was used for well boring of 8mm diameter. 1ml of sterile agar was poured into the bottom of the wells. The different concentration extract were then poured into the wells. Two concentration of 40mg/ml and 8 mg/ml of each extract were used. For diffusion of extracts the plates were kept at room temperature for about 3 hours and then the plates were incubated for 24 hours at 37C⁰. The zone of inhibition around the wells were measured to determine the antimicrobial activity of the extracts.

RESULTS

During this study 100 different food samples were included. The different food samples were 25 each of poultry meat, fish meat, beef and vegetables. For the isolation of different *Salmonella species* from various food products, Bismuth sulphite agar was used. Black colonies on bismuth sulphite agar were considered as *Salmonella species*. We obtained black colonies as shown in figure 1. The gram staining was shown as gram negative rods (Fig 2)

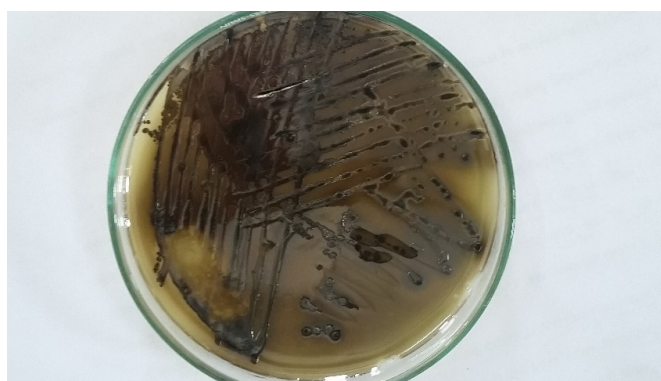


Figure 1: Black colonies of salmonella species on bismuth sulphite agar.

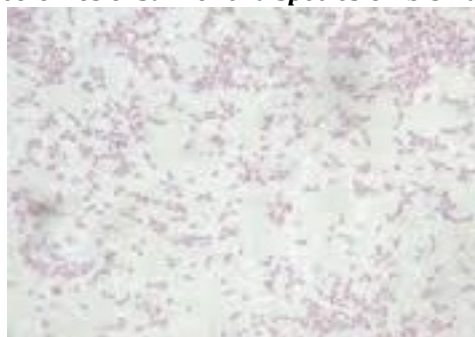


Figure 2: Gram negative rods on gram staining

The results of the biochemical test for the biochemical identification of the *salmonella sppis* given in table 4.

Table 4: *Salmonella spp* biochemical identification tests

Biochemical Test		RESULTS
Oxidase test		-ve
Catalase test		+ve
Indole test		-ve
Urease test		-ve
Citrate test		Differential
Motility test		+ve
Triple sugar iron test	slope	Red and pink
	Butt	Yellow
	H ₂ S gas production	+ve
	Gas production	Differential

A whole of 100 different food samples were handled in our research work, that include poultry meat, beef, fishes and vegetables. 17% prevalence of *salmonella spp* in different food product was observed in our study as a whole. (Table 5) Out of which *salmonella spp* from poultry meat was 24% (6/25), beef 16% (4/25) and from fish it was 20% (5/25) and in vegetables 8% (2/25) of *Salmonella spp* prevalence was observed. Sample. (Table 5) The comparative prevalence between the different vegetables are given in the table 6.

Table 5: Salmonella spp prevalence in different food products

Serial NO	Type of sample	Number of samples	Positive <i>Salmonella</i> species number (%)	Negative <i>Salmonella</i> species number (%)
1.	Poultry meat samples	25	6 (24%)	19(76%)
2.	Beef samples	25	4(16%)	21(84%)
3.	Fish samples	25	5(20%)	20(80%)
4.	Vegetables samples	25	2(8%)	23(92%)
	Total samples	100	17(17%)	83 (83%)

Table 5: Comparative prevalence of salmonella spp in vegetables

Type of vegetable sample	Number of vegetable samples	<i>Salmonella</i> species positive n (%)	<i>Salmonella</i> species negative n (%)
Cauliflower	5	1(20%)	4(80%)
Cabbages	5	00	5(100%)
spinach	5	1(20%)	4(80%)
Onion	5	00	5(100%)
Potato	5	00	5(100%)
Total	25	2(8)	23(92%)

The antibacterial activity of plant extracts against the different isolates of *salmonella spp* from the food products are given in table 7. The zone of inhibition of different plant extract against *salmonella spp* are given in figure 3. Our study observed that n-hexane and methanol extracts of garlic shows more potency than ginger extract against *salmonella spp* showing maximum inhibition zone of 23 mm at 40 mg/ml and 25 mm at 80 mg/ml while n-hexane and methanol extracts of ginger shows less potency than garlic extract showing maximum inhibition zone of 16 mm at 40 mg/ml and 18 mm at 80 mg/ml.

Table 7: Zone of inhibition of different plant extracts against isolates of salmonella spp

<i>Salmonella</i> spp isolate	Zone of inhibition(mm) Methanol extract of garlic		Zone of inhibition(mm) N_hexane extract of garlic		Zone of inhibition(mm) Methanol extract of ginger		Zone of inhibition(mm) N_hexane extract of ginger	
	40mg/ml	80mg/ml	40mg/ml	80mg/ml	40mg/ml	80mg/ml	40mg/ml	80mg/ml
isolate1	14	21	12	14	12	14	10	12
isolate2	16	19	13	15	15	16	12	14
isolate3	14	17	11	8	16	18	14	16
isolate4	18	21	15	17	14	16	12	14
isolate5	22	24	18	21	8	10	6	9
isolate6	15	18	13	16	9	11	8	11
isolate7	17	20	16	18	11	13	8	11
isolate8	13	16	11	15	12	15	9	11
isolate9	21	23	19	21	9	12	8	10
isolate10	23	25	18	21	13	15	11	14
isolate11	20	22	17	21	12	15	12	14
isolate12	21	23	17	22	8	11	6	8
isolate13	18	20	16	19	12	13	11	12
isolate14	17	20	14	17	9	11	8	10
isolate15	12	16	14	18	10	12	8	10
isolate16	15	18	12	16	11	13	10	12
isolate17	18	22	16	19	12	14	10	12



Figure 3: Well diffusion assay of different plant extracts

DISCUSSION

In the world the major cause for food-borne diseases is salmonellosis [39]. A large number of food products are contaminated by this pathogen but poultry is majorly contributed in the human salmonellosis epidemics [40]. A whole of 100 different food samples were handled in our research work, that include poultry meat, beef, fishes and vegetables. 17% prevalence of *salmonella spp* in different food product was observed in our study as a whole out of which *salmonella spp* from poultry meat was 24% (6/25), beef 16% (4/25) and from fish it was 20% (5/25) and in vegetables 8% (2/25) of *Salmonella spp* prevalence was observed. Our study results are in accordance with a study directed in Faisalabad city of Pakistan (41). 25% prevalence of *S. enteritidis* is reported in Iran from poultry product [42]. According to our report the prevalence of *salmonella spp* in beef is 16%. Our results are also similar with the study done by Ahmed OB, *et al* [43] in which they observed that 21.3% of the total samples were Positive for *salmonella spp* and the result were as follow; contamination of beef meat by *Salmonella spp* was 23.4% while chicken meat was 18.6% and fish meat was 33.3%. The prevalence of salmonella Spp in vegetables and fishes are in agreement with the study done by Siala *et al.* [44] while our result of prevalence of *salmonella spp* in poultry and beef is contrasted with the study done by Siala *et al.* [44]. The major transmission vehicle for *Salmonella spp* is food products. This role of food in *salmonella spp* transmission demands for measures to stop transmission. The transmission can be stopped by various measures like suitable practices of agriculture and animal husbandry, feed and water protection from contamination and suitable methods for disposal of wastes along with overall clean environment. To guarantee the safety and quality of foods, it need to understand *salmonella spp* and its behavior. All the isolates shows susceptibility against plant extracts. The n-hexane and methanol extracts of garlic were observed to be more potent against *salmonella spp* with maximum zone of inhibition of 23 mm at 40 mg/ml and 25 mm at 80 mg/ml while n-hexane and methanol extracts of ginger shows less potency then garlic extract showing maximum inhibition zone of 16 mm at 40 mg/ml and 18 mm at 80 mg/ml.

Bioactive components are presents in the methanol and n-hexane extracts of ginger and garlic that give them antimicrobial activity [45]. The extracts of garlic give inhibitory zone than the extract of ginger against all isolates. The antimicrobial activity of these plant extracts might be due to presence of secondary metabolites in these plants [46]. Abdullahi *et al.* reported that solvent is important factor that effect the antimicrobial property of the extracts [47]. The extract of ginger shows less antibacterial activity against all isolates. Our this result is in contrast to the study done by Aliyu *et al.* [48] who reported more antimicrobial activity of ginger. One of the component of garlic called allicin have been found that have antimicrobial property by moderately inhibiting the DNA and protein synthesis while completely inhibiting the RNA synthesis(49). Anionic components such as nitrates, chlorides and sulfates are anionic component present in the garlic which give them antimicrobial characteristics [50].

The antimicrobial activity of garlic have been identified in previous studies against many microorganisms. *Alium* genus have more antibiotic activity against *Streptococcus mutans* [51] and against *Streptococcus agalactiae* [52]. Additionally antimicrobial activity of garlic was also shown against many bacteria like *Streptococcus olaris*, *Streptococcus mitis*, *Staphylococcus aureus* [53]; *Escherichia coli*, *Salmonella typhi*, *Shigella flexineri*, *Proteus mirabali* [50]; and also shows good antimicrobial activity against *Vibrio parahaemolyticus*, *Escherichia coli* and *Staphylococcus aureus* [54]. *Escherichia coli* and *Staphylococcus aureus* show resistance to extracts of garlic in many studies [55].

Antimicrobial activities of the ginger is due to gingerol associated components of the ginger [49]. Ginger have been shown by many studies to have antimicrobial microbial property against various bacteria [56].

The earlier studies shows that ginger have moderate to good antimicrobial characteristics [57]. When ginger is used as antimicrobial agent at the concentration of 1%, it has the ability to stop three log cycle of the bacteria in beef sausages after frozen storage of two to three months [58].

Conversely, the weak antimicrobial property are shown in many reports [53]. One of the study reported that many multidrug resistant bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella Typhi* have no susceptibility towards the ginger extract [59]. Vuddhakul *et al.*, reported that ginger have no antimicrobial activity against *Vibrio parahaemolyticus*, *Escherichia coli* and *Staphylococcus aureus*. [54].

CONCLUSION

Our study concluded that poultry meat, fish, beef and vegetables can be contaminated with *Salmonella spp.* The people of Pakistan are at great risk due to these contaminated foods. Against all isolates of salmonella spp Garlic extract shows excellent antimicrobial activity as compared to ginger extract. Our study encourage to use the medicinal value plants and spices by keeping in mind the various social and economic factors of Pakistan principally the poor people, and poor hygienic condition. This will help to decrease the high cost, side effects and will also help to reduce the emerging drug resistance of many pathogens against currently available antibiotics

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