5. Effect of spices extract in controlling the growth of \textit{S. Enteritidis} in hens, duck and Japanese quail eggs

5.1 Introduction

In recent years, food safety concerns have been focused on pathogens, such as \textit{Salmonella}, which is recognized as one of the leading causes of food borne bacterial diseases. The problem of human salmonellosis following consumption of contaminated foods has increased worldwide. Based on reports from 1973 to 1997, cases of salmonellosis other than typhoid have been reported almost each year. These outbreaks were epidemiologically linked to the consumption of several types of foods including chocolate, egg drink, cuttlefish, mayonnaise, fruit soup, fresh fruits and vegetables, dairy products, and fermented meat products (D'Aoust, 1997; Sauer \textit{et al.}, 1997; Wallace \textit{et al.}, 2000). \textit{S. Enteritidis} is one of the most frequently reported food borne disease in developed and developing countries. According to the World Health Organization (WHO) forecast food borne disease will soon become the second largest cause of morbidity in Europe.

Poultry and eggs are good, nutritious foods. However, there is sometimes a lack of control of pathogens in the environment in which chickens are raised and eggs produced. For example, poultry feed and water becomes contaminated from cockroaches, flies, birds and rodents (mice and rats) in poultry houses. As a result, the poultry becomes diseased, and occasionally eggs become contaminated. Two types of pathogenic bacteria often associated with eggs and poultry are \textit{Salmonella} spp. and \textit{Campylobacter jejuni}. \textit{S. Enteritidis} silently infects the ovaries of healthy appearing hens and contaminate the eggs before the shells are formed. The freshly laid egg has a number of lines of defense against bacterial contamination. Significant bacterial proliferation occurs only within the yolk. The layers of the shell provide a barrier, beginning with the cuticle, which hardens on the outside of the egg shortly after
oviposition and effectively blocks the pores. The eggshell matrix proteins also provide some antibacterial activity although it appears to be fairly weak and short acting against *S.* Enteritidis. The shell membranes, as well as providing a mechanical barrier, contain bacteriolytic enzymes. The egg albumen contains proteins that inhibit bacterial growth: ovotransferrin, lysozyme, ovoinhibitor and cystatin and the high pH of albumen reduces bacterial growth. The perivitelline membrane also provides some degree of protection from bacterial contamination of the yolk. However, in spite of all these lines of defense, under some circumstances, *S.* Enteritidis is able to penetrate into the yolk of the egg.

*S.* Enteritidis can be inside the perfectly normal appearing eggs, and if the eggs are eaten raw or under cooked, the bacterium can cause illness. Consumers should be aware of the disease and learn how to minimize the chances of becoming ill. There has been increasing interest in discovering new natural antimicrobials (Sagdic *et al.*, 2003), this is also has been true in food microbiology. Plant products with antimicrobial properties notably have obtained emphasis for a possible application in food production in order to prevent bacterial and fungal growth (Lanciotti *et al.*, 2004). Plant products are characterized for a wide range of volatile compounds, some of which are important flavor quality factors (Utama *et al.*, 2002). Moreover, plant volatiles have been generally recognized as safe. Extracts of garlic, mustard, ginger, onion, pepper and other herbs exhibit anti microbial activity (Lai and Roy 2004; Suree and Pana 2005; Indu *et al.*, 2006).

Spices active compounds have been included in class of naturally occurring food preservatives and have their inclusion in foods allowed by food production regulator offices (Brull and Coote, 1999). Several scientific reports describe the inhibitory effect of spices on a variety of microorganisms, although considerable variation for resistance of different microorganisms to a given spice and of the same microorganisms to different spices has been observed (Akgul and Kivanç, 1988).
little information is available emphasizing the preservative and antimicrobial role of spices in the prevention of foods of the microbial action (Arora and Kaur, 1999).

Being plant natural foodstuffs, spices appeal to consumers who tend to question the safety of synthetic additives (Farag et al., 1989; Sagdic 2003). Antimicrobial properties of spices have been well documented (El Shami et al., 1985; Akgul and Kivanç, 1988; Cosentino et al., 1999; Ristori et al., 2002; Radhakrishanan-Sridhar and Velusamy-Rajaopal, 2003).

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods (Germano and Germano, 1998). Spices include leaves (bay, mint, rosemary, coriander, laurel, and oregano), flowers (clove), bulbs (garlic, onion), fruits (cumin, red chilli, and black pepper), stems (coriander, cinnamon), rhizomes (ginger) and other plant parts (Shelef, 1983). Although, spices have been well known for their medicinal, preservative and antioxidant properties, they have been currently used with primary purpose of enhancing the flavor of foods rather than extending shelf-life (Aktug and Karapinar 1986, Ristori et al., 2002).

Spices are recognized to stabilize the foods from the microbial deterioration. This could be observed when spices show initially high microbial charge and as time progresses, the microbial growth become progressively slower or it is eventually totally suppressed (Kizil and Sogut, 2003). Antimicrobial activity of spices depend on several factors, which includes: i) kind of spice, ii) composition and concentration of spice, iii) microbial species and its occurrence level, iv) substrate composition and v) processing conditions and storage (Shelef, 1983; Farag et al., 1989).

Gould (1995) has emphasized the possible use of spices and derivatives like alternatives for inclusion in a new perspective of food conservation called "natural antimicrobial system", which could use the synergistic effect of antimicrobial
compounds from animal, plant and/or microbial origin, more physical procedures in order to create an inhospitable environment for microbial survival in foods.

Antibacterial activity of spices has been reported by several researchers. Aktug and Karapinar (1986) observed inhibitory action of thyme (Thymbus vulgaris), mint (Mentha piperita) and laurel (Laurus nobilis) ground leaves and their extracts on S. Aureus, S. Typhimurium and V. parahaemolyticus. Grohs and Kunz. (2000) observed that spices mixtures were able to inhibit the growth of various meat-spoiling microorganisms (Bacillus subtilis, Enterococcus spp., Staphylococcus spp., E. coli K12 and Pseudomonas fluorescens) providing stabilizing effect on colour and smell of fresh portioned pork meat. It is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments (Hora et al. 1994). They are also extensively used, particularly, in many Asian, African and other countries. In recent years, in view of their beneficial effects, use of spices/herbs has been gradually increasing in developed countries also.

Food safety continues to be a major concern for the food industry in recent years. One of the industry's top priorities has been to find alternative ways to preserve their newly developed foods while satisfying the increasing consumer demand to produce safe, all-natural products. In order to achieve this "clean label", much research has been devoted to the use of effective plant-based antimicrobials, such as those from herbs and spices, to replace chemical preservatives.

In the present study have evaluated the antibacterial effect of the extracts of widely used spices in South India such as Allium sativum (garlic), Gingiber officianalis (ginger), Allium sepa (onion) and capsicum annum (green chilli) to control the growth of S. Enteritidis in hens and duck egg.
5.2 Review of literature

Spices have been used for thousands of years to enhance the flavor and aroma of foods that makes delicious. Spices are used for prescribing foods and for their medicinal purposes. In the last nineteenth century scientific experiments have documented the anti microbial properties of some spices and their components. Several medicinal plants have been tried against several pathogenic microorganisms to show their inhibitory activities. The growing concern about safety of foods has recently led to development of natural antimicrobials to control food borne pathogens. Spices are some of the most commonly and pungent stimuli but also provide anti microbial property. The anti microbial property of spices may differ depending on the forms of spices added. In order to uses spices to control Salmonella in foods, it is essential that anti bacterial effects of crude. There for the aim of the present study is to determine the antibacterial property of some spices extracts for future application.

Arora and Kaur (1999) tested different spices for their antibacterial activity. They found that garlic and clove were showed antimicrobial activity. Bibitha et al. (2002) carried out studied a preliminary screening of fifty-eight plants for antibacterial activity. The result showed that fifteen of them posses antibacterial activity, out of which nine showed broad-spectrum activity by disc diffusion method. Both crude and acetone extract of Punica granatim, Tamarindus indica, Garcina gummmgutta, etc were active against all the organisms. The crude extract was found to be more active than acetone extract. Chaiswal et al. (2003) studied the potential for use of medicinal herbs as natural antimicrobial additives for foods. Of the fifteen medicinal herbs studied using agar diffusion method six of them including Zingiber officinale shown high activities in the first screening and six potential medicinal herbs were performed in duplication. All of six herbs exhibited anti microbial activities against Bacillus aereus.
and *Staphylococcus aureus* and all except *Zingiber officinale* exhibited significant activity against *S. Typhi*.

In 1983 Elnima *et al.* studied the antimicrobial activity of garlic and onions by aqueous extract method were tested for gram negative and gram positive organisms. The result indicates that garlic extract showed greater activity as compared to the extract of onion. Harris *et al.* (2001) studied the historical use of garlic and its sulfur chemistry and to provide a basis for further research in to its antimicrobial properties. Six different mixtures of garlic distilled oils containing diallyl disulfide and diallyl trisulfide ranging from 1 to 51% and 88 to 38% respectively have been analysed against a number of yeasts, Gram positive bacteria and Gram negative bacteria. The result obtained support a specific antifungal more than an antibacterial activity and implicates DDS as the active constituent. Incubation of garlic extracts made up of 1% DDS and 85% DTS resulted. Evandro *et al.* (2005) studied the antimicrobial effectiveness of spices. They found that inhibitory activity of spices and derivatives on the growth of bacteria, yeasts, fungi and microbial toxins synthesis has been well reported. So they could be used in food conservation as main or as adjuvant antimicrobial compounds.

The inhibitory activity of garlic (*Allium cepa)* was studied by Kumar and Berwal (1997) against *S. aureus*, *S. typhi*, *E. coli*, and *Listeria monocytogenes* was measured by the turbidity method. Minimum inhibitory concentration of garlic at 80% inhibition level was calculated for these bacteria. *E.coli* was most sensitive and *Listeria monocytogenes* was least sensitive. Mousumi Banerjee and Prabir Sarkar (2003) studied the inhibitory effect of garlic on bacterial pathogens from spices. The aqueous extracts of garlic were found to possess a potent bacteriostatic principle against Gram positive as well as Gram negative bacterial pathogens. Indu *et al.* (2006) studied the antibacterial activity of extract of garlic, nutmeg, ginger onion and pepper has been evaluated against twenty different sero groups of *Escherichia coli* eight serotypes of *Listeria monocytogens*
and Aeromonas hydrophylia. Garlic extracts shown excellent antibacterial activity against all the test organisms. They found that extract of ginger showed inhibitory activity against two sero groups of E. coli. Onion and pepper extract did not show any antibacterial activity against the test organisms.

Although some researchers have studied the antibacterial activity of spices against several species of bacteria, few serotypes of Salmonella have been tested, such as S. Typhimurium (Juven et al., 1994; Kim et al., 1995; Lachowicz et al., 1998; Elgayyar et al., 2001) S. Enteritidis (Tasou et al., 1995), S. Infantis (Alzoreky et al., 2002) and S. Anatum (Swetwiwathana et al., 1999). Antimicrobial activity of spices may differ between strains within the same species of bacteria. The sensitivity of each type of spices against several serotypes of Salmonella has not been reported. In addition, the antimicrobial property of spices may differ depending on the forms of spices added, such as fresh, dried, or extracted forms. In order to use spices to control Salmonella in foods, it is essential that antibacterial effects of crude ethanolic extracts and essential oils of spices against several serotypes of Salmonella be investigated.

Antibacterial action of garlic extract on food poisoning bacteria was studied by Sato et al. in 1990. Aqueous extract of garlic was tested by an agar plate diffusion method for growth inhibitory activity against 15 Gram negative and 8 Gram positive species. The result showed that the extracts had a bacteriostatic activity at low concentration regardless of the bacterial species used. O157:H7 and stored at 4 and 8°C for two weeks. Commercial ginger paste and fresh garlic paste showed the strongest antimicrobial activity with complete inactivation of E. coli 0157:H7 in the paste at 3 days at 4 and 8°C. Carrot and turmeric pastes did not show any antimicrobial activity both at 4 and 8°C. Commercial garlic showed antimicrobial activity at both temperatures in the paste.
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In a study by Kuo et al. (1997) a peroxidase catalyzed compound (PCC) sanitizer was tested to determine its bacterial activity on S. Typhimurium and S. Enteritidis inoculated on egg shell surfaces, a Egg with no treatment were compared to those immersed in either de ionized distilled water, PCC or 200 ppm chlorine treated water for 1, 3 or 5 min. Result showed that egg immersed in PCC or Chlorinated water solutions had lower S. Typhimurium and S. Enteritidis populations than those not exposed to treatments. PCC has potential as an effective shell egg sanitizer.

Satish et al. (1999) studied the anti bacterial activity of aqueous extract from leaves of thirty higher plants; collected from different localities were screened *in vitro* for antibacterial activity against different pathovars of the phytopathogenic bacterium, *Xanthomonas campestris*. Eight plant species showed antibacterial activity was observed in the aqueous extract of *Prosopis juliflora*, *Oxalis corniculata* and *Lawsonia inermis*.

Antibacterial activity of garlic powder against *E. coli* O-157 was tested by Sasaki et al. (1999) The use of powder from fresh garlic was more effective for antibacterial activity than that from old garlic, the 1% solution of fresh garlic powder eradicating the O-157 in 6 hrs. Thus the practical use of garlic powder is expected to prevent bacteria cause food poisoning.

In 2001, a study conducted by Sreenivasan et al. on fifty medicinal plants belonging to twenty six families for their anti microbial activity. They found that about twenty-two plant extracts from fifteen families exhibited activity against both gram positive and gram-negative bacteria. Fourteen plants belonging to eleven families did not show activity against any of the bacteria tested.

Vrinda et al. (2003) studied the inhibitory effect of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extracts at 0.5, 1, 2, 7 and 10 percent level *in vitro* on *E. Coli* and *Listeria monocytogens* by spectrophotometer method. They found that both spices
exhibited bacteriostatic effect against both the test organisms. The inhibitory effect increased with the increase in concentration of extracts.

Effect of storage and temperature of aqueous garlic extract on the growth of certain pathogenic bacteria was studied by Zakaria (2003). The result showed that a concentration of 750-1000µg/ml of the aqueous extract has high antibacterial effect, which reached to 100 % with some exceptions. The temperature of 70-100°C led to loss of the efficacy of aqueous garlic extract.

Lai and Roy (2004) studied the anti microbial and chemo preventive properties of herbs and spices. They found that the commonly used spices and herb such as garlic, clove, cinnamon thyme, mustard rosemary etc. posses antimicrobial properties. Other spices such as saffron, turmeric, tea etc. contain potent phytochemicals.

Pharmacological screening of some medicinal plants as antimicrobial and feed additives, a study done by Thakare and Mohan. (2004). Result suggests that Cinnamon and thyme have antimicrobial activity *invitro*.

Yasar *et al.* (2004) studied the invitro antibacterial effects of single or combined plant extracts of black thyme, fennel, Sage, Wild Tea and Wild Mint were used to evaluate invitro antibacterial activity against common pathogenic and lactic acid bacteria. The inhibition of lactic acid bacteria was weaker by single plant extracts and the combined plant extracts provide an entire antibacterial effect against pathogenic bacteria.

Matan *et al.* (2006) studied the inhibitory activity of Cinnamon and Clove oils against important spoilage microorganisms including four fungal species, four yeast species and two bacteria species of intermediate moisture food by agar plate method. The result showed that higher ratios of cinnamon oil / clove oil were more effective for inhibiting the growth of *Aspergillus flavus*. 
Rajan and Nagarajan (2006) studied the antimicrobial activity of selected spices such as pepper, coriander and cumium was assed against two pathogens namely *E. coli* and *Staphylococcus aureus*. Among the four spices tested the highest degree of antimicrobial effect was exhibited by the extract of the buds of clove *Syzygium aromaticum* and the seeds of pepper, *Piper nigrum*. *E. coli* was susceptible to all the spices.

Antimicrobial effect of two spices (Sumac and Avishan-e Shiraz) used in Iranian traditional medicine were investigated by Mohammed *et al.* (2007) against some pathogenic food borne Gram negative and Gram positive bacteria. The result revealed that Sumac showed better activity against the tested bacteria compared to Avishan shirazi.

### 5.3 Objectives

Review of literature clearly highlights the role of *S. Enteritidis* as a leading food borne pathogen and eggs as the most important food vehicle implicated in these infections. The efficacy of spices to control the growth of pathogenic bacteria in food is seen as an alternative to other chemical preservatives. In the light of such background, the specific objectives of the present study have been drawn as follows:

1. To test the efficacy of crude extracts from commonly used spices such as garlic, onion, ginger and green chilli in controlling the growth of *S. Enteritidis* in the eggs from commercial layer hen, non-commercial layer hen, duck and Japanese quail.

2. To compare the efficacy of the above spices extracts in controlling the growth of *S. Enteritidis* in egg microcosms.
5.4 Materials and Methods

5.4.1 Description of Spices

5.4.1.1 Ginger

Botanical name: *Zingiber officinale*

Family: Zingiberaceae

Ginger is commonly used as a spice throughout the world. Though commonly referred to as a root; it is actually the rhizome of the monocotyledonous perennial plant *Zingiber officinale*. Originating in Southern China it is a herb with tuberous, horizontal and aromatic rootstocks. Stems are leafy and tall. Flowers are white or yellowish in colour. Rhizome posse a warm pungent taste and pleasant odour. The main constituents are sesquiterpenoids with zingiberene as the main component. The pungent taste of ginger is due to non-volatile phenyl proponoids and diarylheptanoids.

Ginger contains up to 3% of essential oil that cause the fragrance of the spice. The volatile oil constitutes chavicol, citral, acetates etc. The mineral present in ginger are iron, calcium and phosphorous. It also containing vitamin such as thiamine, riboflavin, niacin, and vitamin C. It is used as a traditional medicine (Ravindra Sharma, 2003).

5.4.1.2 Garlic

Botanical name: *Allium sativum*

Family: Liliaceae

An annual or perennial, bulbous plant with numerous white bulbs called as cloves enclosed in a common, membranous white or pinkish sheath. It has long, flat leaves and bears small, white flower and bulbils in a globose mass on a solid scape. The bulb contains allicin, allin, scordine, anthocyanins and essential oil. The garlic has proved to be an antiseptic antidiabetic, anti cancer, anti bacterial anti fungal, insecticidal and pesticidal.
Garlic contains at least 33 sulfur compounds, several enzymes, 17 aminoacids and minerals such as selenium (Newall et al., 1996). It contains a higher concentration of sulfur compounds than any other Allium species. The sulfur compounds found in fresh garlic appear to be nearly 1000 times more potent as antioxidants than crude and aged garlic extract (McCaleb, 1993). These compounds are responsible both for garlic’s pungent odor and many of its medicinal effects. One of the most biologically active compounds, allicin, does not exist in garlic until it is crushed or cut; injury to the garlic bulb activates the enzyme allinase, which metabolizes the amino acid, alliin to allicin (Block, 1985).

5.4.1.3. Onion

Botanical name: Allium cepa

Family: Liliaceae

Onion a pungent edible bulb of the family Liliaceae is one of the oldest cultivated vegetable. It is a biennial herb, usually cultivated as annual it occurs mainly in central Asia. All its parts produces a strong onion odor when crushed. It has superficial root system, a very short flattened stem at the base of the plant. Leaves are long, linear and hollow. The fruit is a globular capsule.

Compared with other fresh vegetables, it is relatively high in food value, moderate in protein content and is rich in calcium and riboflavin. The odor in onion is due to organic sulfur compounds. Onion contains 86.6% moisture, 47mg calcium, 1.2% protein, 11.1% carbohydrates and 11mg Vitamin C.

5.4.1.4. Green chilii

Botanical name: Capsicum annum

Family: Solanaceae

An annual much branched herb with ovate, elliptic, pointed leaves. Flowers are white and the fruit, a berry is of variable size and shape red on ripening. The fruit is eaten raw or cooked food for its fiery hot flavor which is concentrated along the
top of the pod. The stem end of the pod has glands which produce the capsaicin. Capsaicinoids are known to control or even eliminate certain parasites in the gastrointestinal tract. Chilli is rich in nutritional value, as well as delicious. It is rich in Vitamin A and C, when eaten fresh with salads. Green chilies are reported to retard cancer.

5.4.2 Preparation of the inoculum

The \textit{S. Enteritidis} strains aseptically taken from the vials, inoculated into 10ml sterile Tryptone Soya Broth and incubated at $37^\circ$C for 16-18 hours. The cells were harvested by centrifugation at 3000 rpm for 10 minutes. Supernatant was removed and the cells were resuspended in 10ml isotonic saline. The process was repeated thrice (washing and resuspending). Cell density was determined by serial dilution and plating in hektoen enteric agar (HEA).

5.4.3 Preparation of spice extract

Spices obtained from the market are cleaned using water and descaled if it necessary and washed in sterile distilled water. After that it is rinsed again using ethanol or acetone. To obtain the extract a known amount of spice (50gm) were crushed with sterile mortar and pestle. Then the extracts were sieved through a sterile muslin cloth and filtered using filter paper. This extract is considered as 100% concentration. From this different volume such as 0.5ml, 1ml and 2.5ml were taken for inoculation into the egg microcosm.

5.4.4. Preparation of egg microcosm

After thorough scrubbing of the egg shell surface using soap and water, they were surface sterilized by immersing the eggs in 70% ethyl alcohol for 30 min., air dried in sterile chamber for 10 minutes. After the surface sterilization, the egg was cracked aseptically and the contents of each egg were transferred into sterile conical
flasks and mixed thoroughly. An aliquot (4.5 ml) of homogenized content was taken into a sterile test tube.

5.4.5 Inoculation of egg microcosm and treatment with spice extracts

Microcosm, prepared as above was inoculated with 0.5 ml S. Enteritidis cells (around \(10^6\) cells/ml). Different concentrations of spices extract (garlic, onion, ginger and green chilli) were introduced in to test the tube containing egg microcosm and allowed to react for 15 minutes at room temperature. After 15 minutes of reaction time 0.5 ml of samples were taken from each test tube serially diluted using sterile isotonic saline and spread plated on sterile hektoen enteric agar plates. The numbers of S. Enteritidis cells in each reaction mixture were estimated after incubating the plates for 24 hours at 37°C. Egg microcosm without spices extract served as control.

5.5. Results

In this study the extract of four different spices namely garlic (\(Allium sativum\)), ginger (\(Zingiber officinale\)), onion (\(Allium cepa\)), and green chilli (\(Capsicum annum\)) were screened for their antibacterial activity against the important food borne pathogen S. Enteritidis in egg microcosm. Four different types of eggs such as eggs of commercial layer hen, non commercial layer hen, and duck and from Japanese quail were used in this study.

5.5.1 Effect of different spices on growth of S. Enteritidis in each type of egg microcosm.

The antibacterial activity of extract of four different spices against S. Enteritidis in non commercial egg microcosms is presented in the Figure 5.1. It can be inferred that the garlic extract showed more antibacterial activity than the other three spices. Garlic extract at 50\% concentrations showed excellent antibacterial activity. While growth of S. Enteritidis in this egg microcosm ginger and onion showed moderate effect and chilli had no effect.
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The antibacterial activity of extract ginger, garlic, onion, and green chili in commercial layer hen egg microcosm is represented in Figure 5.2. The results revealed that garlic at 50% concentration was able to reduce the *S. Enteritidis* cells in commercial layer hen egg microcosm significantly. *S. Enteritidis* were able to show growth in onion extract at 20% while green chilli had no effect on the test organism.
Figure 5.2 Effect of various spices extract on the growth of *S*. Enteritidis in commercial layer hen egg microcosm

Figure 5.3 illustrates the antibacterial activity of different spices extract against *S*. Enteritidis in the duck egg microcosm. The results revealed that almost all the spices were effective against the test organism. In this case onion and chilli showed good antibacterial activity. Garlic extract was able to significantly reduce *S*. Enteritidis cells, while ginger extract showed moderate effect.
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Figure 5.3 Effect of various spices extract on the growth of S. Enteritidis in duck egg microcosm

Effect of various spices extract on the growth of S. Enteritidis in Japanese quail egg microcosm illustrated in Figure 5.4. In Japanese quail egg all the spices were effective against the test organism.

Figure 5.4 Effect of various spices extract on the growth of S. Enteritidis in Japanese quail egg microcosm
5.5.2 Effect of each spices on growth of \textit{S. Enteritidis} in different type of egg microcosm.

Growth of \textit{S. Enteritidis} was inhibited by all the spices extracts. While the extracts of ginger, onion and green chilli had not much effect beyond 20% concentration. Effectiveness of garlic extract found to increase with concentration.

The results of garlic extract on different egg microcosm are presented in Figure 5.5. It showed that garlic extract had good inhibitory activity against \textit{S. Enteritidis} in almost all eggs, though the activity was better non-commercial layer hen egg microcosm.

Figure 5.5 Effect of garlic extract on the growth of \textit{S. Enteritidis} in egg microcosm prepared from different types of eggs
The antibacterial activity of ginger against *S. Enteritidis* in four different eggs microcosm showed in Figure 5.6. It indicates that in non commercial layer hen egg, ginger had no antibacterial activity. Ginger extract showed a good reduction in the number of *S. Enteritidis* cells in commercial layer hen egg and Japanese quail egg.

Figure 5.6 Effect of ginger extract on the growth of *S. Enteritidis* in egg microcosm prepared from different types of eggs.
Different concentrations of onion extract showed moderate antibacterial activity against *S. Enteritidis* in all the four types of egg microcosms. Onion extract showed moderate reduction of *S. Enteritidis* in Japanese quail egg and good reduction in duck egg (Figure 5.7).

![Figure 5.7 Effect of onion extract on the growth of *S. Enteritidis* in egg microcosm prepared from different types of eggs](image-url)

Studies on *Salmonella* contamination in the eggs of layer hens, ducks, and Japanese Quail...
Figure 5.8 represents the antibacterial activity of green chilli extract against *S. Enteritidis* in different egg microcosms. Results revealed that antibacterial activity of chilli extract was well pronounced in Japanese quail egg and in duck egg when compared to other. In the case of commercial layer hen egg and non commercial layer hen egg microcosm chilli extract was unable to control growth of *S. Enteritidis*.

![Figure 5.8](image)

Figure 5.8 Effect of green chilli extract on the growth of *S. Enteritidis* in egg microcosm prepared from different types of eggs
5.6 Discussion

In the present study, an attempt has been made to study the effect of crude extract of four commonly used spices such as garlic, ginger, onion and green chilli on the growth of S. Enteritidis in four different types of eggs. Experiments were carried out using egg microcosm.

Of the four species used garlic extract showed significant activity against S. Enteritidis. Good antibacterial activity of garlic extract has been reported previously (Indu et al., 2006) who had evaluated the antibacterial activity of extract of garlic, nutmeg, ginger onion and pepper against twenty different serogroups of Escherichia coli, eight serotypes of Salmonella, Listeria monocytogenes and Aeromonas hydrophila.

In the present study, various concentration of onion extract showed moderate to good antibacterial effect against S. Enteritidis. The observation of Sreenivasan et al. (2001) agreed with our findings, they reported the moderate antibacterial activity of onion extract against E. coli, S. Paratyphi and S. Typhimurium. However, the finding were also contradictory to the findings of Indu et al. (2006), who had reported that onion had no antibacterial activity. This may be due to differences in the variety of onion used. Suresh et al. (2004) had reported good antibacterial activity of garlic extract against S. Enteritidis even at very low concentration.

Onion and ginger showed only moderate levels inhibitory effects. Our result also comparable with the findings of Elnima et al. (1983), who studied the antimicrobial activity of garlic and onions by aqueous extract method were tested for gram negative and gram positive organisms. The result revealed that garlic extract showed greater activity as compared to the extracts of onion.
A study conducted by Lai and Roy (2004) also found out that the commonly used species such as garlic, clove etc posses good antibacterial activity against the organism tested. Our study also agreed with the findings of Kumar and Berwal (1988) and Arora and Kaur (1999). The antibacterial activity of garlic is reported to be due to the action of diallyl disulfide (Harris et al., 2001; Avato et al., 2000). Muhsin et al. (2007) studied the susceptibility of some multiple resistant bacteria to garlic extract. His study assessed the antibacterial potentiality of garlic extracts using modern micro plate based antibacterial assay reported good antibacterial activity of fresh garlic extract. However, none of the three commercial preparations tested had any significant activity.

The result of our study revealed moderate antibacterial properties of ginger extract against S. Enteritidis in eggs. The result obtained from the present study agrees with the result of Vrinda et al. (2003) who studied the inhibitory effect of ginger and garlic on E. coli and Listeria monocytogenes. The result revealed that the antibacterial activity was directly proportional to the concentration in case of garlic and ginger. Chaisawadi et al. (1999) they found out that Zingiber officinale shown high inhibitory activities against S. typhi. Our results were also comparable to the results obtained from Sato et al., (1990), Sasaki et al. (1999), Zakaria (2003) and Evandro et al. (2005). They found out that species have good antibacterial activity. Shivani and Sadhana (2005) reported strongest antimicrobial activity of ginger, garlic, and turmeric and carrot paste against E. coli 0157:H7. Commercial ginger paste and garlic paste with complete inactivation of E. coli 0157:H7 in the paste after three days at 4°C and 8°C.

In our study extracts of green chilli was found to be ineffective in controlling the growth of S. Enteritidis in different types of eggs. Much literature on the antibacterial activity of green chili is not available.
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Herbs and spices are generally considered safe and prove effective against certain pathogenic organisms. The sensitivity of each type of spices against several serotypes of *Salmonella* has not been reported. In addition, the antibacterial property of spices may differ depending on the forms of spices added, such as fresh, dried or extracted forms.

The traditional cooking practice followed in South India uses various concentrations of the spices used in this study in most of the dishes. The results of the study support the use of these spices in marinating/ mixing with different food items for various time intervals. Though the antibacterial effect of the spices varies, none of them supported the growth of S. Enteritidis in egg microcosms. Egg being a potential vehicle for food borne salmonellosis, use of spices extracts in egg based dishes is recommended.