



EFFECT OF GINGER (*ZINGIBER OFFICINALE*) EXTRACT ON *SALMONELLA TYPHI*

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Abstract

This study was conducted to statement the effect of ginger (*Zingiber officinale*) extract in *Salmonella typhi* bacteria which is the etiologic agent of typhoid fever. A total of 50 blood specimens were collected from suspected typhoid patient, their ages (1-60) years during the period from Jun 2018 to December 2018, they attended to Al Fayhaa hospital in Basra city. Several criteria have been measured during the study. Firstly, widal test was used to identified of typhoid patient, and bacteriological methods including culturing of specimens and all bacterial isolates were biotype. The susceptibility of bacterial isolate antibiotics was used to show level of resistant. Preparation of plant extracts from ginger, fractionation with soxholet extractor, evaluation its potency on *Salmonella typhi* isolates by using well diffusion and disk diffusion methods. The results showed that 17(34%) were positive result to widal test, while 5 (10 %) of isolates were recovered from blood of patient, the positive isolates of *Salmonella typhi* identified by cultured method, biochemical test and Vitek system. The antibiotic susceptibility test revealed all *Salmonella typhi* isolates were sensitive (100%) for chloramphenicol, ceftriaxone, cefepime, cefazolin and imipenem with significantly differences ($p < 0.05$). On the other hand, all *Salmonella typhi* showed non-significant differences ($p > 0.05$) resistant (100%) for tetracycline and erythromycin. The effect of Plant extracts showed that highest inhibition zone diameter of *Salmonella typhi* growth (23.1) mm by action of ginger extract

Keywords: Ginger (*Zingiber officinale*), *Salmonella Typhi*.

Introduction

Salmonella typhi is an important intracellular pathogen. Among the more than 2,300 closely-related *Salmonella serovars* bacteria recognized, *S. typhi* is the only one that is pathogenic exclusively for humans, in whom it causes typhoid or enteric fever (Zhang *et al.*, 2008). Salmonellosis is seen in two kinds of viz. enteric fever which can be typhoid or paratyphoid and gastroenteritis which is non-typhoidal. Typhoid fever is an acute, life-threatening febrile illness caused by the bacterium *S. typhi* and Paratyphi, and there are estimated 20 million cases and 200,000 deaths worldwide each year (Crump *et al.*, 2004).

The greatest incidence of infection is in children of age less than 5 years (Brooks *et al.*, 2007). The illness may last from three to four weeks and death rate ranges between 12% and 30%. Although the global burden of typhoid fever has reduced, emergence of multi drug resistant *S. typhi* (MDRST) is still a threat to public health. *S. typhi* gained resistance to antibiotics like ampicillin, ceftriaxone, and co-trimoxazole, besides developing resistance to efficacious drugs like ciprofloxacin. The emergence of multidrug resistance to the commonly used antibiotics has further complicated the treatment and management of enteric fever and this is recognized as one of the greatest challenges in the management of this disease (Sehra *et al.*, 2013).

Plant derived medicines have been part of traditional health care in most parts of the world for ages and there is increasing interest in them as sources of agents to fight microbial diseases (Ajayi and Akintola, 2010). *Zingiber officinale* plant, commonly known as ginger, has more than 1200 species in 53 genera. It has been used as a medication since ancient times. According to the Chinese Pharmacopoeia, the medicinal uses and indications of ginger include epigastric pain, vomiting, diarrhea, weak pulse, dyspnea, cough, and sputum production (Torkzadeh *et al.*, 2014). Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potential for introducing new templates into modern medicine (Akinyemi

et al., 2005). This study aimed to evaluate the antibacterial activity of the ginger on microorganism *Salmonella typhi*. the work ascertained whether these spices could affect growth inhibition on the test organism *in vitro*.

Materials and Methods

Plant Extracts

Ginger (*Zingiber officinale*) Plant used in the study was collected from the market fresh, the Ginger was cleaned, and was crushed into small pieces for drying at 40°C until constant weight was reached. The pieces were further ground to powder. A total of 50 g of the plant powder was dissolved in 250 ml of 70% methanol for its in percentage (1:5) with stirring at 70°C-60°C. for 6 h. The extracts were then filtered using Whatman's No 1 filter paper and evaporation of the solvent was done using a rotary evaporator at 40°C, the extracts are stored in 4°C until to use. dilutions are done by dimethyl sulphoxide 10% to different concentration (10, 20, 30, 40 and 50 %) of extracts by dissolving (100, 200, 300, 400 and 500) mg of extract with 1 ml of organic solvent as mentioned by (Nanasombat & lohasupt-hawee, 2005).

Patients

The study enrolled 50 patients, admitted at the Al-Fayhaa hospital in Basra city, and Clinical signs of typhoid patient were recorded by physician.

Blood Specimens Collection

Fifty blood specimens (10 ml) have been obtained from suspected typhoid fever patients aged (1-60) who of attended Al Fayhaa Hospital–Al Basra city during the period extended from Jun 2018 to December 2018. 5ml of collected blood samples was injected in to the prepared sterile brain heart infusion broth with 2% sodium citrate, incubated for 24 hrs at 37°C after that sub cultured on blood agar and incubated aerobically at 37°C (willke *et al.*, 2001). The rest of blood samples were centrifuged to obtain serum for serological assay (widal test) as described by Anduaem *et al.* (2014).

Widal Test

Widal testing was done using O Somatic Antigens and H Flagellar Antigens, by using widal slide agglutination method (Andualem *et al.*, 2014).

Bacterial Isolation

For specific isolation of *S. typhi* from blood specimens, 5 ml of blood were cultured on Brain heart infusion broth then incubated at 37°C for 24 hr, then purified by sub cultured on blood and MacConkey agar. The identification tests for the isolate, including cultural, morphological, biochemical characteristics and Vitek system were done for each isolate.

Anti-microbial Susceptibility test

In this study, the choice of antibiotics (chloramphenicol, ceftriaxone, cefepime, cefazolin, imipenem, tetracycline and erythromycin). The disc diffusion of these antibiotic agents was determined by as it is suggested through (CLSI, 2012).

Statistical Analysis

Data were analysed using MINITAB version 16. Statistical differences between the samples and the controls were evaluated by one-way analysis of variance (ANOVA). Results are expressed as the mean of three determinations \pm standard deviation (SD). Mean values differences at $P < 0.05$ was considered significant statistically.

Result and discussion

Demographic study of *Salmonella typhi* according to the age groups

According to age groups of patient, the lowest isolation is among the (41-50) and (51-60) age groups (5.88%). Whereas the highest isolation is among (21-30) ages group (47.05%). As based on F-test the cases between age groups distribution positive for typhoid fever were significant ($P < 0.05$) table (1).

Table 1 : Distribution relation of typhoid fever with age group

Age group	Widal test (+)	%	Culture (+)	%
1-10	2	11.76	-	-
11-20	3	17.64	2	22.22
21-30	8	47.05*	5	55.60*
31-40	2	11.76	1	11.10
41-50	1	5.88	1	11.10
51-60	1	5.88	-	-
Total	17		9	

*Significant ($P < 0.05$)

The result study showed (47.05%) of case with typhoid fever are in aged (21-30 years old), this may be due to the fact that this is working age-group who are exposed to infection early in the community (Bultar *et al.*, 2004), regarding the age -group, which are affected by this disease. This may be consumption of un hygienic food and water in works and colleges Sur *et al.* (2007). In addition, the little cases are in age groups (41-60) years old. The reason may be related to frequent boosting of immunity (Bultar *et al.*, 2004).

Widal Test

A total of fifty blood samples are collected from patients with suspected typhoid fever, 34% are positive for

widal test, 5 isolates (10 %) recover from blood of patients table (2). typhoid fever is a global health problem, typhoid fever, caused by *S. typhi*. is a major cause of morbidity and mortality worldwide? Obviously, the disease has a very high social and economic impact (Punjabi, 1998). Despite recent studies in public health and sanitation, typhoid fever continues to be a major cause of morbidity and mortality. In Iraq (AL-Tememy, 2001).

Table 2 : Widal test for typhoid fever patient.

Blood samples	Widal test	
	Positive case	Negative case
	17	33
Total	50 patients	

Blood Culture

The results of the present study showed that out of 17 widal test positive cases. From 10 salmonella isolate only 5 isolates are diagnosed as *S. typhi*. All these bacterial isolations are identified based on colonial morphology and comparison of the biochemical characteristics. Similar finding is obtained by Tarrish *et al.* (1997), who reported that blood yield more positive culture during first week of fever, delayed hospital admission may have contributed to the low salmonella isolation rate from blood, this low rate may be due to pre-hospital antibiotic administration (Bulter & Scheld., 2004).

Identification of *Salmonella typhi*

Morphological identification: *Salmonella typhi* cultured are recognized based on colonial morphology which appeared smooth 2-3 mm with irregular edges. No lactose fermenter, non hemolytic, produced H₂S on S.S agar and red colonies with black center on XLD. Also, grew on tetrathionate broth. *S. typhi* organism first recognized when staining with gram stain, which appeared as gram negative cocco bacilli, motile and non-spore forming bacteria (Figure 1 and 2). The present results like that mentioned by Wain & Hosoglo, (2008) who reported that the colonial morphology of *S. typhi* on MacConkey agar, blood agar and S.S agar appeared as small, round, smooth, pale color, convex and non-hemolytic on blood agar, non-lactose fermenter on MacConkey agar and produce red colonies with black centre on XLD.

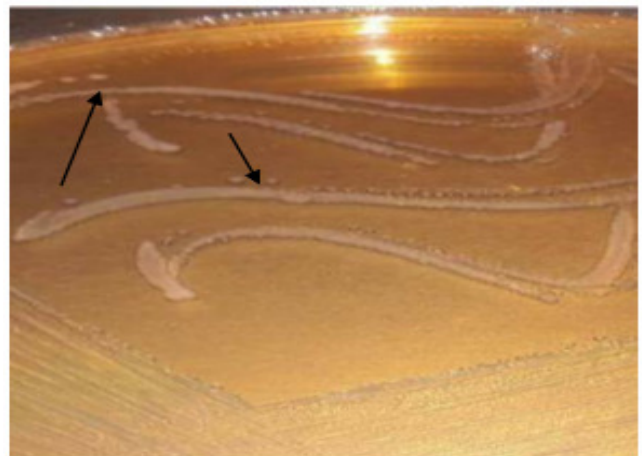


Fig. 1 : Colonial morphology of *Salmonella typhi* on MacConkey agar showing round, smooth, small, pale yellow colonies and non-lactose fermenter.



Fig. 2 : Colonial morphology of *Salmonella typhi* on XLD agar showing round, smooth, small, pale yellow colonies and non-lactose fermenter.

Biochemical Identification: To confirm diagnosis of *S. typhi* with biochemical test that are used table (3), *S. typhi* is positive for catalase and negative for oxidase and urease, produce acid with H₂S on TSI also negative for indole and VP, while was positive for methyl red, citrate utilization and H₂S production. The current results of this study are agreement with Subhei, (2010) who reported that *S. typhi* is identified by gram staining, positive catalase reaction, negative oxidase reaction, non-urease production and phenylalanine deaminase production, ferment glucose, maltose and sorbitol and produce H₂S and acid, while non-lactose and sucrose fermenter, nitrate reductase, non-liquefy of gelatine, negative for indole and positive for methyl red also positive for decarboxylase of amino acid.

Table 3 : The biochemical results of *S. typhi*

Organism	Glucose	H ₂ S	Motility	Indole	Urease	Citrate
<i>S. typhi</i>	A	+	+	-	-	-
<i>S. paratyphi A</i>	AG	-	+	-	-	-
<i>S. paratyphi B</i>	AG	+	+	-	-	+

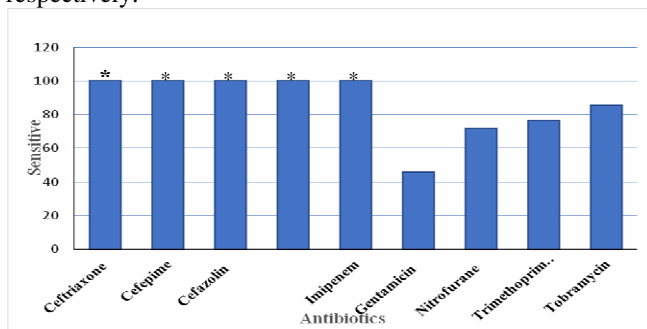
S: *Salmonella*

Vitek system identification:

Among the biochemical test methods which were used to the identification of bacteria, vitek system for identification of *S. typhi*. The analytical profile index of this system shows probability 98% identification percentage.

Antibiotic susceptibility test

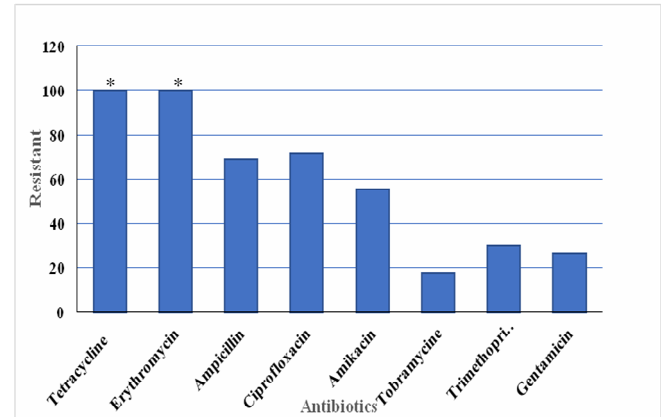
The results that are obtained from this study found in figure 3. Reveal that all *S. typhi* isolates are sensitive (100%) for ceftriaxone, cefepime, cefazolin, chloramphenicol, imipenem, while other *S. typhi* isolates was showed resistance rates (45.8%) for Gentamicin also *S. typhi* isolates were resistance (71.56%) for Nitrofurane, (76.42%) for trimethoprime-sulfa and (85.71%) for tobramycin respectively.



*Level of significant (P<0.05)

Fig. 3 : *Salmonella typhi*. antibiotics sensitivity

All *S. typhi* isolates are resistance (100%) for tetracycline and erythromycin. According to the results of F-test, the differences in sensitivity for isolates are significant at level (P<0.05), while there are no significant differences (P >0.05) in resistance among isolates for ampicillin, ciprofloxacin, penicillin G, amikacin, figure 4.



*Level of significant (P<0.05)

Fig 4. : *Salmonella typhi*. antibiotics resistant

The result of study was agrees with study of Arora and arora, (2011) which finds that typhoid fever responds slowly to ampicillin, amoxicillin, cotrimoxazole or trimethoprim alone of the third generation cephalosporin, ceftriaxone, cefotaxime and cefoperazone are effective therapeutic alternative in multi-drug resistant *S. typhi* infected cases, the fluoroquinolones (ciprofloxacin and ofloxacin), third generation cephalosporin (ceftriaxone and cefixime) and azithromycin came up as the second choice of treatment for multi-drug resistant strains. aztreonam and imipenem are also potential third line drugs that have been used recently in serious infections. The azalide antimicrobial Azithromycin is also an option in the treatment of multi drug resistant enteric fever (Raveendran *et al.*, 2010).

Effects plant extracts in growth *Salmonella typhi*

Ginger is used worldwide for different purposes such as cooking spice, condiment, it is used as an effective medicine for diarrhea (Spicer *et al.*, 2007).

The results of ginger extract was summarized in figure (5-6). Which illustrate their effect on *S. typhi* isolates ginger extract develop the highest inhibition zone with diameter reached (23.1) mm, the highest inhibitory zone effected was attained with ginger at 50% concentration, whilst the lowest effect was at 10 % concentration (14.80 mm).

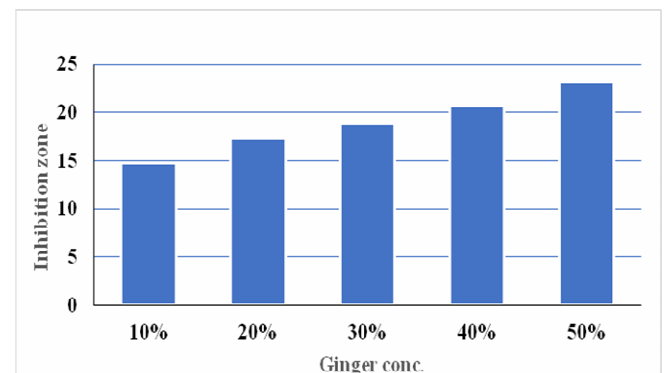


Fig. 5 : Sensitivity of *Salmonella typhi* for plant extracts

The results show that ginger extract was more effective against most pathogenic bacteria like *Staph. aureus*, *E. coli*, *Klebsiella pneumonia*, *Streptococcus pyogenes*, *Enterococcus faecales*, and *Pseudomonas aeruginosa*. When tested by well diffusion method. Ginger has been studied extensively with animal and invitro models, leads to speculation for its use as an antioxidant, antimicrobial, antifungal, antineoplastic, and antihypertensive agent. (chaiyakunapruk *et al.*, 2006).

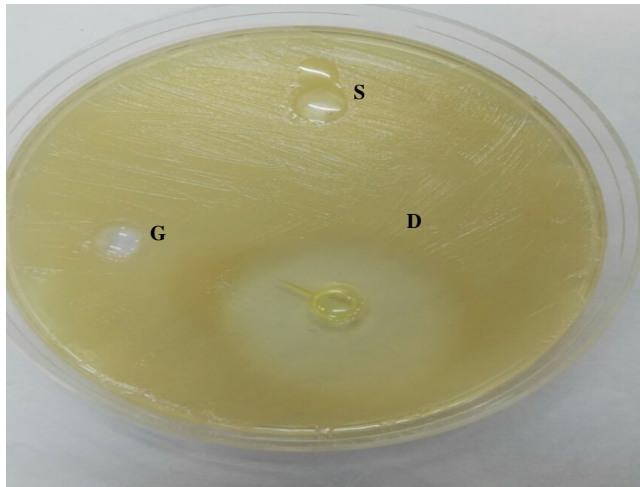


Fig. 6 : Effect of ginger extract on *Salmonella typhi*. G: ginger extract, D: Distal water (control), S: Solvent (dimethyl sulphoxide).

James *et al.*, (1999) clarified that some constituents of ginger inhibit the growth of some colon bacteria like *E. coli*, *Proteus spp.*, *Staph.*, *Streptococcus* and *Salmonella*. It has been found that out of 29 plant extracts. Ginger extract has the broadest range of antifungal activity measured either by the fungal inhibited or as the average diameter of the zones of inhibition.

Conclusion

The present study showed that ginger as alternative treatment rather than antibiotics *S. typhi* showed good effectivity in different concentration and the most effective concentration was 50% which showed the greatest inhibition zone, and as conclusion, Ginger (*Zingiber officinale*) plant can be considered as alternative treatment mainly for highly resistant strains of *S. typhi* against antibiotics. The results indicated the antibacterial activity of ginger extracts that have high potential for medicinal uses to reduce the typhoid fever for patients and can be used clinically due to its lack of toxicity on human as recommendation.

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