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Original Article

Antibacterial Effect of Ginger against Entero-pathogenic Organism: *Escherichia coli*

Rahman M A[1], Shah S K[2], Hena K.[3], Nahar D N[4], Akhiruzzaman[5], Sarkar M[6]

Abstract

The study was conducted during the period of July 2016 to June 2017 in the Department of Pharmacology and Therapeutics with the collaboration of Department of Microbiology, Mymensingh Medical College, Mymensingh to determine the profile of antibacterial effect of Crude Ginger Extract (CGE), Ethanolic Ginger Extract (EGE) and standard antibiotic Amikacin against standard strains of *Escherichia coli*. It was an exploratory study based on laboratory experiment.

The objectives of the study were a) Determination of inhibitory effects of crude ginger extract by incorporation into Nutrient agar media against *Escherichia Coli.* b) Antibacterial sensitivity testing of ethanolic ginger extract against *Escherichia Coli* by using disc diffusion method. **c)** Determination of minimum inhibitory concentration (MIC) of ethanolic ginger extract against the test organism by broth dilution technique. d) Determination of minimum inhibitory concentration (MIC) of antibiotic Amikacin against test organism by broth dilution technique and e) Subculture studies of materials from effective CGE, EGE and Amikacin preparations for confirmation of respective results of Experiments I, III and IV.

It was revealed that the growth of *Escherichia coli* started to be inhibited from 50% CGE incorporated media and even no complete inhibition of growth occurred at 100%. In case of Ethanolic Extract in disc diffusion method sensitivity was seen against *Escherichia coli* zone of inhibition was 7 mm at 25 µg/10 µl, 22 mm at 50 µg/10 µl and, 30 mm at 100 µg/10 µl concentrations respectively. The broth dilution technique was performed to determine the MICs of EGE and Amikacin. The MIC of EGE was 500 µg/ml against *Escherichia coli* and the MIC of Amikacin 1 µg/ml against *Escherichia coli*. The subculture study showed the same results with that of previous experiments.

The study confirmed antibacterial effect of ethanolic ginger extract (EGE) against *Escherichia coli.* The crude ginger extract (CGE) also had its inhibitory effects against the organism studied. The finding highlights the need for further extensive study to detect and isolate the active ingredients present in the Ginger extract responsible for antibacterial effect.

Key word: Crude ginger extract, Ethanilic ginger extract, Antibacterial effect, Escherichia Coli.

Introduction

The emergence of bacterial resistance is creating a global health issue. Undertaking this global problem various kinds of synthetic antimicrobials are being introduced by the various pharmaceutical companies. The newer generations of antimicrobials are costly and adverse effects are notable. In this regard one of the herbal spice *Zingiber officinale* (Ginger) was undertaken to investigate the antibacterial effect against the commonly encountered pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi)* causing various type of infections for its low cost, availability throughout the year and less adverse effects.

The microbial infection represents a critical problem to health and they are the major causes of morbidity and mortality of developing countries[1]. Antimicrobial agents are available for the treatment and management of infectious diseases[2]. In order to overcome the effects of chemical drugs, the World Health Organization have advised researchers to investigate possible use of natural products.

This subcontinent is a fertile soil for growing of various medicinal plants and herbs. In this context the name of Bangladesh is to be mentioned because our country is enriched with medicinal plants and herbs. Notable amount of modern medicines like cardiac glycosides, morphine, atropine, castor oil, aspirin, quinine etc. are obtained from various types of medicinal plants[3].

1. Dr. Md. Abdur Rahman
Assistant Professor and Head, Dept. of Pharmacology and Therapeutics, Ad-din Akij Medical college, Boyra, Khulna
2. Prof. Dr. Shyamal Kumar Shah
Professor and Head, Dept. of Pharmacology and Therapeutics Mymensingh Medical College, Mymensingh
3. Dr. Kamrunnahar Hena
Assistant Professor and Head, Dept. of Pharmacology and Therapeutics, Merin City Medical College, Chittagong
4. Dr. Dewan Nilima Nahar
Assistant Professor, Dept. of Pharmacology and Therapeutics, City Medical College, Gazipur
5. Dr. Akhiruzzaman
Assistant Professor, Dept. of Community Medicine Diabetic Association Medical College, Faridpur
6. Dr. Mohasin Sarkar
Medical Officer, UHC, Dimla, Nilfamari

Correspondence to:
Dr. Md. Abdur Rahman (MBBS, Mphil)
Assistant Professor and Head, Department of Pharmacology and Therapeutics, Ad-din Akij Medical College, Boyra, Khulna.
Email: dr.rana3939@gmail.com

More than 80% of the people of this country live in the rural area that's why they are far away from the modern treatment. On the other hand the existing antimicrobial agents have declined in effectivity due to resistance of organism to those agents[4]. This resistance is particularly evident in enteropathogenic bacteria e.g. *Escherichia coli, Shigella,* and *Salmonella* species[5]. Because of the magnitude of the problem of drug resistance, some researchers have chosen to develop alternative strategies[6]. Ginger (*Zingiber officinale*) is a perennial herb belonging to Zingiberaceae family and widely grown in Asia and Africa[7]. The active ingredients of ginger are, phenolic compounds: shogaols and gingerols; sesquiterpenes: bisapolene, zingiberene, sesquiphellandrene, curcurmene; others: 6-dehydrogingerdione, galanolactone, gingesulfonic acid, zingerone, geraniol, ginger glycolipids[8]. The active ingredients in ginger are thought to reside in its volatile oils, which comprise approximately 1-3% of its weight[9]. Ginger's active ingredients have a variety of physiologic effects. For example, the gingerols have antioxidant, anti-inflammatory, anti-tumor, analgesic, sedative, antipyretic and antibacterial effects in vitro and in animals[10,11]. Active constituents of ginger inhibit multiplication of bacteria by membrane disruption[12]. Ginger is a strong antibacterial agent against *Escherichia coli*[10]. Because of the increasing resistance of bacteria to antibiotics, herbal products are looking for new leads to develop better antibiotics[13]. Therefore the aims of this study are to investigate the antibacterial effectiveness of crude paste and ethanolic ginger extract.

Materials and Methods

This laboratory based exploratory study was carried out in the Department of Pharmacology and Therapeutics in collaboration with the Department of Microbiology, Mymensingh Medical College, Mymensingh, during the period from July 2016 to June 2017. Ginger was used as a material for experiment which was collected from local market of Mymensingh, Bangladesh. Another important material Aminoglycoside antibiotic (Injectable form) was bought from local market. Standard reference strains of *Escherichia coli.* ATCC 25922 was use for testing and collected from Microbiology Department of Mymensingh Medical College. Five experiments were conducted during this time period to determination of inhibitory effects of Crude Ginger Extract (CGE) by incorporation into nutrient agar media against *Escherichia Coli.* (experiment-1), antibacterial sensitivity testing of Ethanolic Ginger Extract (EGE) against *Escherichia Coli* by using disc diffusion method (experiment-2), determination of Minimum Inhibitory Concentration (MIC) of ethanolic ginger extract against the test organism by broth dilution technique (experiment-3), determination of Minimum Inhibitory Concentration (MIC) of antibiotic Amikacin against test organism by broth dilution technique (experiment-4) and subculture studies of materials from effective CGE, EGE and Amikacin preparations for confirmation of respective results of Experiments I, III and IV (experiment-5).

Procedure of Experiment- I:

Inhibitory effects of CGE against *Escherichia coli* into Nutrient Agar (NA) media. Ginger (1000gm) was washed initially by distilled water and then by 95% ethanol and homogenized by using sterile mortar and pestle. Then sieved through double layer of sterile fine mesh cloth to make crude extract. This CGE was considered as 100% crude ginger extract (Table-1).

Table 1: Composition of different concentration of CGE incorporated into NA media

Set No	CGE (ml)	Distil water in NA media to make 100ml	Percentage of CGE incorporated into NA media	Test organism
Set-I	5	95	5	One loopful*
Set-II	10	90	10	One loopful
Set-III	15	85	15	One loopful
Set-IV	20	80	20	One loopful
Set-V	30	70	30	One loopful
Set-VI	40	60	40	One loopful
Set-VII	50	50	50	One loopful
Set-VIII	60	40	60	One loopful
Set-IX	70	30	70	One loopful
Set- X	80	20	80	One loopful
Set-XI	90	10	90	One loopful
Set-XII	100	00	100	One loopful
Control				
Set XIII	-	100	-	One loopful

* One loopful =20 µl

Bacterial (*Escherichia coli*) Suspension was prepared by 3-5 similar colonies from 18-24 hours old agar plates and mixed with normal saline. The turbidity of the suspension was adjusted with 0.5 Mc Farland standards (1.5×10^8 organisms/ml). A cotton swab was dipped in the bacterial suspension and inoculated into CGE containing NA media as well as control plates. Then all the plates were placed in the incubator at 37^0C for 24 hours.

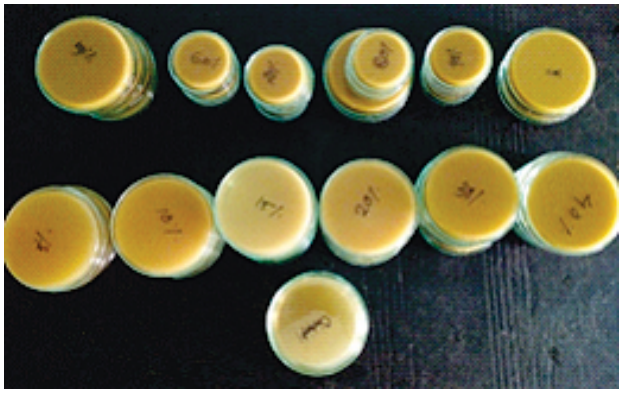

Figure 1: Petri dish contains prepared different concentration of CGE**.**

Procedure of Experiment- II:

Antibacterial sensitivity testing of Ethanolic Ginger Extract (EGE) against *Escherichia coli* by disc diffusion method and all materials were sterilized accordingly (same as procedure I). Ethanolic Ginger Extract was prepared by using 10 grams of the grounded ginger mixed with 200 ml of 95% ethanol and left in room temperature for 24 hours. After that it was filtered by using gauze pad to remove the large particle then centrifuged at 3000 rpm for 10 minutes. Secondarily by filter paper to obtain a clear solution which was dried at 40°C in hot water bath and stored in the refrigerator until use. For preparation of parent solution, 1gm powder extract mixed with 10 ml ethanol. Then filtered by gauze pad and centrifuged at 3000rmp for 10 min then filtered by filter paper. This solution was the source of preparing different concentrations with adding ethanol. The extract was stored at 4°C in refrigerator.

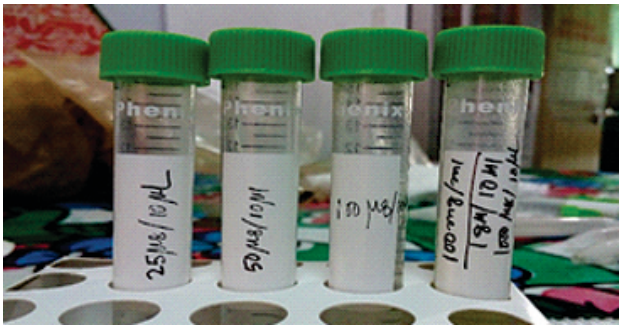

Figure 2: Prepeared Ethanolic Ginger Extract

A sterile cotton swab was dipped into bacterial suspension (Prepared as per procedure I) and inoculated into NA plates then left 5-10 minutes on room temperature. By using a sterile forceps the blank discs were placed on the surface of the plates and with the help of micropipette different concentrations of EGE were put over the blank discs and left for five minutes. Then the plates were incubated at 37°C for 24 hours then the zone of inhibition were measured in mm by using ruler.

Procedure of Experiment- III:

Determination of Minimum Inhibitory Concentration (MIC) of Ethanolic Ginger Extract (EGE) against *Escherichia coli* by broth dilution technique where instruments were sterilized and medium was prepared accordingly (as per procedure- I)

Stock EGE was prepared by mixing 1 gm of powdered ginger extract in 10 ml ethanol. (Parent Solution) So, 1 ml Solution contains 100 mg EGE. This solution was marked as Stock EGE Solution-I. To prepare more diluted working solution, 1:100 dilution was done of the stock EGE solution -I by adding 99 ml of Ethanol.

So, 100 ml of working solution contains 100 mg of EGE.
So, 1 ml of working solution contains 1 mg of EGE, This solution was marked as EGE Solution-II. This solution (EGE Solution-II) was used for determination of MIC of EGE by making different working solution of different concentrations. (Table-2)

Table 2: Composition and different concentrations of working EGE solutions with controls

No of Sets	EGE solution-II (ml)	Nutrient broth medium (ml)	Total (ml)	Concentration of EGE(µg/ml)	Test organism (µl)
Set- I	9	1	10	900	20
Set- II	8	2	10	800	20
Set- III	7	3	10	700	20
Set- IV	6	4	10	600	20
Set- V	5	5	10	500	20
Set- VI	4	6	10	400	20
Set- VII	3	7	10	300	20
Set-VIII	2	8	10	200	20
Set-IX	1	9	10	100	20
Set- X Control-1	10	0	10	1000	20
Set- XI Control-2	-	10	10	-	20
Set-XII Control-3	-	10	10	-	-

With each 10 ml preparation except control-3 (set XII) 20 µl bacterial suspension was added after matching its opacity with that of 0.5 McFarland Standard. After matching the turbidity of bacterial suspension with 0.5 McFarland standards, 20 µl or one drop (0.02 m1) of bacterial suspension of *Escherichia coli* was separately added with each concentrations of working EGE in separate test tubes. These inoculum was also added to the controls (I and 2) except Control-3. The test tubes were marked set wise with black marker and were placed in the incubator at 37 ^{0}C for 18 -24 hours. Then growth of test organism in each preparations of EGE were examined and compared against that of controls by matching their turbidity. The clear preparations were considered as no growth of bacteria and turbid ones, as growth of bacteria. The MIC was reported as lowest concentration of EGE required to prevent the visible growth of test organism.

Procedure of Experiment- IV:

Determination of MIC of Amikacin against *Escherichia coli* by Broth dilution. All the materials were sterilized by hot air oven and autoclaving.

Nutrient broth medium was prepared accordingly and stock solution of Amikacin was prepared by mixing five hundred (500) mg of Amikacin injection with 500 ml of sterile D/W. So, 1 ml solution contains 1 mg Amikacin. (Stock Amikacin solution-I) Then 1 ml of stock Amikacin solution-I was mixed with 99 ml of sterile D/W. This 1:100 dilution of stock Amikacin solution-I had the concentration of 10 µg/ml. This solution was marked as Stock Amikacin Solution-II which was used as stock solution for the determination of MIC of Amikacin. (Table-3)

Table-3: Composition and different concentrations of working Amikacin solutions and the controls.

No. of Sets	Stock Amikacin solution-II (ml)	NB media (ml)	Total (ml)	Concentration of Amikacin (µg/ml)	Test organism (µl)
I	0.25	9.75	10	0.25	20
II	0.5	9.50	10	0.5	20
III	0.75	9.25	10	0.75	20
IV	1	9	10	1	20
V	1.5	8.5	10	1.5	20
VI	2	8	2	2	20
VII	Control-1	10	10	-	20
VIII	Control-2	10	10	-	-

With each 10 ml preparation except control-2 (set VIII) 20 µl bacterial suspensions were added after matching its opacity with that of 0.5 McFarland Standard. After 18 to 24 hours of incubation at 37°C, the growth of *Escherichia coli* in each preparations of Amikacin were examined and compared against that of controls by matching their turbidity. The clear preparations were considered as no growth of bacteria and turbid ones, as growth of bacteria. The MIC was reported as lowest concentration of Amikacin required to prevent the visible growth of test organism.

Procedure of Experiment- V:

Subculture studies of materials from effective CGE, EGE and Amikacin preparations for confirmation of respective results of Experiments I, III and IV

The materials from last two sets of growth and all sets of no growth of CGE incorporated into NA media were subculture in the pure NA (solid) media plates (without any incorporation of CGE). After 18 to 24 hours of incubation at 37°C, the growth of test organism was examined.

The materials from last two sets of growth and all sets of no growth of *Escherichia coli* from dilutions of EGE and Amikacin preparations were sub cultured in the pure NA (solid) media plates (without any EGE and antibiotic mixed with the media). After 18 to 24 hours of incubation at 37°C, the growth of test organism was examined.

Observation and Results

Observation of experiment- I:

There was no inhibition of growth of *Escherichia coli* from 5% to 40% CGE incorporated medium. The growth of *Escherichia coli* started to be inhibited from 50% CGE incorporated media and even no complete inhibition of growth occurred at 100%.

Results of experiment- I:

Crude Ginger (*Zingiber officinale)* Extract (CGE) incorporated into nutrient agar media had a definite inhibitory effect against growth of *Escherichia coli* (Table 4)

Table 4: Inhibitory effect of Crude Ginger Extract (CGE) into Nutrient agar medium against growth of *Escherichia coli.*

No of Sets	Percentage of CGE in NA media	Amount of inoculation	*Escherichia Coli*
Set-I	5	One loopful	Growth not inhibited
Set- II	10	One loopful	Growth not inhibited
Set-III	15	One loopful	Growth not inhibited
Set-IV	20	One loopful	Growth not inhibited
Set-V	30	One loopful	Growth not inhibited
Set- VI	40	One loopful	Growth not inhibited
Set-VII	50	One loopful	Medium growth
Set-VIII	60	One loopful	Medium growth
Set-IX	70	One loopful	Medium growth
Set-X	80	One loopful	Medium growth
Set- XI	90	One loopful	Medium growth
Set-XII	100	One loopful	Medium growth
Set-XIII (Control)	Without CGE	One loopful	Huge Growth

Observation of experiment- II:

In case of Ethanolic extract in disc diffusion method sensitivity was seen against *Escherichia coli* Zone of inhibition 7 mm at 25 µg/10 µl, 22 mm at 50 µg/10 µl and 30 mm at 100 µg/10 µl concentration (Figure 3).

Results of Experiment- II:

According to Zone of diameter interpretation chart it is clearly observed that there is definite antibacterial effects of ethanolic ginger extract (EGE) against *Escherichia coli as* zone of inhibition was 30 mm at 100 µg/10 µl concentration.

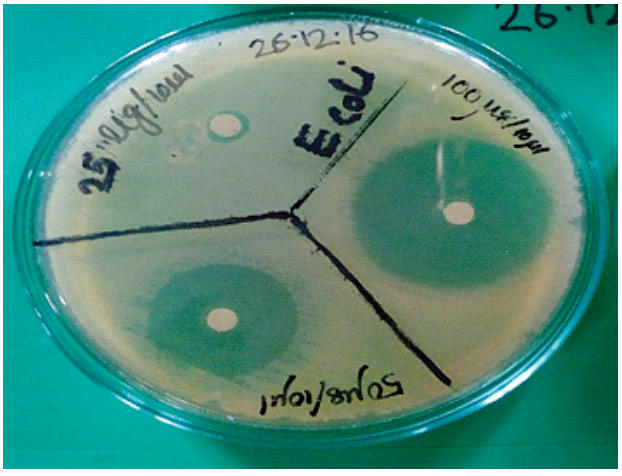

Figure 3: Disc Diffusion showing *Escherichia coli* is sensitive to EGE.

Observations of experiment- III:

In case of *Escherichia coli* the visible growth was in set- IX to Set-VI. Their growth was not visible in Set-V to Set-I. So the MIC of EGE against *Escherichia coli* was 500 µg/ml (Set-V).

Results of Experiment- III:

The Minimum Inhibitory Concentration (MIC) of Ethanolic Ginger Extract (EGE) against 500 µg/ ml at Set-V for *Escherichia Coli*. (Table 5).

Table 5: Minimum Inhibitory Concentration (MIC) of Ethanolic Ginger Extract (EGE) against *Escherichia coli*

No. of Sets	Concentration(EGE) (µg/ml)	*Escherichia Coli*
Set- I	900	No Growth
Set- II	800	No Growth
Set- III	700	No Growth
Set- IV	600	No Growth
Set- V	**500**	**No Growth**
Set- VI	400	Growth
Set- VII	300	Growth
Set- VIII	200	Growth
Set- IX	100	Growth
Set- X Control- 1	1000 (Pure stock EGE+Bacteria)	No Growth
Set- XI Control- 2	N/A Media+Bacteria	Huge Growth
Set- XII Control- 3	N/A media+No Bacteria	No Growth

Table 5 showed the test organisms failed to grow in **control-1** containing pure stock solution of EGE with bacterial inoculum, **control-2** containing nutrient broth medium with inoculum of bacteria showed their visible huge growth and **control-3** containing nutrient broth medium without any bacterial inoculum showing no visible growth of test organisms.

Observation of experiment -IV:

Visible growth of *Escherichia coli* observed at Set-I to Set-III. But the organisms failed to growth at Set-IV to Set-VI. So the MIC of Amikacin against *Escherichia coli* was 1µg/ml (Set IV).

Results of Experiment-IV:

The MIC of Amikacin against *Escherichia coli* was 1.5 µg/ml at set V

Table 6: Minimum Inhibitory Concentration (MIC) of Amikacin against *Escherichia coli*

No of Sets	Concentration (µg/ ml)	*Escherichia coli*
Set-I	0 .25	Growth
Set-II	0.5	Growth
Set-III	**0.75**	Growth
Set-IV	**1**	**No Growth**
Set-V	**1.5**	No Growth
Set-VI	2	No Growth
Set-VII Cintrol-1	(NB medium+No bacteria inoculation)	No Growth
Set-VIII Control-2	(NB media+Bacterial inoculation)	Growth

Table 6 also showed **control-1** containing nutrient broth medium without any bacterial inoculum had no visible growth and **control-2** containing nutrient broth medium with bacterial inoculum observed their visible growth.

Observation of experiment- V:

It was observed from table 7 that the lowest percentages of CGE showing complete inhibition of growth of partial inhibition of *Escherichia coli in* subculture plates were coincided with previous lowest percentages of CGE incorporated into NA media showing inhibitory effects against the test organisms (as found in Experiment-I). The minimum inhibitory concentrations (MICs) of EGE and Amikacin were also coincided with results of their subculture in NA media against the test organism as the found in experiment -III and IV (Table 7).

Results of experiment- V:

Results of subculture study of materials from effective CGE, EGE and Amikacin in NA media were coincided with the respective results of previous experiments.

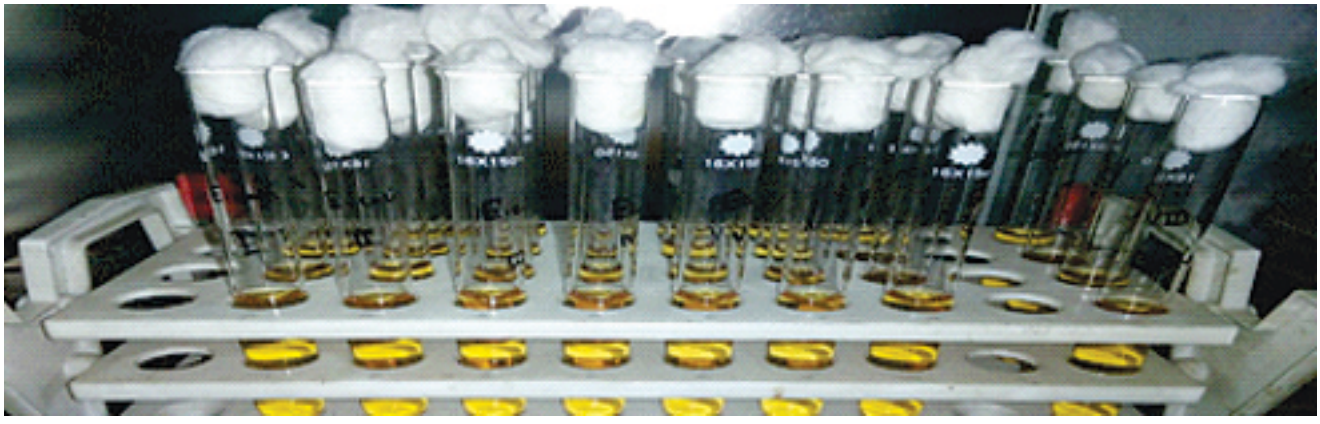

Figure 4: Determination of Minimum Inhibitory Concentration (MIC) of amikacin by broth dilution technique.

Table 7: Subculture study of materials from effective Crude Ginger Extract (CGE), Ethanolic Ginger Extract (EGE) and Amikacin in NA medium for Confirmation of respective result of previous experiment.

Test organisms	Concentration of CGE		Concentration of EGE		Concentration of Amikacin	
	Inhibitory effect against growth	Observed effect in subculture plate	MIC	No growth in subculture Plate	MIC	No growth in subculture plate
	ml/100 ml		µg/ml		µg/ml	
Escherichia coli	100	partial inhibition	500	500	1	1

Discussion

In this study it is found that 50%- 100% CGE has moderate inhibitory effect against *Escherichia coli*. Shah P. 2012[14] and Neihaya HZ. 2015[15] also found that crude ginger extract has moderate antibacterial activity against *Escherichia coli* which is almost similar to this study.

Karuppiah P. 2012[16], determined the antibacterial effect of Allium sativum cloves and Zingiber officinale rhizomes against multi-drug resistant clinical pathogens with the help of disc diffusion method. In that study the zone of inhibition against *Escherichia coli was* 8.50 mm at 25µg/ml, 18.00 mm at 50 µg/ml and 13.50 mm at 100µg/ml. In this study it was 7 mm at 25µg/ml, 22 mm at 50µg/ml and 30 mm at 100µg/ml, which is almost similar with this study.

Yusha MU. 2008[17], determined the antimicrobial activity of ethanolic ginger extract against Gram-negative bacteria with the help of agar well diffusion method. They found that zone of inhibition against *Escherichia coli* was 34 mm at 100% concentration of EGE. In this study the zone of

inhibition against *Escherichia coli* was 30 mm which is almost similar with this study. In both studies it was found that EGE is sensitive against those test organisms.

Ekwenye UN., Elegalam NN. 2005[18], determine the antibacterial activity of ginger extracts on *Escherichia coli* with the help of paper disc diffusion method. In that study the zone of inhibition (ZOI) against *Escherichia coli* was 10 mm at 75% concentration. In this study ZOI of EGE against *Escherichia coli was* 22 mm at 50% concentration. In both studies it was found that EGE is sensitive against those test organism.

Karuppiah P. 2012[16], determined the MIC of ethanolic ginger extract against *Escherichia coli* was 75 µg/ml. But in this study the MIC of EGE was against *Escherichia coli* 500 µg/ml. This is bit different with this study. This is may be due to the species difference or the ginger difference in different biologic condition.

Conclusion

From this study it is clearly observed that there is definite antibacterial effects of ethanolic Ginger extract (EGE) against *Escherichia coli.* The crude Ginger extract (CGE) also has its definite inhibitory effects against *Escherichia coli.* Further studies are required to detect and isolate the active ingredients present in the Ginger extract responsible for antibacterial effect. Then their effects against the studied organism should be studied in vivo separately and their toxicity profiles should also be taken into account. Only then the Ginger extracts will fulfill the criteria for its therapeutic use. Until then ginger may be used in gastrointestinal tract infection, respiratory tract infection, skin infection and urinary tract infection along with the conventional antibiotics which are used in those conditions.

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