Print ISSN 2307-7689
Online ISSN 2616-6054
Mediscope
The Journal of GMC

ORIGINAL ARTICLE

DOI: https://doi.org/10.3329/mediscope.v7i1.47137

Antibacterial effect of Ginger *(Zingiber officinale)* against *Staphylococcus aureus*

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Abstract

This experimental study was carried out to determine the antibacterial effect of Crude Ginger Extract (CGE) and Ethanolic Ginger Extract (EGE) against standard strain of Staphylococcus aureus in the Department of Pharmacology and Therapeutics with the collaboration of Department of Microbiology, Mymensingh Medical College, Mymensingh from July 2016 to June 2017. The growth of Staphylococcus aureus started to be inhibited from 70% CGE incorporated media and complete inhibition of growth occurred at 100% CGE. In case of Ethanolic Extract, sensitivity was seen against Staphylococcus aureus using disc diffusion method. Zones of inhibition were 8, 13 and 19 mm at 25, 50 and 100 µg/10 µl respectively. The minimum inhibitory concentration (MIC) of EGE was assessed by broth dilution technique. The MIC of EGE was 400 µg/ml against Staphylococcus aureus. From the study it is clearly observed that there is definite antibacterial effect of crude ginger extract (CGE) and ethanolic ginger extract against Staphylococcus aureus. Further studies are required to detect and isolate the active ingredients present in the Ginger extract responsible for antibacterial effect.

Introduction

Medicinal plants are found to be useful as pharmaceuticals, nutraceuticals, cosmetics and food supplements.[1] Plant derived products have been used for medicinal purposes for centuries. In traditional Indian medicine or Ayurveda, Zingiber officinale and many other herbs have been used as medicine.[2] With an increase in the antibiotic-resistant strains of microorganisms, traditional plants are being investigated for their antibacterial and medicinal values. Traditional uses of plants have led to investigating their bioactive compounds, which have resulted in the detection of a significant number of therapeutic properties.[1] Ginger has been used as medicine from Vedic period and is called 'maha- aushadhi' which means the great medicine.[2] Ginger is easily available, universally acceptable and relatively inexpensive and well tolerated by

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most of the people.The ginger has been listed in'Generally Recognized as Safe' (GRAS) document of the US FDA.[2] Ginger (Zingiber officinale) belongs to Zingiberaceae family.1 The Zingiberaceae plants are characterized by their tuberous or non-tuberous rhizomes, which have strong aromatic and medicinal properties.[3] 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.[4] The active ingredients in ginger are thought to reside in its volatile oils, which comprise approximately 1-3% of its weight.[5] 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.[6,7] Active constituents of ginger inhibit multiplication of bacteria by membrane disruption.[8] Ginger is a strong antibacterial agent against Staphylococcus aureus.[6] Because of the increasing resistance of bacteria to antibiotics, herbal products are looking for new leads to develop better antibiotics.9 Therefore the aims of this study are to investigate the antibacterial effectiveness of crude paste and ethanolic ginger extract.

Materials and methods

This experimental 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 the main 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 Staphylococcus aureus ATCC 25923 was collected from Microbiology Department of Mymensingh Medical College.

Preparation of crude ginger extract:

Ginger (1000 gm) was washed initially by distilled water and then by 95% ethanol and homogenized by using sterile mortar and pestle. Then it was sieved through double layer of sterile fine mesh cloth to make crude extract. This CGE was considered as 100% crude ginger extract.

Table 01: Composition of different concentrations of CGE incorporated into Nutrient agar (NA) media

Set No.	CGE (ml)	Distilled water in NA media to make 100 ml	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 (Staphylococcus aureus) 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 McFarland standards (1.5×108 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.

Preparation of ethanolic ginger extract:

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 and then centrifuged at 3000 rpm for 10 minutes. Secondary filtration was done 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 was mixed with 10 ml ethanol. Then it was filtered by gauze pad and centrifuged at 3000 rpm for 10 min and again filtered by filter paper. This solution was the source of preparing different concentrations of ethanolic ginger extract. The extract was stored at 4°C in refrigerator.

Calculation of concentration of different EGE Disc Diffusion solutions:

Powdered ginger extract 1gm in 10 ml ethanol. This solution was marked as Parent solution. 10 ml ethanol contains 1gm = 1000 mg ethanolic ginger extract. So, 1 ml Solution contains 100 mg EGE. This solution was marked as Stock EGE DD (Disc Diffusion) Solution-I. Then 1:10 dilution was done of stock EGE DD solution-I by adding 9 ml of ethanol.

So, 10 ml solution contains 100 mg of EGE.So, 1 ml=1000 µl solution contains 10 mg=10×1000 µg of EGE=10000 µg. Thus, 10 µl solution contained 100 µg of EGE; this solution was used in Disc Diffusion Method and different lower concentration solutions (25 µg and 50 µg per 10 µl) were made from this by adding ethanol. In case of making higher concentration of disc Diffusion solution same procedure was applied but the difference was done in making Parent solution. Instead of 1gm of powdered ginger extract in higher concentration Disc Diffusion Solution, 2 gm, 4 gm, and 8 gm powdered ginger extract was mixed with 10 ml ethanol. So the concentrations were 200 µg, 400 µg and 800 µg per 10 µl respectively.

Antibacterial sensitivity test by disc diffusion method:

Antibacterial sensitivity test was performed by Modified Kirby-Bauer disc diffusion technique as following. After matching with 0.5 McFarland standards, a sterile cotton swab was dipped into bacterial suspension and streaked in three directions on the surface of Mueller-Hinton agar plates and then left for 5-10 minutes in room temperature. By using sterile forceps the blank paper discs (6 mm in diameter) were placed on the surface of the plates. Then with the help of micropipette 5µl amount of 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. After that zone of inhibition for respective organisms were measured in mm by using ruler.

Determination of Minimum Inhibitory Concentration (MIC) of Ethanolic Ginger Extract (EGE) by broth dilution technique:

The minimum inhibitory concentration (MIC) is the concentration giving the least inhibitory activity and below which there is no further inhibition.

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 02)

Table 02: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 C-1	10	0	10	1000	20
Set- XI C-2	-	10	10	-	20
Set-XII C-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 Staphylococcus aureuswas 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.

Results

Inhibitory effects of crude ginger extract

Table 03 shows that there was no inhibition of growth of Staphylococcus aureus from 5% to 60% CGE incorporated medium. The growth of Staphylococcus aureus started to be inhibited from 70% CGE incorporated media and complete inhibition of growth occurred at 100%.

Table 03: Inhibitory effect of CGE incorporated into Nutrient agar medium against growth of Staphylococcus aureus

No of Sets	Percentage of CGE in NA media	Amount of inoculation	*Staphylococcus aureus*
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	Growth not inhibited
Set-VIII	60	One loopful	Growth not inhibited
Set-IX	70	One loopful	Medium growth
Set-X	80	One loopful	Medium gro wth
Set- XI	90	One loopful	Medium growth
Set-XII	**100**	**One loopful**	**No growth**
Set-XIII (Control)	Without CGE	One loopful	Huge Growth

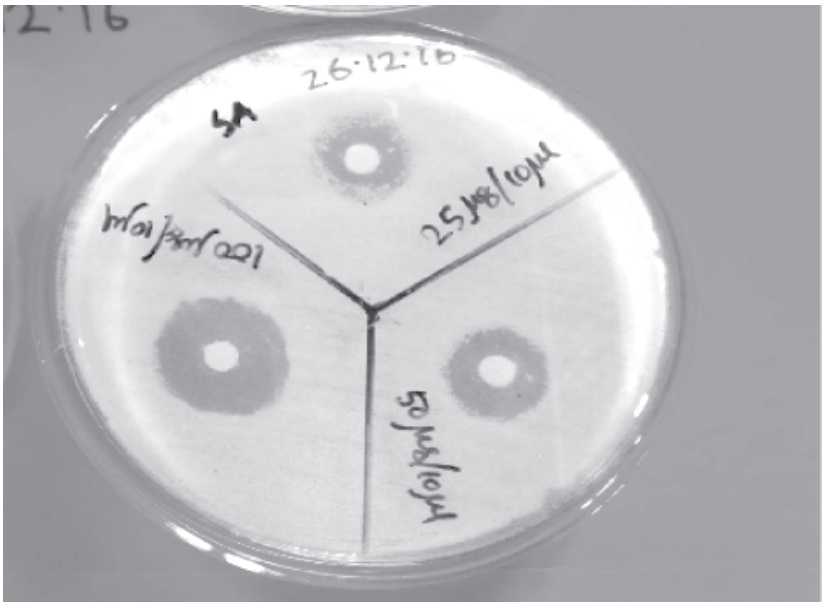

Figure 01: Disc Diffusion showing Staphylococcus aureus is sensitive to EGE.

Antibacterial sensitivity testing of ethanolic ginger extract

In case of Ethanolic extract in disc diffusion method sensitivity was seen against Staphylococcus aureus Zone of inhibition 8 mm at 25 µg/10 µl, 13 mm at 50 µg/10 µl and 19 mm at 100 µg/10 µl concentration (Figure 01).

Determination of minimum inhibitory concentration (MIC) of ethanolic ginger extract

Table 04 shows that visible growth of Staphylococcus aureus was from Set-VII to Set-IX. And no growth was visible from Set-I to Set-VI. So, the MIC of EGE against Staphylococcus aureus was 400 µg/ml (Set-VI).

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Table 04: MIC of EGE against *Staphylococcus aureus*

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**	**No 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-XIControl- 2	N/A Media + Bacteria	Huge Growth
Set-XIIControl- 3	N/A media + No Bacteria	No Growth

Discussion

In this study it is found that 100% CGE has complete inhibitory effect against Staphylococcus aureus. Shah P10 also found that crude ginger extract has antibacterial activity against Staphylococcus aureus which is almost similar to this study. Karuppiah P11 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 Staphylococcus aureus was 9.30 mm at 25µg/ml, 11.55 mm at 50 µg/ml and 12.52 mm at 100µg/ml. In this study it was 8 mm at 25µg/ml, 13 mm at 50µg/ml and 19 mm at 100µg/ml, which is almost similar with that study. Kaushik P12 determined the antibacterial effect of ginger with the help of agar well diffusion method against Staphylococcus aureus with the use of various solvents. In ethanolic extract Staphylococcus aureus was sensitive and zone of inhibition was 14 mm. In this study Staphylococcus aureus is sensitive to ethanolic ginger extract. Neihaya HZ13, determine the antibacterial effect of ginger and black pepper extracts (alone and combination) with sesame oil on some pathogenic bacteria at different concentration. In that study zone of inhibition was 13 mm, against Staphylococcus aureus at 10% concentration. But in this study zone of inhibition was 8mm at 25µg/ml. In both studies Staphylococcus aureus was sensitive to ethanolic ginger extract. Karuppiah P14 determined the MIC of ethanolic ginger extract against Staphylococcus aureus was 67 µg/ml. But in this study the MIC of EGE was against Staphylococcus aureus 400 µg/ml. This is bit different with this study. This 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 Staphylococcus aureus. The crude Ginger extract (CGE) also has its definite inhibitory effects against Staphylococcus aureus. 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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