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Antibacterial Effect of Ginger *(Zingiber officinale)* against *Pseudomonas aeruginosa*

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Abstract

Background: *Historically, medicinal plants have been a source of novel drug compounds. Plants derived products have made large contributions to human health and wellbeing. Green pharmacy may become the base for the development of medicines by providing a pharmacophore which could be used for the development of new drug with novel mechanisms of action. Many scientists across the globe have reported antimicrobial properties of several medicinal plants but still a very few portion of this tremendous potential drug-repertoire has been scientifically screened. Because of the increasing resistance of some bacteria to antibiotics, herbal products are looking for new leads to develop better antibiotics.* ***Objectives:*** *In this regard one of the herbal spice Zingiber officinale (Ginger) was undertaken to investigate the antibacterial effect against the commonly encountered pathogens (Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Salmonella typhi) causing various type of infections forits low cost, availability throughout the year and less adverse effects.* ***Materials and Methods:*** *The study was conducted from July 2016 to June 2017 in the Department of Pharmacology and Therapeutics with the collaboration of the Department of Microbiology, Mymensingh Medical College, Mymensingh, to determine the profile of the antibacterial effect of Crude Ginger Extract (CGE), Ethanolic Ginger Extract (EGE) and standard antibiotic Amikacin against standard strains of Pseudomonas aeruginosa. It was an experimental study. Five separate experiments were done e.g.* ***Experiment-I****, Determination of inhibitory effects of crude ginger extract by incorporation into Nutrient agar media against Pseudomonas aeruginosa.* ***Experiment-II****, Antibacterial sensitivity testing of ethanolic ginger extract againstPseudomonas aeruginosaby using disc diffusion method.* ***Experiment-III****, Determination of minimum inhibitory concentration (MIC) of ethanolic ginger extract against the test organism by broth dilution technique.* ***Experiment-IV****, Determination of minimum inhibitory concentration (MIC) of antibiotic Amikacin against test organism by broth dilution technique and* ***Experiment-V****, Subculture studies of materials from effective CGE, EGE and Amikacin preparations for confirmation of respective results of Experiments I, III and IV.* ***Results:*** *The growth of Pseudomonas aeruginosastarted to be inhibited from 90% CGE incorporated media and complete inhibition of growth occurred at 100%. In case of Ethanolic Extract in disc diffusion method sensitivity was seen against Pseudomonas aeruginosa zone of inhibition was 14 mm at 25 µg/10 µl, 16 mm at 50 µg/10 µl and, 18 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 600 µg/ml against Pseudomonas aeruginosa and the MIC of Amikacin 1.5 µg/ml against Pseudomonas aeruginosa. The subculture study showed the same results with that of previous experiments.* ***Conclusion:*** *From the study it is clearly observed that there is definite antibacterial effect of ethanolic ginger extract (EGE) against Pseudomonas aeruginosa. The crude ginger extract (CGE) also has its definite inhibitory effects against the organism studied. Further studies are required to detect and isolate the active ingredients present in the Ginger extract responsible for antibacterial effect.*

Keywords: *CGE, EGE, MIC, pseudomonas aeruginosa.*

Introduction

Historically, medicinal plants have been a source of novel drug compounds. Plants derived products have made large contributions to human health and well-being. Green pharmacy may become the base for the development of medicines by providing a pharmacophore which could be used for the development of new drug with novel mechanisms of action. Many scientists across the globe have reported antimicrobial properties of several medicinal plants but still a very few portion of this tremendous potential drug repertoire has been scientifically screened (1). A number of medicinal plants have been screened for antimicrobial activity in recent years (2) and efforts have been done to identify their active constituents (3). The plants extracts possessing bioactivity are essentially evaluated for toxicity and the extracts are usually tested for short or long term toxicity in animal models (4, 5). Nontoxic extracts possessing good bioactive principles may provide potential antimicrobial leads.

Ginger (Zingiber officinale) belongs to Zingiberaceae family (6). The Zingiberaceae plants are characterized by their tuberous or non-tuberous rhizomes, which have strong aromatic and medicinal properties (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 Pseudomonas aeruginosa(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 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 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 Pseudomonas aeruginosa ATCC 27853 was use for testing and collected from Microbiology Department of Mymensingh Medical College. Five experiments were conducted during this time period, they are as follows

Experiment-I:

Pregnant women having gestational diabetes Patients who had incomplete data regarding clinical and laboratory profile

Experiment-II:

Antibacterial sensitivity testing of Ethanolic Ginger Extract (EGE) againstPseudomonas aeruginosaby using disc diffusion method.

Experiment-III:

Determination of Minimum Inhibitory Concentration (MIC) of Ethanolic Ginger Extract (EGE) against test organism by broth dilution technique.

Experiment-IV:

Determination of MIC of Amikacin against test organism by Broth Dilution Technique.

Experiment-V:

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

Procedure of Experiment-I:

Inhibitory effects of CGE against Pseudomonas aeruginosainto 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).**

* One loopful =20 µl

Bacterial (Pseudomonas aeruginosa) 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.

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

Fig 1. Petri dish contains prepared different concentration of CGE

Procedure of Experiment- II:

Antibacterial sensitivity testing of Ethanolic Ginger Extract (EGE) againstPseudomonas aeruginosaby 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.

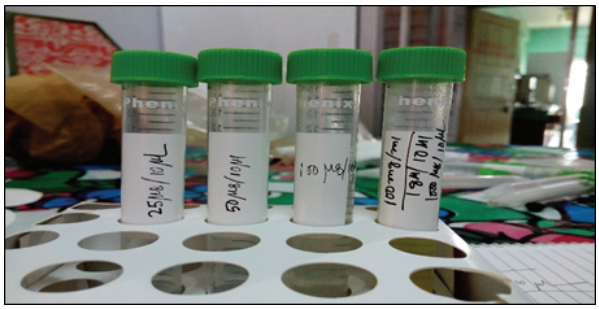

Fig 2. Prepared Ethanolic Ginger Extract

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.

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 Pseudomonas aeruginosaby broth dilution technique where instruments were sterilizedand 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 o 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-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 Pseudomonas aeruginosain 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- IV:

Determination of MIC of Amikacin against Pseudomonas aeruginosa 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 DW. 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 Pseudomonas aeruginosain 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 testorganism.

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 ofPseudomonas aeruginosafrom 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.

Results

Results of the experiment- I:

It was observed that the growth of Pseudomonas aeruginosa was started to be inhibited from 90% Crude Ginger (Zingiber officinale) Extract (CGE) incorporated media and complete inhibition of growth occurred at 100% **(Figure 3).**

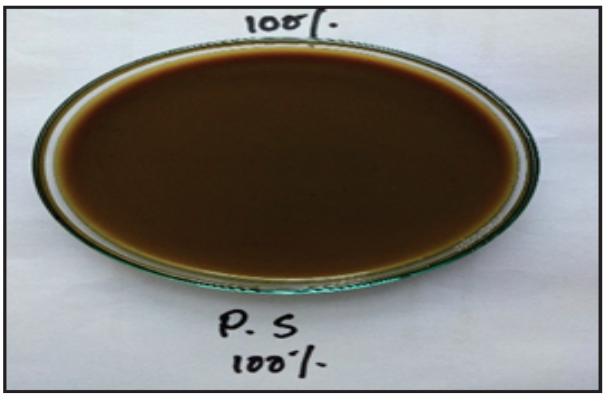

Figure 3: Inhibitory effect of CGE at 100% concentration

The inhibitory effect of Crude Ginger (Zingiber officinale) Extract (CGE) incorporated into nutrient agar media against the growth of Pseudomonas aeruginosa is shown in **Table 4.**

Table 4: Inhibitory effect of CGE incorporated into Nutrient Agar (NA) medium against the growth of Pseudomonas aeruginosa

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

Results of the experiment- II:

In case of Ethanolic extract, in disc diffusion method, sensitivity was seen against Pseudomonas aeruginosa with a maximum zone of inhibition of 18 mm at 100 µg/10 µl concentration (Figure 3). According to the Zone of diameter interpretation chart, it was clearly observed that there is an antibacterial effect of ethanolic ginger extract (EGE) against Pseudomonas aeruginosa as zone of inhibition was 18 mm at 100 µg/10 µl concentration.

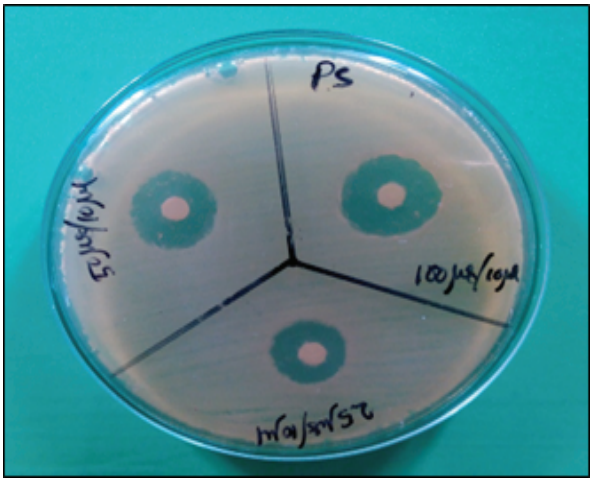

Figure 4: Disc Diffusion showing the sensitivity of Pseudomonas aeruginosa to EGE.

Results of the experiment- III:

Visible growth of Pseudomonas aeruginosa was observed in set-IX to Set-V (Table 5). Their growth was not visible in Set-IV to Set-I. So, the MIC of EGE against Pseudomonas aeruginosa was 600 µg/ml (Set-IV). The inhibitory effect of ethanolic ginger extract (EGE) against the growth of Pseudomonas aeruginosa is shown in **Table 5.**

Table 5: IInhibitory effect of EGE *against Pseudomonas aeruginosa*

No. of Sets	Concentration of EGE (µg/ml)	*Pseudomonas aeruginosa*
Set-I	900	No Growth
Set-II	800	No Growth
Set-III	700	No Growth
Set-IV	600	No Growth
Set-V	500	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

Results of the experiment- IV:

Visible growth of Pseudomonas aeruginosa was observed at Set-I to Set-IV but the organisms failed to grow at Set-V to Set-VIII (Figure 5). So, the MIC of Amikacin against Pseudomonas aeruginosa was 1.5µg/ml (Set V). The inhibitory effect of Amikacin against the growth of Pseudomonas aeruginosa is shown in **Table 6.**

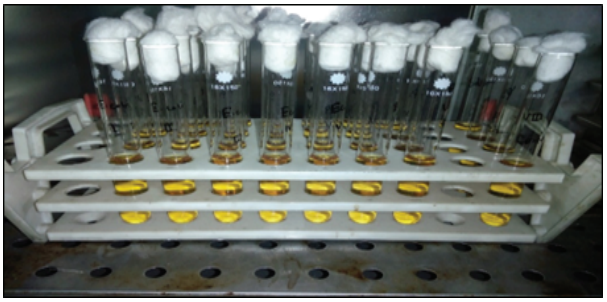

Figure 5: Determination of MIC of amikacin by broth dilution technique

Table 6: MIC of Amikacin against *Pseudomonas aeruginosa*

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

Results of the experiment- V:

It was observed that the concentration of CGE in subculture plates showing complete inhibition of growth of Pseudomonas aeruginosa was 100 ml/100 ml which coincides with the findings of the experiment- I (Table 7). The MICs of EGE and Amikacin found in the subculture plates were 600 µg/ml and 1.5 µg/ml respectively were also coinciding with results of experiment- III and IV **(Table 7)**.

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

Material	Minimum inhibitory Concentration (MIC)	Observed effect in subculture plate
CGE (Crude Ginger Extract)	100 ml/100 ml	Complete inhibition
EGE (Ethanolic Ginger Extract)	600 µg/ml	No growth
Amikacin	1.5 µg/ml	No growth

Discussion

In this study it is found that 100% CGE has complete inhibitory effect against Pseudomonas aeruginosa.Shah P. 2012 (14) also found that crude ginger extract has antibacterial activity against Pseudomonas aeruginosawhich is almost similar to this study.

Karuppiah P. 2012 (15), 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 Pseudomonas aeruginosawas 10.4 mm at 25µg/ml, 13.5 mm at 50 µg/ml and 14.1 mm at 100µg/ml. In this study it was 14 mm at 25µg/ml, 16 mm at 50µg/ml and 18 mm at 100µg/ml, which is almost similar with this study.
M. Yusha. U et al. 2008 (16), determined the inhibitory effect of garlic and ginger extracts on some respiratory tract isolates of gram negative organisms. In that study the zone of inhibition against Pseudomonas aeruginosawas 21 mm at 25µg/ml, 23 mm at 50 µg/ml and 25 mm at 100µg/ml. In this study it was 14 mm at 25µg/ml, 16 mm at 50µg/ml and 18 mm at 100µg/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.

H.Z. Neihaya 2015 (17), 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 00 mm, against Pseudomonas aeruginosaat 10% concentration. But in this study zone of inhibition was 14 mm at 25µg/ml. This is bit different with this study.

Karuppiah P. 2012 (15), determined the MIC of ethanolic ginger extract againstPseudomonas aeruginosawas 75 µg/ml. But in this study the MIC of EGE was against Staphylococcus aureus600 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.

Conclusionon

From this study it is clearly observed that there is definite antibacterial effects of ethanolic Ginger extract (EGE) against Pseudomonas aeruginosa. The crude Ginger extract (CGE) also has its definite inhibitory effects against Pseudomonas aeruginosa. 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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