Antibacterial activity of ginger extracts and its essential oil on some of pathogenic bacteria

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Abstract:

The antimicrobial activity of ginger extracts (cold-water, hot-water, ethanolic and essential oil) against some of pathogenic bacteria (*Escherichia coli , Salmonella* sp *, Klebsiella* sp *, Serratia marcescens, Vibrio cholerae , Staphylococcus aureus , Streptococcus* sp*)* was investigated using Disc diffusion method , and the results were compared with the antimicrobial activity of 12 antibiotics on the same bacteria .

The results showed that the ginger extracts were more effective on gram-positive bacteria than gram-negative . *V. cholerae* and *S. marcescens,*were the most resistant bacteria to the extracts used , while highest inhibition was noticed against *Streptococcus* sp (28 mm) . The ethanolic extract showed the broadest antibacterial activity (11 to 28 mm) , in comparison with moderate activity of essential oil , it was observed that the cold-water extract was more effective on the bacteria than hot-water extract .

Ginger ethanolic extract presented higher diameter of inhibition zone for *Streptococcus* sp than in Ciprofloxacin , Cefotaxime , Cefalotin , Cephalexin and Cephaloridine , also it was found a similarity between the higher inhibition zones of ethanolic extract of ginger and some antibiotics for *S. aureus , E. coli , Salmonella* sp and *Klebsiella* sp . *V. cholerae* and *S. marcescens,*also highly resistant to antibiotics .

Phytochemical analysis of ethanolic extract of ginger revealed the present of glycosides, terpenoids, flavonids and phenolic compounds

Key words: Antibacterial activity, ginger extracts.

Introduction :

Ginger (*Zingiber officinale*) is member of the zingiberaceae family of plant that include cardamom and turmeric. The strong aroma of ginger is the result of pungent ketones including gingerol [1].The medicinal use of ginger rhizome dates 2,500 years in china and India, where it was prescribed to treat headaches, nausea, rheumatism and colds [2].Ginger has been shown to have antimicrobial activity against pathogenic bacteria such as *Escherichia coli, Proteus sp.*, staphylococci, streptococci *and Salmonella [3,4]* **.**The ginger has the capacity to eliminate harmful bacteria responsible for most of the diarrhoea, especially in children . It has been shown to reduce the stickiness of blood platelets, hence may help reduce risk of arthrosclerosis[5]**,** antimicrobial activity of spices and herbs has been known and described for several centuries. At present, its estimated that about 80% at world population rely on botanical properties of medicines to meet their health need . Herbs and spices are generally considered safe and proved to be effective against certain ailments [6].

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Since the introduction of antibiotics there has been tremendous increase in the resistance of diverse bacterial pathogenes [7]. The enterococci have intrinsic resistance to multiple antimicrobials, most drug resistance in enteric bacteria is attributed to the wide spread transmission of resistance plasmid among different genera [8]In the present study we have evaluated the antibacterial effect of the extracts of ginger against some of pathogenic bacteria. The inhibitory effect of ginger was compared with the effect of 12 antibiotics and the results are discussed and also find out the phytochemical active constituents.

Materials and Methods:

The bacterial isolates *E.coli*,*Salmonella* sp, *klebsiella* sp, *S. marcescens*, and *V. choierae* were isolated from gastrointestinal infections, also *S. aureus* and *Streptococcus* sp were isolated from respiratory tract infections. All the bacteria were obtained, as clinical isolated, from Al-Yarmook teaching hospital in Baghdad. Bacterial cultures were maintained on Nutrient agar(NA)Slopes. They were subcultured monthly and subsequently stored at 4^{o}C.

Culture preparation

Aloopfull of 24 hr. surface growth on a NA slope of each bacterial isolate was transferred individually to 5ml of Brain heart infusion broth(PH 7.6)and incubated at 37^{o}C for 24 hr, bacterial cells were collected by centrifugation at 3000rpm for 15 min., washed twice and resuspended in 0.1% pepton water. Turbidity was adjusted to match that of as Mcfarland standared (10^{8} CFU/ml). Then 1:10 dilution of the cell suspension was performed to give an inoculum concentration of 10^{7}CFU /ml.

Ginger extraction

The ginger rhizomes were washed with clean sterile distilled water and allowed to air-dry for one hour , then the outer covering of the ginger were manually peeled off and the ginger was washed again and extracted using the following procedures:

A-cold-water extraction

Exactly 20g of fresh ginger rhizomes were blended into fine powder and soaked in 100ml of distilled water for 24 hr .The pulp obtained was left in aclean ,sterile glass container and shaken at 150 rpm for 8 hr vigorously to allow for proper extraction and it was filtered using asterile muslin cloth after which the extract was obtained, air-dried and stored below ambient temperature until required[9]

B-Hot-water extraction

Exactly 20g of fresh ginger rhizomes were blended and soaked in 100 ml of hot water at 80 C (shaker water bath) at 150 rpm for 24hr.,the resulted juice was extracted air- dried and storied as in above [9].

C-Crude ethanolic extraction.

20g of small pieces of fresh ginger rhizomes were soaked in 100ml of 95% ethanol ,and shaken at 150rpm for 24 hr at ambient temperature .the mixture then filtered. The filterates were evaporated using vacuum rotary evaporator,and frozen at -20^{o}C .Stock solutions of crude ethanolic extracts were prepared by diluting the dried extracts with 10% dimethyl sulphoxide (DMSO)solution[9].

D-Essential oils

300g of small pieces of fresh ginger rhizomes with distilled water (1L) were placed in flask (2L) together after steam distillation, the essential oils were collected, dispensed into dark bottles, and stored at 4^{o} C until used [10].

Antibacterial screening test of extracts using disk diffusion method.

The disk diffusion test was performed using standard procedure by Jorgensen *et al.* [11].The inoculum suspension of each bacterial isolate was swabbed on the entire surface of Muller-Hinton agar (MHA)(pH7.3).Sterile 6mm filter paper discs (Watman No.3) were aseptically placed on MHA surface, and crude ethanolic extract, essential oil , hot –water extraction and cold – water extraction were immediately added to discs in volume of 20 ml. A 20ml aliquot of 10% DMSO and distilled water were also added to a sterile paper discs as a negative control, whereas an antibiotic screening by disc method used as a positive control.

The plates were left at ambient temperature for 15 min. to allow excess predifferent of extraction prior to incubation at 37º C for 24 hr. Diameters of inhibition zone were measured each experimental was done in duplicate.

Inhibition zone with diameter less than 12mm were considered as having no antibacterial activity ,diameter between 12 and 16 mm were considered moderately active, and these with >16mm were considered moderately active[12].

Antibiotic sensitivity testing (antibiogram).

The test microorganisims were also tested for their sensitivity by Disc-diffusion method (Kirby-Bauer method) [13] against the antibiotics manufactured by Bioanalyse and Oxoid in 2008 with the concentration (**µ**g/disc) , Penicillin G(10), Ampicilin(10), Cefotaxime(30), Cephalexin(30), Cefalotin(30), Cephaloridine(30), Trimethoprime sulphamethoxazole(1.25+23.75), Tetracyclin(30), Erthromycin(15), Kanamycin(30), Vancomycin(30) and Ciprofloxacin(10)

Phytochemical anylysis

The identification tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids, flavonids , phenolic compounds , reducing sugars and tannins by the procedures as described by Siddiqui and Ali (1997)[14]

Result and Discussion:

The results of antibacterial activity of ginger extracts (cold-water, hot-water, crude ethanolic and essential oil)on the pathogenic bacterial isolates (*E. coli, Salmonella sp, klebsiella sp, S. marcescens, V. cholera ,S. aureus and Streptococcus sp*) are shown in table(1).

Table (1): Antibacterial activity of crude extracts of ginger against some of pathogenic bacteria.*

No.	pathogenic bacteria	Diameter of inhibition zone (mm)			
		cold -water extract(100 %)	hot -water extract(100 %)	Ethanolic extract(100 %)	Essential oil(100 %)
1-	*Escherichia coli*	16	10	18	12
2-	*Salmonella* sp.	18	N.I.**	20	17
3-	*Klebsiella* sp.	14	10	18	14
4-	*Serratia marcescens*	N.I.	N.I.	11	N.I.
5-	*Vibrio cholerae*	N.I.	N.I.	10	8
6-	*Staphylococcus aureus*	18	8	24	18
7-	*Streptococcus* sp.	14	11	28	20

*Data are means of two replications. **
N.I.:No. Inhibition.

The results of this work indicates that the ginger extracts were more effective on gram –positive bacteria (the widest zone of inhibition was 28 mm) than on gram negative bacteria (the widest zone of inhibition was 20mm). This is probably due to the differences in cell wall structure of gram-positive bacteria and gram negative bacteria. These results agree with observations of theAkoachere *et al*. [15], who had reported that the extracts of ginger exhibited antibacterial activity against the pathogens *S.aureus* and

Streptococcus pyogenes .Highest inhibition was noticed against *Streptococcus* (with highest inhibition zone 28mm).

In gram negative bacteria it was observed that ginger extracts(except hot-water extract) had activity on *E. coli , Salmonella* sp *and Klebsiella* sp (the range of inhibition zone was 12 to 20mm) , while *S.marcesces* and *V.cholerae* were the most resistant bacteria to all extracts used . These results are contradictory to the observations of Indue *et al*.[12], who had reported that the ginger extracts did not show any antibacterial activity against all serogroups of *E.coli* and *Salmonella* sp.The differences may be due to a difference in the variety of the ginger used in this study , the difference in the strains of pathogenic bacteria and the source of samples .

The ethanolic extract of ginger showed the broadest antibacterial activity by inhibiting growth of all bacterial isolate tested (the diameter of inhibition zone,11-28mm).This credit to ethanol extraction was supposed to ethanol being an organic solvent and will dissolve organic compounds better, hence liberate the active component such as zingerone, gingerol and shogaol required for antimicrobial activity [5]

It was observed that the cold –water extract of ginger was more effective on the bacteria than hot-water extract, this may be explained by the fact that the antimicrobial substances in the ginger extract are destroyed by heat from the hot-water which might have raised the temperature of the extracts inactivating them[16].Nelson *et al*. [5] explain that the antimicrobial substance in the extract are mainly phenolic compounds were destroyed or inactivated by heat . Ginger essential oil possessed moderate antibacterial activity in this study. The major pungent compound of ginger are gingerone and gingerol which have strong inhibitory activity against pathogenic bacteria [17].These result disagree with observations of Suree and Pana [18], who obtained that the inhibitory activity of essential oil was greater than that of ethanolic extract . The greater effect of ethanolic extract compared to the other may be due to that plant extract in organic solvents provided more cnsistent antimicrobial activity , also we think that the ginger oil has therapeutic properties such as analgesic , antiemetic and antispasmodic more than antibacterial properties .

The antimicrobial susceptibility results for the pathogic bacterial isolates against commonly used antibiotics are summarized in table (2).

Table (2) antimicrobial susceptibility of some pathogenic bacteria to antibiotics.*

no	*Anitbiotic*	*E.coli*	*Salmonella*	*Klebsiella*	*S.marcescens*	*V.cholerae*	*S.aureus*	*Streptococcus*
1-	Penicillin G	R*	R	R	R	R	R	R
2-	Ampicillin	R	R	R	R	R	R	R
3-	Cephalexin	R	16**	R	15	R	20	18
4-	Cefalotin	22	R	R	R	17	16	19
5-	Cephaloridine	R	R	15	R	R	16	18
6-	Cefotaxime	R	18	R	24	R	24	20
7-	Trimetoprime-sulphamethaxozol	18	R	20	R	R	R	R
8-	Tetracyclin	R	R	R	R	R	16	R
9-	Erthromycin	R	R	R	R	R	R	R
10-	Kanamaycin	20	18	R	R	17	20	14
11-	Vancomycin	R	R	R	R	10	14	12
12-	Ciprofloxacin	22	R	18	R	16	25	22

*The results of sensitivity to antibiotics were performed in accordance with NCCLS guidelines (19).

R: Resistant

** The number is mean the inhibition zone in mm .(the bacteria intermediate sensitive or susceptible to antibiotic)

The results showed that all isolates were resistant to 3 or more antibacterial and defined as multidrug resistant. Ginger extract presented higher diameter of inhibition zones for *Streptococcus* sp than in Ciprofioxacin, Cefotaxime, Cefalotin, Cephalexin, and Cephaloridin*e.*

It was found there is a similarity between the higher inhibition zones of ethanolic extract of ginger and some antibiotics for *S.aureus, E.coli, Salmonella* sp *,and Klebsiella* sp*,* also observed that *V.cholerae* and *S. marcescens* highly resistant to antibiotics as in their resistant to ginger extracts and this had led to the suggestion that there may be the presence of multiple plasmids in the mutants or plasmid carrying multiple resistance determinants[20].Also as in the effect of antibiotics it was suggested that the antimicrobial action of spices is due to the impairment of variety of enzymes systems involving in the production of energy or synthesis of structural components in microbial cells [21].

Phytochmical analysis of ethanolic extract of ginger revealed the presence of glycosides, terpenoids, flavonids and phenolic compounds (table 3) . It has been observed that there is a possibility of synergism between the active compounds in the crude extract than in isolated constituents [22] . Acetone and ethanol extracts of ginger contains pungent substances namely Oleoresin (gingerol and shagaol) , phenols(zingerone and gingeol) and paradol [23]. Hydroethanolic ginger extract exhibited potent antibacterial activity against gram positive and gram negative bacteria, this effect may be due to gingerols and phenolic compounds[24]

Table (3) chemical constituents of ethanolic extract of ginger.

The type of extract	Alcaloids	Glycosides	terpenoids	Flavonids	phenolic compounds	Reducing sugars	Tannins
Ethanolic extract	-ve	+ve	+ve	+ve	+ve	-ve	-ve

+ve=detected -ve=not detected

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الفعالية المضادة للبكتريا لمستخلصات الزنجبيل وزيته العطري في بعض البكتريا الممرضة

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الخلاصة:
بحثت الفعالية المضادة للبكتريا لمستخلصات الزنجبيل(الماء البارد، الماء الحار، الايثانول،الزيت العطري)تجاه بعض البكتريا الممرضة (*Streptococcus* sp, *Staphylococcus aureus, Vibrio cholera,* , *Klebsiella* sp، *Escherichia coli, Salmonella* sp , *Serratia marcescens*) باستخدام طريقة الانتشار بالقرص وقورنت النتائج مع تأثير 12 مضاد في هذه البكتريا .
اظهرت النتائج ان مستخلصات الزنجبيل اكثر تأثيرا في البكتريا الموجبة لصبغة كرام منها في السالبة لصبغة كرام وان بكتريا *V. cholerae* و*S. marcescens* كانت الاكثر مقاومة للمستخلصات المستخدمة ، في حين ان اكبر منطقة تثبيط لوحظت مع بكتريا *Streptococcus* sp (28ملم)، أظهرمستخلص الايثانول فعالية عالية ضد البكتريا (11-28 ملم) مقارنة بفعالية متوسطة للزيت العطري، كما لوحظ ان مستخلص الماء البارد اكثر فعالية ضد البكتريا من مستخلص الماء الحار.
أعطى مستخلص الايثانول أكبر قطر تثبيط مع بكتريا *Streptococcus* sp مقارنة بتأثير المضادات المستخدمة Ciprofloxacin، Cefotaxime، Cefalotin، Cephalexin وCephaloridine ، ووجد ان هنالك تشابها بين مناطق التثبيط للمستخلصات و بعض المضادات تجاه بكتريا *S. aureus* , *E. coli* , *Salmonella* sp و*Klebsiella* sp بينما اظهرت العزلات البكتيرية *V. cholerae* و*S. marcescens* مقاومة عالية تجاه المضادات والمستخلصات النباتية على السواء . اختبارات الكشف الكيمياوي النوعي عن بعض المركبات الفعالة في مستخلص الايثانول اظهر وجود الكلايكوسيدات ، التربينويدات ، الفلافونات والمركبات الفينولية .