

Antibacterial activity of *Zingiber officinale* alcoholic and aquatic extracts Against *Escherichia coli* isolated from children with diarrhea In Al-Refaee hospital /Iraq

Alaa Yaqoob Rahi

Department of Science, College of Basic Education, University of Sumer, Iraq

Alaterialaa102@gmail.com

Abstract:

The effect of alcoholic and aquatic extracts of *Zingiber officinale* and essential oils was done against isolates of *E. coli*. Alcoholic and aquatic extracts were present inhibitory effectiveness against *E. coli*. The range of diameter area was between (16-18) mm at diameter area was between (16-18) mm at concentration of 400 mg/ml. Essential oils Present inhibitory effectiveness ranging diameter area between (20-24) mm at concentration of 250mg/ml. The minimal inhibitory concentration MIC of essential oils ranges (250 - 400) mg/ml. Also, the study showed that the effect of extracts had approximation for the effect of antibiotics such as: Tetracycline, Erythromycin Kanamycin and Amoxicillin.

Keywords: *Zingiber officinale*, *Escherichia coli*, Antibacterial activity

1- Introduction

There are many types of plants that contain, impart or all of their plant, substances of therapeutic value for humans and animals. These are known as medicinal plant (1). Ginger is considered one of these medicinal plants. Scientific name of ginger is *Zingiber officinale*. Its family is Zingiberaceae. It is a perennial plant with an aromatic rhizome. Ginger has green flowers mixed with a layer of violet(2) The benefits of ginger include strengthening memory, treating headaches and coughs, relieving nervous tension, reducing respiratory disorders and treating some cases of diarrhea (3). *Escherichia coli* live naturally in the intestines of healthy humans and animals. Most types of these bacteria are harmless or cause diarrhea for a relatively short period, but these are a few dangerous strains, including *E. coli* (O157:H7) that can cause severe stomach cramps, diarrhea and vomiting. We may be exposed by to *E. coli*, from water or food contaminated with bacteria especially Vegetables and undercooked meat. Indication usually begins to appear three or four days after exposure to the bacteria. The most important symptoms are mild. Watery to severe and bloody diarrhea, in addition to stomach cramps or feeling of pain. This is all due to the bacteria secreting strong toxin that destroys the lining of the intestine (4). The benefits of ginger are due to the presence of a group of different

chemical compounds, including volatile compounds. Ginger contains eleven compounds that have healthy effects (5). These are compounds found as metabolic substances in plants and are sometimes called volatile oils these compound are capable of stabilizing bacteria, as it has been shown that the inhibitory effectiveness of some Plants lies in their essential oils. Researchers have mentioned that there are several disorders in the digestive system that ginger can be taken to alleviate or eliminate. Therefore, this study aimed to extract essential oils from the ginger plant and test their inhibitory effectiveness against *E. coli* isolated from some cases of diarrhea in children aged between (1 - 12 year) and thus, the Possibility of using these oils as medicinal alternatives to treat cases of diarrhea caused by *E. coli* instead of medical drugs with side effects, after conducting clinical Studies

2-Materials and Methods

2-1 study plant

Ginger was purchased from the local market, ground in an electric grinder and the powder was stored in glass bottle until used.

Bacterial isolates used in the study2– 2

A total of 60 bacterial isolates, 10 clinical isolates were randomly selected and diagnosed in Al-Rifai General hospital laboratory based on nutrient broth medium and stored in nutrient according to (7)

Preparation of aqueous extract3-2

20 gm. of ginger Powder was mixed with 200 ml of sterile distilled water and the solution leaved with continuous stirring using lab-shaker for 24 hours at room temperature. Then, the Solution was filtered through several layers of gauze, and then put the extract in petri dish, leaved it uncovered to dry at 37c° for a week. The Powder was kept in sterile glass bottles to test its antibacterial activity later (8).

4 Preparation of alcoholic extract-2

The same way that used in the preparation of aquatic extract was used to prefer alcoholic extract, except for replacing water with 70% ethanol (9).

Testing the antibacterial activity of alcoholic and aqueous extracts 2-5

The isolates of *E. coli* at this study activated in nutrient broth media prepared according to the instructions of the manned manufacturer. In this study we used agar diffusion method according to the method of (10). Muller-Hinton agar was prepared according to the Prepared Company's instructions. The medium was poured into Petri dishes After solidification, the medium is inoculating with a 1ml of bacterial Suspension at a concentration of 1×10^6 bacterial cell/ml, measured with spectrophotometer at wave length of 540nm. The suspension of bacteria was spread using sterile cotton swabs. To absorb the suspension in the culture media-Two holes made in each dish with 6mm diameter, using sterilized flange drill. 10 microliters of the extract were added at a concentration of 0.5 mg/ml of both alcoholic and aqueous extracts using micropipette Plates incubate at 37° for 24 hours. Results were recorded by measuring the inhibition zone diameters in millimeters (Table 1) this method was prepared with all the isolates at study. There was a control dish contain a nutrient media without extract (6).

2-6 Isolation of essential oils from ginger using continuous extraction method

There was 40g of ginger powder weighted and placed in Thimble. The sexulet continuous extraction was conducted for 16-24 hour using 400 ml of solvent N-Hexane. Then the solution was dried at Petri dish and drying it at 37 c° the viscous liquid was obtained (10), and the weight of the extract oils was 2-14g.

2-7 Qualitative analysis of ginger essential oil extract

Qualitative analysis was carried out by using specific reagents to detect the active compounds in the oil extract (Table 2) (11).

2-8 Testing the antibacterial activity of o its isolated from ginger

The same method that mentioned in Paragraph (5) was followed to test the effectiveness of oils isolated from the ginger plant, by using two concentrated) 0.5mg/ml), and (1g/ml). The results were recorded by measuring inhibition Zone diameters in millimeters (Table 3).

2-9 Determination of the minimum inhibitory concentrations (MIC)

For isolated extracts and oils MIC of crude ginger extracts and oils isolated from it against *E. coli* at this study were determined according to the method of (12) this method included Preparing stock solution for each extract by dissolving (2g) of the extract in a series of dilutions were prepared) 5ml of Dimethyl sulfoxaide (DMSO mg/ml, and then 1 ml of each) ,400(10,25,50, 100 , 150, 200, 250,300 dilution was mixed with 2ml of Muller- Hinton agar. Then, 10 microliters as drops of the inoculum added to each dish whose density is of measured by us spectrophotometer at a wavelength of 540 nm which is equivalent to (1×10^6) cell/ml the dishes were incubated at 376° for 24 hour. The result was recorded at the MIC was determined for each extract (Table 4).

2-10 Testing the sensitivity of *E. coli* against antibiotics

Muller-Hinton agar medium was used for measuring the biological effectiveness of four types of antibiotics (Tetracycline, 30mg, Erythromycin 15 mg, Kanamycin 30 and Amoxicilline 30) prepared by Bicanlyas company atm 0.1 ml of the bacterial broth culture was spread in the culture medium using sterile swabs, Then the cultured dishes were left for30 minutes to absorb the added liquid. The antibiotic Saturated discs were placed in their places on the dish Dishes inoculated at 37c° for 24-hour (13) Results were recorded by measuring inhibition Zone diameter in millimeters (Table- 5)

3- Results

3-1 Antibacterial activity of Alcoholic and aquatic ginger extracts against *E. coli*

Table (1) explains the antibacterial activity of alcoholic and aquatic ginger extract the effect was similar for both extracts. The inhibition Zone diameters were (14-18) mm for all isolates at this study.

Table (1) Antibacterial activity for alcoholic and aquatic extract of ginger against *E. coli*

Number of isolate	Inhibition zone diameter (mg/ml)	
	Alcoholic	Aquatic extract
50	17	16
37	15	16
32	15	16
40	18	17
20	16	14
45	17	16



Figure (1) Inhibition Zone Alcoholic and aquatic extracts against *E. coli*

3-2Qualitative analysis for essential oils extract

Table (2) explains the results of qualitative statements of essential oils extract for ginger this table explains that’s the-extract contains Tertiary terfenes Terpenoids, Phenols and flavonoids. These compounds have an antibacterial activity against *E. coli*

Table (2): Qualitative analysis for essential oils extract

revealed	Chemical reagent	result
Flavonoids	Alcoholic potassium hydroxide	+
Phenols	Mercuric chloride 1%	+
Terpens	Lieberman Burch ard	-
Trepenoids	Concentrated sulfuric acid	+
Sabonin	Aquatic mercuric	-

3-3 Antibacterial activity of essential oils extract for ginger.

Table (3) explains the antibacterial activity for essential oils extract against *E.coli*

Table (3): Antibacterial activity for essential oils extract against *E. coli*

No of isolate	Inhibition zone diameter	
	concentration	
	0.5 mg\ ml	1 mg\ ml
50	22	23
37	20	21
32	21	24
40	21	23
20	20	24
45	22	23



Figure (2): Inhibition zone of essential oils extract against *E. coli*

3-4 MIC of essential oils extracts isolated from ginger

Table (4) explain the MIC for alcoholic and aquatic essential oils extracts isolated from ginger against *E. coli*

Table (4): MIC of Alcoholic and aquatic essential oils extracts.

Name of extract	MIC mg\ml
Aquatic	400
Alcoholic	400
Essential oils	250

3-5 Sensitivity of *E-coli* against Antibiotics

Table (5) Pointing to the sensitivity of *E. coli* against some antibiotics.

Table (5): Sensitivity of *E-coli* against antibiotics

Antibiotics	Inhibition zone MIC diameter (mm)	No. of isolate					
		45	20	40	32	37	50
Tetracycline (TE)	30	21	22	23	25	23	23
Erythromycin (E)	15	17	16	16	17	16	16
Amoxicillin (AMC)	30	R	R	R	R	R	R
Kanamycin (K)	30	18	16	16	16	15	15

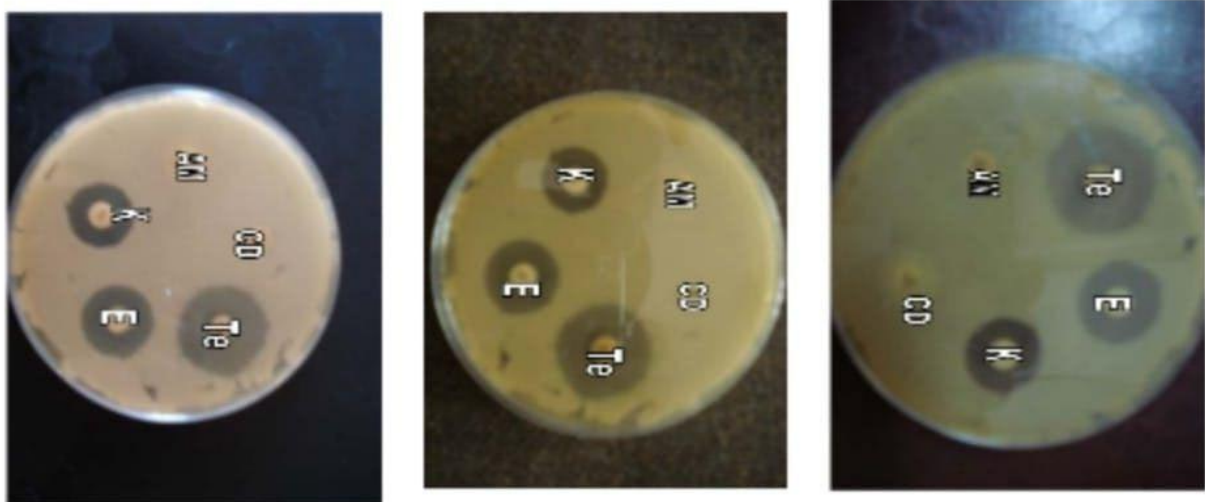


Figure (3) Sensitivity of *E. coli* against antibiotics

4- Discussions

The results at table (1) appear the Presence of antibacterial activity for all of Alcoholic and aquatic extract of ginger. Inhibition zone diameter for Alcoholic and aquatic is (18 mm), because that the ginger sufficiency with active chemical compounds such as Volatile oils, organic acids, Aminic acid

and some vitamins and salts (14). Therefore, bioactivity of plant does not appear because of the contradiction may occur between compounds - Nonactivity components affect in the absorption and spread of active components (15). This is What A this study showed, so the station isolated oils from ginger gave higher antibacterial activity than alcoholic and aquatic extracts inhibition zone diameter for the isolates ranging between (20-24) ml, while the crude extract gave (18ml) activity. This is consistent with what mentioned [16, 17]. This mentioned that the antibacterial activity of some plants lies in its essential oils, as mentioned at (18) that the antibacterial activity for ginger lies in its volatile oils, these oils were effective in reducing diarrhea. The reason of isolated oils effectiveness from ginger to its containment many of compounds such as Texpenes, Terpenoids, phenols and Flavonoids. The antibacterial activity of terpenes and Treprenoids lies in its functional properties are lipophilic, interact with lipid parts of bacterial cell membranes, so it affects the ion exchange mechanism (19). Essential oils of ginger contain Limonen and Zingebrane, these are Hydrocarbonate Trefenoids, also Sesquiterpenoidis,

Phenets have an antibacterial activity)it is asulfe terpenid compound (20 against gram negative and positive bacteria, it causes complete destruction of membranes and call walls bacteria (21). The activity of ail referred to the phenolics of Tolar phenolics aromatic ring Presence of Tolar phecave the interact containing (OH) that's have the interact ability and bonds using hydrogen bonds with active groups coenzyme of bacteria (22). Essential oils of ginger contain Zingerane, its Pheny Propanoid-derived called Vanillyl acetone.

References

- [1] WHO. World Health Organization. (1996). Supplementary guidelines for the manufacture of herbal medicinal products. WHO Technical Report Series, Geneva, Annex 8, pp. 109-113.
- [2] Almayah, Abd Alrida Alwan. (2001). Medicinal plants and herbal medicine. Taez University - Basra University. Retrieved from <http://fashion.azyya.com/639-3>.
- [3] Baranowska, K., Makekmar, D., & Marrian, W. (2002). Antifungal activity of the essential oils from some species of the genus Pinus. Zeitschrift für Naturforschung, 57, pp. 478-482.
- [4] Kivance, M. (1988). Antimicrobial activity of Cortuk species: Its essential and methyl eugenol. Die Nahrung, 32(6), pp. 635-637.
- [5] Holt, J.C., Kriej, H.R., Sneath, P.H., Staley, J.T., & William, S.T. (1994). Bergey's Manual of Determinative Bacteriology.
- [6] Alwan, Z.W., Siwan, F., & Noori, F.J. (2005). Testing the biological effectiveness of Asfr flower extract against bacteria and fungi. Basra University Jour.(Scientific), 3(31), pp. 39-47.
- [7] WHO. World Health Organization. (1987). Manual for laboratory investigation of acute enteric infections. CCD, 183, pp. 83-93.
- [8] Plummer, D.I. (1971). Introduction to Practical Biochemistry. McGraw Hill Book Co. LTD., England, pp. 186-190.
- [9] Harborne, J.B. (1984). Phytochemical Methods. 2nd ed. Chapman and Hall, London, UK.
- [10] National Committee for Clinical Laboratory Standards. (1997). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4, Wayne, PA, USA.
- [11] Aljoburi, M.M. (1990). Medical Bacteriology. Ministry of Higher Education and Scientific Research, University of Mosul.

- [12] Al-Sanafi, A.A.O. (2009). Ginger and its therapeutic properties. College of Pharmacy, University of Tikrit.
- [13] Balchin, M., Simmonds, M., Hart, S., & Deans, S.G. (1999). Agrochemical and medicinal usage of essential oils of representative species of the Geraniaceae. *Journal of Applied Microbiology*, 67(3), pp. 187-195.
- [14] Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Review*, 12(4), pp. 564-582.
- [15] Dorman, H.J. & Deans, S.G. (1999). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88, pp. 308-316.
- [16] Knobock, K., Pauli, A., Iberi, B., Wegand, H., & Weis, N. (1989). Antibacterial and antifungal properties of essential oil components. *Journal of Essential Oil Research*, 1, pp. 119-128.
- [17] Smith Palmer, A., Stewart, J., & Fife, L. (1997). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Applied Microbiology*, 26, pp. 118-122.
- [18] <http://www.ibtesam.com/ub/showthread-145/99/HTML>.
- [19] <http://www.cobarty.com>.