

Study the Antibacterial Activity of *Zingiber officinale* roots against Some of Pathogenic Bacteria

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الخلاصة

تم دراسة الفعالية التثبيطية للمستخلص المائي لجذور نبات الزنجبيل *Zingiber officinale* تجاه أنواع من البكتريا الموجبة والسالبة لصبغة كرام (*Klebsiella pneumoniae* , *Proteus vulgaris* , *Streptococcus pyogenes* and *Staphylococcus aureus*) باستخدام طريقة الانتشار في الاقراص، حيث أظهر المستخلص فعالية تثبيطية واضحة ضد البكتريا المرضية وقد ازدادت هذه الفعالية بزيادة التركيز حيث أعطى أعلى فعالية تثبيطية بالتركيز (٠.٤ ملغم/مل) من المستخلص المائي ضد *Klebsiella pneumoniae* , *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus* . وقد تم مقارنة هذه الفعالية التثبيطية للمستخلص بعدد من المضادات الحيوية (*Nalidixic acid*, Trimethoprim, Chloromphenicol, Gentamicin and Erythromycin) حيث تفوق التركيز الأخير للمستخلص على المضادين Chloromphenicol, Gentamicin اللذين أعطى أعلى قدرة تثبيطية ضد *Klebsiella pneumoniae* , *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus*.

ABSTRACT

In this study aqueous extract of ginger (*Zingiber officinale*) Roots were used for antibacterial activity against various Gram-negative and Gram-positive bacteria (*Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus*). growth inhibition was evaluated by the disc diffusion. The extract of ginger (G) showed clear antibacterial activity against pathogenic bacteria, which this activity was enhanced with the increasing of concentrations belongs to them. The latest concentration (0.4mg/ml) of the extract gave highest activity against *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus* . Antimicrobial activity of ginger extract was compared with a number of antibiotics (A) that known for their ability include, nalidixic acid, trimethoprim, chloromphenicol, gentamicin and erythromycin by using antibiogram test. Antimicrobial activity of latest concentration of ginger was better than that to Chloromphenicol, Gentamicin against *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus* .

Keywords: Antibacterial Activity, *Zingiber officinale*, Pathogenic Bacteria

INTRODUCTION

Medical plants have a long history of use and their use is widespread in over world countries. According to the report of the World Health Organization 80% of the words population rely mainly on traditional therapies which involve the use of plant extracts or their active substances[1]. The herbal medicines may be in form of powders, liquids, or mixtures, which may be raw, or boiled, ointments, liniments, and incisions[2].

Nutrient Composition Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fiber and 12.3% carbohydrates.

The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C[3].

Ginger (*Zingiber officinale*) belongs to *Zingiberaceae* family. The part of the plant used is rhizome[4]. In the fresh ginger rhizome, the gingerols were identified as the major active components and gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one] is the most abundant constituent in the gingerol series. The powdered rhizome contains 3-6% fatty oil, 9% protein, 60-70% carbohydrates, 3-8% crude fiber, about 8% ash, 9-12% water and 2-3% volatile oil[5].

In dried ginger powder, shogaol a dehydrated product of gingerol, is a predominant pungent constituent upto biosynthesis[3-5]. Oleoresin, which is isolated by acetone and ethanol extraction, contains 4-7.5% of dried powder, pungent substances namely gingerol, shogaol, zingerone and paradol[6].

In vitro studies have shown that active constituents of ginger inhibit multiplication of colon bacteria, These bacteria ferment undigested carbohydrates causing flatulence, this can be counteracted with ginger[7]. It inhibits the growth of *Escherichia coli*, *Proteus* sp, *Staphylococci*, *Streptococci* and *Salmonella*[8,9].

Ginger has strong antibacterial activity and to some extent antifungal properties [10]. Ginger inhibits *Aspergillus*, a fungus known for production of aflatoxin, a carcinogen[11].

Fresh ginger juice showed inhibitory action against *Aspergillus niger*, *Sacharomyces cerevisiae*, *Mycoderma* spp. And *Lactobacillus acidophilus* at 4, 10, 12 and 14%, respectively at ambient temperatures [12].

This study aimed to investigate the antibacterial effect of *Zingiber officinale* roots against some pathogenic bacteria. This is in pursuance of the efforts to search for drugs from plants and the verification of the scientific basis of some known practices in traditional medicine.

MATERIALS AND METHODS

Preparation of extract

Fresh rhizomes of Ginger (G) were collected, washed thoroughly in tap water and peeled, cut into pieces and dried at dark room temperature for one week. The dried ginger was ground using an electric blender, 25g of the ground material (Ginger) placed in a conical flask and 100 ml of distilled water was added to the flask and put on a rotary shaker at 220 rpm for 72 h. The crude extracts were obtained by filtration through Whatman No.1 filter paper. The filtrate was reduced to 25 ml and then autoclaved at 121°C and 15 lb pressure for 20 min. The extract was cooled and immediately assayed for antibacterial activity [14].

Preparation of bacterial solution

The tested microorganisms (*Klebsiella pneumoniae* , *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus*) were obtained from Microbiology Laboratory of Biotechnology department – Applied sciences - University of Technology . The organisms were inoculated onto Nutrient Broth (Hi-media) and incubated at 37°C for overnight and were stored at 4°C and sub-cultured fortnightly. The bacterial cells were harvested by centrifuging at 5000g for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline), (10 mM Sodium Chloride, pH 7.4) and the cells were counted by haemocytometer (*neubauer counting chamber*) as follow: clean up counting chamber with 70% alcohol and let air dry, mix culture well and apply a single drop to counting chamber with Pasteur pipette, examine the counting chamber using high power, oil immersion objective, make a preliminary estimation of the concentration of cells from the overnight culture of using the following formula:

$$\text{cells/ml} = \text{Total cells counted} \times 2.0 \times 10^7 \times \text{dilution factor} / \# \text{ small squares counted}$$

The bacterial cells were diluted to approximately 10^5 CFU/ml before use [15].

Antibacterial activity assay

The antibacterial activity was determined by agar disc diffusion [16]. Agar plates were inoculated with 0.1 ml broth culture of tested organisms and was spreaded with sterile an L-shaped rod glass spreader. Sterile paper disks (Whatman No. 1 filter paper) of 5mm diameter were impregnated with different concentration of crude extracts and dried in a hot air oven at 60°C for 5 min. The disc in the center of agar plate which impregnated with sterile distal water was used as control.

Antibiogram test

In which small discs containing different antibiotics are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in which bacteria can grow. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lysis will become visible.

RESULTS AND DISCUSSION

The results of our experiments showed that different bacterial species exhibited different sensitivities towards the extract of ginger. The sensitivities of bacterial species against phenolic compounds of ginger showed more activity against gram positive bacteria compared to gram negative bacteria under study.(Table-1).

Table-1: The mean of inhibition zone of the aqueous extract of Ginger (G) against *Klebsiella pneumoniae* , *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus* .

Concentration(mg/ml)	0.1	0.2	0.3	0.4
bacteria strain	Zone of inhibition(mm)			
<i>Klebsiella pneumoniae</i>	-	10	12	22
<i>Proteus vulgaris</i>	-	10	20	24
<i>Streptococcus pyogenes</i>	-	-	12	20
<i>Staphylococcus aureus</i>	-	8	14	24

Gram negative bacteria were also more resistant than Gram positive bacteria, as also shown by [17]. These variations in inhibition may be because of differences in the composition and structure surface between Gram positive and Gram negative bacteria [18]. In addition to the cell wall and cell membrane, Gram negative bacteria have an outer membrane composed of a phospholipid bilayer, which may be a protective barrier against these phenolic compounds [19]. (Fig-1).

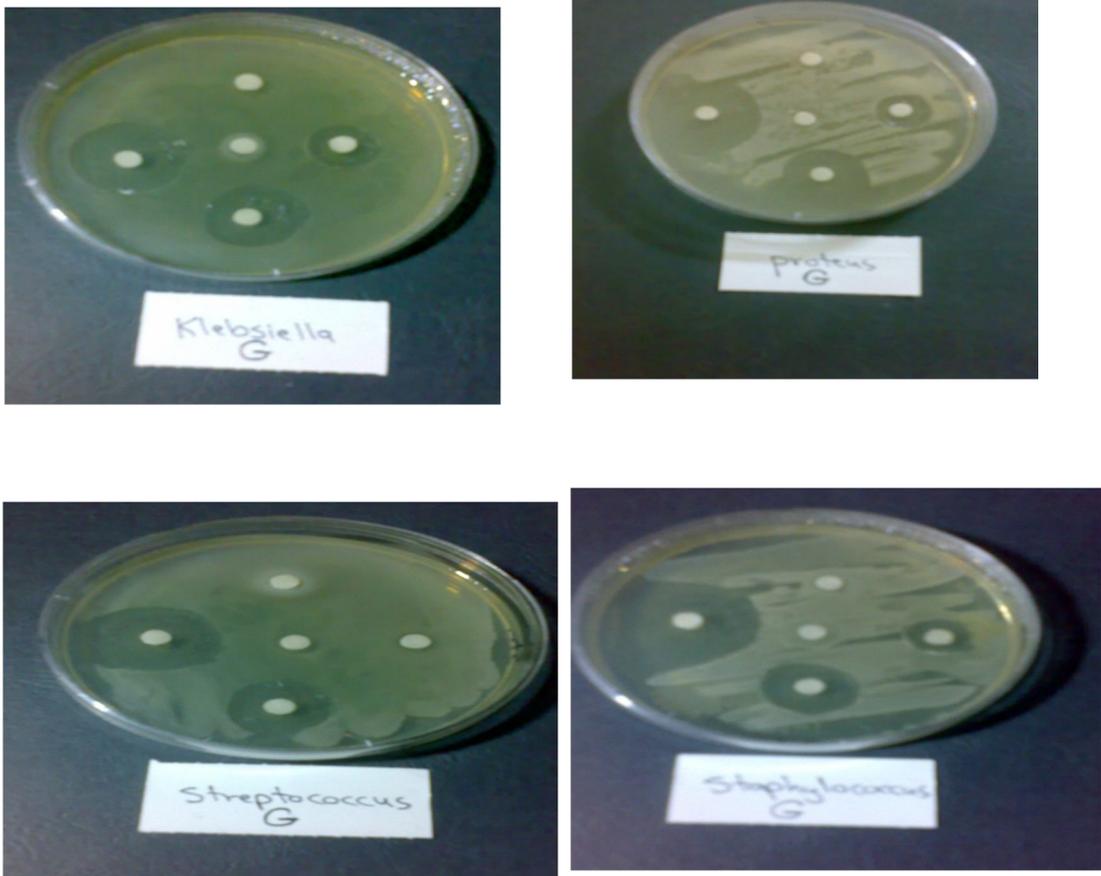


Fig-1: The antimicrobial activity of aqueous extract of Ginger (G) against *Klebsiella pneumoniae* , *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus* .

Moreover, the cell walls of Gram positive bacteria have a large amount of peptidoglycan and a small amount of lipid, while in the case of Gram negative bacteria, due to the presence of an outer membrane, a large amount of lipid and a small amount of peptidoglycan is found [20].

Most of the phenols are protein denaturing agents; they can change the cell permeability, which may lead to swelling and rupture of the bacterial cells, most of them are metal chelators that attach to the active site of metabolic enzymes, reducing enzyme activities and therefore slowing bacterial metabolism and reproduction[21]. As Gram negative bacteria have an additional outer membrane on their cell wall, the entry of phenols may be interrupted and its effects are lesser of the cell serious. However, Gram positive bacteria lack the outer membrane and therefore they are more susceptible to, easily entering phenols [20]. Antibacterial activity of ginger roots extract was compared a number of antibiotics (A) that known for their ability included, nalidixic acid, trimethoprim, chloromphenicol, gentamicin and erythromycin by using antibiogram test (Table-2).

Table-2- The mean of inhibition zone of some antibiotic against *Klebsiella pneumoniae* , *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus*

bacteria strain	Zone of inhibition(mm)				
	Nalidixic acid	Trimethoprim	Chloramphenicol	Gentamicin	Erythromycin
<i>Klebsiella pneumoniae</i>	R	R	18	24	R
<i>Proteus vulgaris</i>	10	14	14	20	10
<i>Streptococcus pyogenes</i>	R	R	22	18	R
<i>Staphylococcus aureus</i>	R	R	20	18	R

R = Resistance

Antimicrobial activity of latest concentration of ginger was better than that to chloromphenicol, gentamicin against *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus* .

The resistance of pathogenic bacteria to nalidixic acid, trimethoprim and Erythromycin was noticed.(Fig.2) the frequent use of antibiotics stimulates the emergence of new strains of pathogenic bacteria, show resistance to these antibiotics and this findings in our study and in other studies.

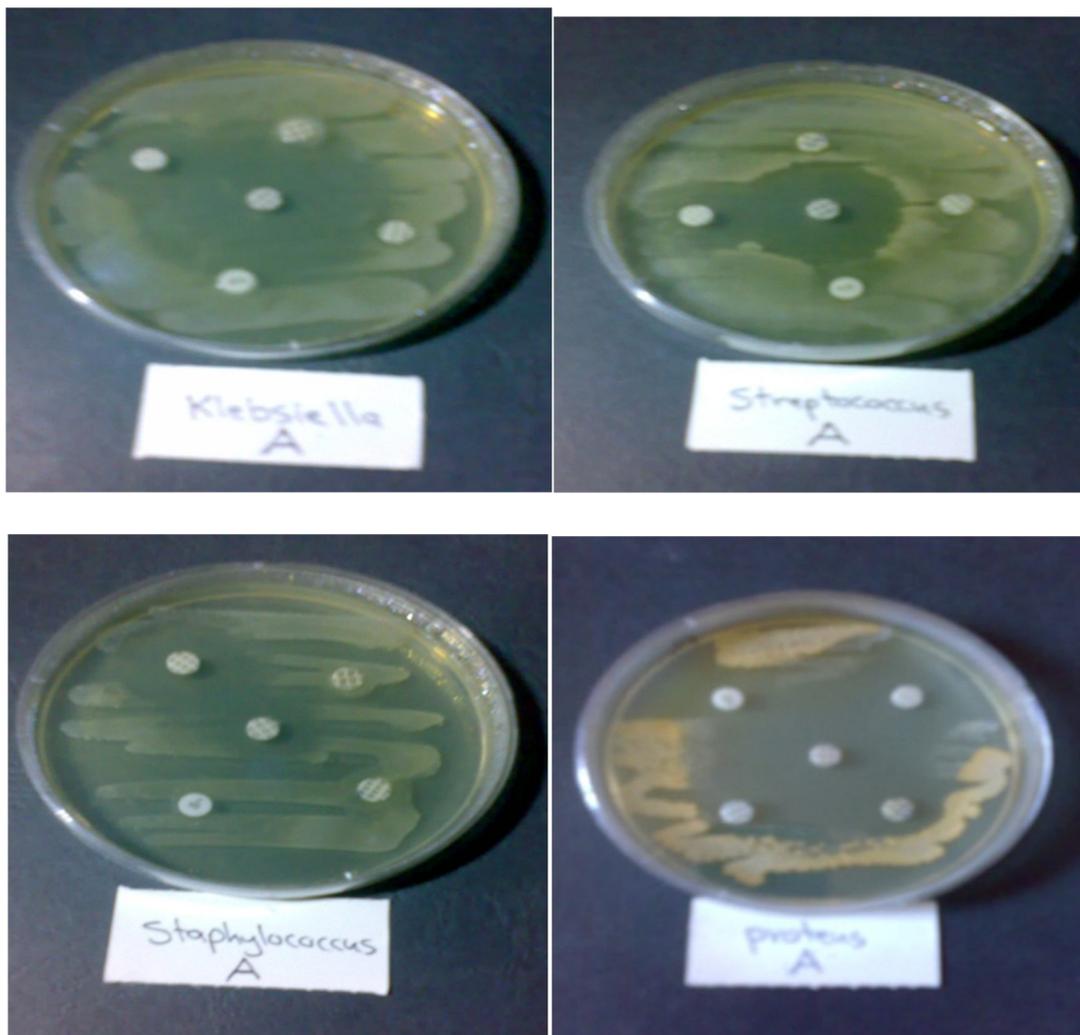


Fig.-2: The antimicrobial activity of some antibiotics (A) against *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus*. A = antibiotic disc

Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Most of the spices extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent drugs. Thus spices, which are normal ingredients of our routine food preparations, can provide protection to a certain extent against our natural enemies like bacterial pathogens.

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