

The Effect of Some Plant Extracts Against *Proteus* spp and *Klebsiella* spp Bacteria and Comparing Them with Antibiotic

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Abstract

The study was conducted to determine the inhibitory effectiveness of different concentrations of lemon (*Citrus lemon*) and ginger (*Zingiberofficinale*) extracts on the *Klebsiella* and *Proteus* Bacteria. The outcome of this work was that the *Klebsiella* showed the highest inhibition at 75% concentration, 24mm of lemon extract and 20mm of ginger extract, while the *proteus* recorded the highest inhibition at 75% concentration of 21mm of lemon extract and 18mm of ginger extract. This conclusion was evaluated using Agar well-diffusion method; the results were compared with the effect of some antibiotics, mix lemon-ginger extract in equal proportions with the same previous concentrations, Different diameters were given and this was evident on *Klebsiella* and *Proteus* bacteria. The extract gave a strong efficacy at 75% concentration 28.25mm over the antimicrobial action that was used in int the study by respectively the highest inhibitor of Erythromycin 20mm on *Klebsiella*, while anti-Amikacin was 18mm on *Proteus*. The reached results were encouraging to conclude that in spite of the difference in the extent of their effect in inhibiting the bacteria growth. The lemon/ginger extract had a larger effect for inhibiting.

Keywords: lemon, ginger, extract, antibiotic, inhibiting bacteria.

DOI: 10.7176/JBAH/9-6-06

Publication date: March 31st 2019

Introduction

Antimicrobial drugs were of interest to researchers in the 20th century due its effect to reduce the mortality rate and to treatment of the resulting problems that caused by microorganisms, but at the end of the century studies have proven that the bacteria can develop antibiotic resistance when adapt and grow under presence of these antibiotics for several generations, and show this resistance through the interchange of genetic material of resistance among different types of bacteria. Scientists concluded that the excessive use of these drugs is the main reason to arise resistant medicine strains (Uwaezuoke and Ariatu, 2004)

Medicinal plants have a long history of use around the world, According to the World Health Organization (W.H.O.) about 80% of the world's nation rely mainly on folk therapies which involve the use of plant extracts (Sofowora,1999).

Ginger *Zingiber officinale* Roscoe, belong to *Zingiberaceae* family is one of the famous spices all over the world. Ginger is the underground rhizome spread cultivation in South East Asia, West Africa and the Caribbean (Weiss, 1997; McGee, 2004). In addition to the nutritional values Ginger has a medicinal important (Kumar et al, 2011) .Many research has been showed that the ginger extract also have antimicrobial activity and anti-oxidant properties against both positive and negative gram bacteria and fungi. Ginger has inhibition actin to the growth of *Escherichia coli*, *Proteus* sp, *Salmonella* *Staphylococci*, and *Streptococci* (White, 2007). Ginger is a plant used in the treatment of diseases by conventional methods. Studies and research have shown that it has been effective in preventing and treating a wide range of diseases, including various infections and its ability to prevent cancerous growth (Akram et al., 2011).

Lemon *Citrus limon* (L.) Osbeck belong to *Rutaceae* family grown all over the world, consider a medicinal plant which it having anticancer activities and the antibacterial potential in crude extract of different its parts against significant bacterial strains has been confirmed (Kawaii,2000). Citrus flavonoids have a big spectrum of biological activity including antibacterial, antifungal, and anticancer activities (Burt, 2004). Thus, ginger and lemon consider one of these protection lines against natural enemies like bacterial and fungal pathogens. This has been led many of antimicrobial researchers to refer to the use medicinal plants for many reasons including their availability, easily obtained, low cost, and safe with rare side effects (Deboer et al., 2005). Lemon acid is not less important than ginger in medical treatments that has been used additionally it is a natural source of vitamin C, which causes scurvy reduction and is an immune stimulant (Ncube et al, 2008).

Hence, the idea of this study where it aims to investigate new therapeutic alternatives against bacteria and the selection of plant extract which has the strongest effects on these germs so it focuses two types of plants

Ginger and lemon.

Materials and Methods

Plant collection

1. Preparing of plants

Ginger and lemon was obtained from the local markets, cleaned, washed with distilled water, ginger peeled and sliced and then dried at room temperature for three weeks, then grinded with an electric grinder. The powder was stored in a glass bottle in the refrigerator, while lemon's fruits were cut into halves and its juice was extracted by squeezing, then dried and packaged in a glass bottle and kept at a temperature until use.

2. Preparation of the Extract

10g of dry ginger powder mixed with 200ml of sterilized water. The mixture was blended with a magnetic mixer for 15 minutes, leaving the solution for a period to precipitate. The solution was filtered and the sediment was neglected. The strained substance was separated by a centrifuge at 3000 rpm for 10 minutes to precipitate the suspended plant parts and obtain pure solution. The solution was concentrated using rotary evaporator at 40°C until it became dry and then stored in the refrigerator. The same process was repeated with lemon powder using the same method. (Boham, Kocipai, 1974).

3. Preparation of Concentrations.

Aqueous extract of ginger and lemon were prepared individually dilution with different concentrations are: 25%, 50%, and 75% (Boham, 1974). Prepare concentration without dilution from stock ginger and lemon extract with 50:50g from each sample, then prepared 25%, 50% and 75%.

4. Pathogenic Bacterial Isolates

Isolate *Klebsiella* and *Proteus* bacteria they are negative gram stain at the laboratories of Baqouba Instructional Hospital, from the infected patients as urine isolation. Nutrient broth and Nutrient agar were used in the development and activation of pathogenic bacterial isolates. The Muller Hinton agar medium was used to determine the effect of plant extracts on the bacteria after activating the bacterial plantations for 24 hours using the diffusion method and three replicates were applied for each plate, incubated at 37°C for 24 hours. The effectiveness of the plant extracts was determined by measuring the diameter of the inhibition zone, which is free of bacterial growth.

5. Antibiotics examination

To study the effect of antibiotics in bacterial growth, the method that was used to determine the effect of antibiotics. Tetracycline, Amikacin and Erythromycin were used according to Reeves (1987)

6 Chemical statements

In order to identify the most important chemical components of the crude extract of ginger and lemon plants, a chemical analysis was carried out by taking 100g of dry powder for each sample with adding 200ml of distilled water and then dried at 50°C for 12 hours and the precipitate was diluted in 200ml of distilled water again and was used in the chemical examinations for each gram of Alkaloids, Flavonoids, Terpenoids, Steroids, Saponins, Tannins according to dependable Harborne (1973) method.

Results and discussion

The results of the experiment showed different effects of the active compounds of the ginger and lemon plants in the growth of *Klebsiella* and *Proteus* bacteria isolates from urine infected patients. There is significant inhibition in the bacterial species under study, which was revealed by One Way Anova analysis with a probability level of 0.05.

As shown in Table (1), the results clarify the effect of Aqueous extracts of ginger and lemon against the bacteria under study, where the higher the concentration of the extract used, the greater the inhibitory effect. This effect was evident against *Klebsiella* bacteria rather than *Proteus* bacteria where the bacterial growth inhibition of *Klebsiella* and *Proteus* bacteria was increased by (24, 21) mm respectively using lemon extract (20, 18)mm respectively. This is the highest concentration used in the study compared with the concentration of 25%, the lowest concentration used. The results showed a difference in the inhibitory activity of the lemon extract and the ginger extract. Where the diameter of the fixation *Klebsiella* and *Proteus* were reduced by (17, 14) mm respectively with 25% concentration of lemon extract while the inhibitory diameter of *Klebsiella* and *Proteus* was (12, 10) mm respectively for the same concentration of ginger extract, which is less effective than lemongrass extract.

This variation in the inhibitory effect of the extract may be due to the fact that they contain varying levels of acid, especially lemons, so that the pH value will be acidic and it will affect the charge of amino acids that are constituent proteins associated with the peptidoglycan, which is a component of the bacterial cell wall. It also affects the active sites of the wall enzymes, leading to a defect in enzyme functions, as agreed by Atlas (1995) and Albayati (2010).

Table (1) Diameters Areas inhibiting the growth of bacteria treated with different concentrations of plant extracts

Isolation	Bacteria Inhibition Diameter(mm)					
	Ginger Extract			Lemon Extract		
	25%	50%	75%	25%	50%	75%
Klebsiella	12	16	20	17	20	24
Proteus	10	14	18	14	17	21

The results of the study showed that the mixture of the extracts was highly effective against the growth of Klebsiella and Proteus bacteria. The bacteria started with the sensitivity at the concentration 25%. As shown in Table (2) where the diameter of the bacterial inhibition Klebsiella and Proteus by (20, 18) mm in the order of 25% concentration. In comparison with the same concentration of lemon extract and ginger extract in Table 1, there is a difference between the inhibitory force of the extract together more than the inhibitory force of the extracts. 75% of the extract when combined gave the strength of the installation (28, 25) mm for Klebsiella and Proteus respectively, while the same concentration gave a 20 to 18 mm inhibitory strength to the same bacteria. This may be due to the fact that lemon and ginger contain high alkaloids that interfere with the metabolic reactions (Tegos et al.2002), as shown in Table 3, as well as the containment of lemons on the tannins that are characterized by their inhibitory activity for bacterial growth (Al-Rawi&Chakravarty, 1988). This may be due to the combination of active substances In the extract of lemon and ginger and their interaction together leading to the formation of a compound with strong effective effects on the bacterial growth of the species under study and this is consistent with (Grzanna et al.2005). The results explain that there is no significant differences among all the extracts used in the study against the Klebsiella bacteria. There were significant differences in lemon extract with ginger in Proteus bacteria at level 0.05, which is consistent with other studies in the same field (Albayati 2010).

Table .2 The biological efficacy of ginger and lemon blend at 50:50%

Isolation	Bacteria Inhibition Diameter(mm)			Average
	25%	50%	75%	
Klebsiella	20	24	28	24
Proteus	18	21	25	21.3

The results in table (2) showed that when the mixture ginger /lemon extract 50:50% gave high results in the bacterial inhibition of the species under study. The rate of inhibited diameter of the concentrations in the Klebsiella bacteria gave the highest value 24mm compared with the rate of diameter inhibition of Proteus bacteria at a value of 21.3mm. This may be due to the similarity of the active substances in the extracts and their interaction together, leading to the formation of a compound with strong effects on the bacterial growth of the species. This is consistent with Grzannaetal.(2005).

Table (3) The qualitative data of the active ingredients of plant extracts

Active Substance	Ginger Extract	Lemon Extract
Alkaline	+	+
Tannins	-	+
Phenol	+	+
Turbine	+	+
Volatile Oiles	+	+

Table (3) shows the most important qualitative chemical statements of some active ingredients in ginger and lemon extract ingredients as proven study containing extracts on alkaloids and tannins, phenols and terpenes and others which are effective materials anti-bacterial, this is consistent with the (Chrubasik, etal.2005), as these substances as inhibitors of the types of bacteria isolated from satisfactory injuries.

Table (4) Comparison of the biological efficacy of the extract blended with some antibiotics

Bacteria	Erythromycin	Amikacin	Tetracycline	Extract75%
Klebsiella	20	10	10	28
Proteus	8	18	15	25

The inhibitory effect of the mixed extract of ginger and lemon was compared with 75% concentration with antagonists TE(Tetracycline), AK(Amikacin), and E(Erythromycin) which is used extensively in the treatment of most bacterial infections. Table (4) shows that the mixed extract had a clear inhibitory effect of Klebsiella and Proteus, against the bacteria Klebsiella with a diameter of inhibitory (28)mm as superior to the work of antibiotics, TE, AK, E. In equal Proteus bacteria, where the extract recorded a high inhibitory effect of 25mm inhibition compared to the inhibitory of antibiotics, TE, AK, E,. The efficiency of plant extracts exceeds antibiotic when it is used in a healthy and precise way, this is agree with (Kawail, etal.2000).

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