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The Vibration Effect of Shaker System (Vortex) and Medicinal plants on *Escherichia coli* and *Klebsiella pneumoniae* Gram-negative Bacteria

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ABSTRACT: The prepared research has been performed to effects of *Gundelia tournefortii, ferulago angulata* and *Rheum rib*es as well as the vibration effect on *Escherichia coli* and *Klebsiella pneumoniae* gram-negaive bacteria at Yasuj University of Medical Sciences, in 2014. Firstly different concentrations of each bacteria were prepared. Then, they were affected by vibrations, and finally, after finishing every vibration with using Spread Plate method it was prepared culture from resulting suspension. To study antimicrobial effects, it was used Plate method after preparing *Gundelia tournefortii, ferulago angulata* and *Rheum ribes*. Our results showed that *Gundelia tournefortii, Ferulago angulata* and *Rheum ribes* extracts had not significant effect on the gram-negative *E. coli* and *K. pneumoniae* bacteria. Furthermore, the results indicated that vibration is no significant change in the number of grown bacteria on culture plate for none of desired concentrations of bacteria and also increasing the time of vibration and frequency.

Keywords: *Escherichia coli, ferulago angulate, Gundelia tournefortii,* herbal extracts, *Klebsiella pneumoniae, Rheum ribes*.

INTRODUCTION

Bacteria are the most important and diverse microorganism on earth. In general, lack of their activities disturbs the life on earth. Only a few bacteria like Chlamydia and Rickettsia are necessarily intracellular parasites. Bacteria are different from Eukaryotes in some aspects. Bacteria consist of S80 Ribosomes, pelliculate organelles such as the nucleus, mitochondria and circular chromosomes without cover. Bacteria (other than Mycoplasmas) have cell walls. Vibration is applicable in electrical circuit and dental drill in medical field. Vibration Operating Systems are classified to different categories. This categorization has been done based on the impact and outcome of education or treatment (Marien, 2010). Due to the increasing resistance of bacteria to anti biogenic, the prepared study considers the effects of medical plants extracts and vibrations on *Staphylococcus aureus* gram-positive bacteria.

It is certain that Eukaryotic organisms have come from living organisms like bacteria. Since bacteria consist of simple structures, many of them can easily be cultured and controlled under laboratory conditions (Taran *et al.,* 2010).

Basic parameters that determine the optimum vibration transmitted to the human body include vibration frequency, amplitude (intensity) in order to enter vibration to body according to the physical body as well as the time duration exposure to it. Arbabien *et al* (2009) studies on a few bacteria and fungi were showed that the Ethanol and methanol extracts of organs have inhibitory effects on micro-organisms growths. The stem and edible organs have the most effect on *Staphylococcus aureus* and the least impact on Bacillus cereus (Arbabien *et al.,* 2009).

Tabatabai Yazdi *et al* (2013) showed in an essay entitled "The study of the antimicrobial effects of *ferulago angulata* aqueous and ethanol extracts on *Escherichia coli*" that all ethanol extracts concentrations have inhibitory effects on *Escherichia coli* bacteruim, but the mentioned effect was not seen in 20 mg/ ml concentration. The least inhibitory effect and Fungicidal concentration of aqueous and ethanol extracts was calculated respectively 32, 64 mg/ml as well as 64,128 mg/ml. According to the bactericidal effects of *ferulago angulata* extracts, it can be used as a new and natural preservative in food industry (Tabatabai Yazdi *et al* 2013).

Kazemi (2014) discussed his findings in an essay entitled "Antimicrobial effects of leaves, stems and roots of *Rheum ribes* extract" and found that *Rheum ribes* have antimicrobial effects on *Escherichia coli* and Klebsiella pneumoniae (Kazemi, 2014). Taren *et al* (2010) showed his findings in an essay entitled "The study of the antimicrobial effects of *ferulago angulata* Oil extract" on a few number of bacteria such as *Staphylococcus aureus*, defining that the mentioned extracts have antimicrobial effects (Taren *et al* 2010). Park *et al* (2007) showed that vibratory waves cause abnormal eye movements (Park *et al* 2007). Vibratory effect has only applied in multi-cellular Eukaryotes such as human, rat and rabbit so far and the effects on Prokaryotes have not been studied yet. The prepared research considers the frequency rate and the time duration hampering the growth of bacteria.

MATERIALS and methods

Nutrient agar Medium was used in this study. In order to prepare pure colonies compared to Escherichia coli and *Klebsiella pneumoniae* gram-negative bacteria cultivation, a striking method was applied. After cultivation, the bacteria are placed in the incubator with 37 degrees for 24 hours for the sake of being developed. In order to check and sterile the colonies, they are removed from the 37 degree environment next day and then placed in autoclave with 121° C and pressure of 15 atm for 15 minutes. After this step, they are removed out of the autoclave and poured to plates in a proper amount to become less hot.

Nutrient broth medium was applied in order to prepare suspension. Different concentrations of bacteria were applied in order to check the vibration effects on bacteria. The following steps show how the concentrations are prepared:

4 gr of nutrient broth is weighted by using the carriage scales and poured to 500 ml of distilled water to form a solution. Then it is heated over a flame to boil. Afterward, 6.3 cc of physiological serum is poured into test tubes by using a calibrated pipette and sterile cotton placed on the test tubes containing nutrient broth and placed inside the sterilized autoclave. After this step, they are removed from the autoclave to become less hot. Seven test tubes are prepared and named from 1 -7. Tube Number 1 has concentration of 102 × 5.1 and the last tube includes concentration of 108 × 5.1, so that the volume of the liquid inside the tube is about 3.6 cc. The amount of 0.4 is removed from the first tube containing the standard suspension by the use of sampler and then added to the next sample to reach the next tube. In order to study the effect of 20λ vibration, each of the concentrations are poured on Agar medium and cultivated through the spread plate method and placed inside the incubator. Then, without placing the samples on the shaker machine, we pour 20λ suspension of each concentration of the liquid medium on a solid medium plate and cultivate it by the use of a sterilized L- shaped rod and air conditioning system under sterile conditions. Afterwards, we spread it to reach to the last tube containing the suspension. The characteristics of each plate are written on it. Meanwhile, to pour aqueous medium suspension on solid agar plates, first it is started from low concentration, then is continued to high one. All the tubes containing liquid medium are graded to fully specify the concentrations. Then the suspension tubes containing the bacteria are placed on shaker (vortex) machine for 2 minutes and at a frequency of 2, for 3 minutes and at a frequency of 3, for 4 minutes and at a frequency of 4, for 5 minutes and at a frequency of 5, for 6 minutes and at a frequency of 6 and for 10 minutes and at a frequency of 6. Next, we pour 20λ suspension of each concentration on the plate and spread it by the use of a sterilized L- shaped rod.

The antimicrobial effects of Gundelia tournefortii, ferulago angulata and Rheum ribes extracts on Escherichia coli and Klebsiella pneumoniae gram-negative bacteria

The mentioned plants for testing were prepared in Yasuoj city and then dried in the shade, away from sunlight. When the plants dried, eatable parts of *Gundelia tournefortii*, *ferulago angulata* leaf and *Rheum ribes* are crushed and powdered. Then 50 g is weighted by using carriage scales. After making the Erlenmeyer flask, graduated cylinders and distilled water sterilized, the amount of 225 cc alcohol (ethanol 96%) is poured into a cylindrical. Then, 225 cc distilled water is added to it. Afterwards, various powders of three types of plants such as *Gundelia tournefortii*, *ferulago angulata* and *Rheum ribes* are poured into three sterilized Erlenmeyer flasks. In next step, the amount of 150 cc water-alcohol mixture in equal proportions, meaning 75 cc ethanol and 75 cc water, are poured into graduated cylinders in each Erlenmeyer flask and then the gates are closed by Parafilm. Then the plants types and experiments dates are specified on them. The flasks need to be stirred every two days so that the extract properly separated from it. After a few days, they need to be placed in a suitable environment. Then the extracts are well prepared for filtration. Next, a few Erlenmeyer flask, beaker, filter paper, gas, Pyrex, funnel and some distilled water are placed in autoclave. All these actions must be performed under sterile conditions. After this step, the gas in the funnel are put in the beaker and the extracts of each plants are poured separately into the flask and beaker specified for that plant. After one week, the final extraction is done by using filter paper and vacuum pumps. This work is carried out with the utmost precision.

Then the extracts are poured into a specific container (Pyrex) and placed in an oven with 37ºC for a few days resulting in water and alcohol evaporation. The resulting extracts are remained as a solid. Then the extract is separated from the container by the use of a sterilized spoon and weighted on a carriage scales by using a sterilized foil. Thus, the resulting extracts are prepared. Results of studying the effects of *Gundelia tournefortii*, *ferulago angulata* and *Rheum ribes* extracts on *Escherichia coli* and *Klebsiella pneumoniae* gram-negative bacteria.

In order to study the antimicrobial effects of *Gundelia tournefortii*, *ferulago angulata* and *Rheum ribes* extracts, the 0.97, 2.9, 15.62, 50, 62.5, 100 Mg/ml concentrations are applied in this experiment in accordance with the following method:

The extract is dissolved in distilled water to ratio 250 mg per 1 cc water and then the antimicrobial effects are considered after bacteria cultivation on a Mueller-Hinton Agar medium through the Diffusion Well method. Then a few wells are created with 5 mm diagonal under sterile condition. The amount of 60λ of each extracts is poured to the well specified for that extract. It should be noted that this work is done for each of extracts. Significant concentration of each extract is added to wells as follows:

The concentration amounts of 0.97, 1.56, 6.25, 15.62, 25, 50, 62.5, 100, 125 Mg/ml of *Rheum ribes* are added to each well for the sake of checking the effects on *Escherichia coli* and *Klebsiella pneumoniae* gram-negative bacteria**.**

The concentration amounts of 1.56, 6.25, 25, 50, 100 Mg ml of *Gundelia tournefortii* are added to each well at the amount of 60λ by using a sampler for the sake of checking the effects on *Escherichia coli* and *Klebsiella pneumoniae* gram-negative bacteria.

The concentration amounts of 0.97, 2.90, 15.62, 62.5, 125 Mg/ml of *ferulago angulata* are added to each well at the amount of 60λ by using a sampler for the sake of checking the effects on *Escherichia coli* and *Klebsiella pneumoniae* gram-negative bacteria.

After incubation, the plates are placed in 37° for 24 hours and the inhibition zones are considered around the wells. Furthermore, the sensitivity of mentioned bacteria toward gentamicin and tetracycline antibiotics is considered through the Disk Agar Diffusion method (Barfar, 2014).

RESULTS

A. The effects of shaker machine (Vortex) on Escherichia coli

According to the obtained results, the increase in time vibration and frequency do not influence the number of bacteria colonies in various concentrations (Table 1).

Table 1: Effect of Vibration of Shaker Device (Vortex) on *Escherichia coli.*

Concentration	10^2	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	1	**CN**	5	**CN**	2	**CN**	7	**CN**	3	**CN**	4	**CN**	3	**CN**	2
	10^3	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	23	**CN**	20	**CN**	23	**CN**	15	**CN**	10	**CN**	17	**CN**	18	**CN**	15
	10^4	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	171	**CN**	129	**CN**	109	**CN**	65	**CN**	60	**CN**	78	**CN**	58	**CN**	60
	10^5	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN
	10^6	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN
	10^7	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN
	10^8	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN

T: Time of Period **F: Frequency** **CN: Colony Number** **IN: innumerable**

B. The effects of shaker machine (Vortex) on Klebsiella pneumoniae

According to the obtained results, the increase in time vibration and frequency do not influence the number of bacteria colonies in various concentrations (Table 2).

C. The effects of medicinal plants various concentrations on Escherichia coli bacteria

Different concentration effects of the plants *Gundelia tournefortii, ferulago angulata* and *Rheum ribes* on *Escherichia coli* bacteria are studied through the Well Diffusion method. The results show that the various concentrations of *Gundelia tournefortii, ferulago angulata* and *Rheum ribes* do not effect on *Escherichia coli* bacteria and the inhibition zone is unseen (Table 3).

Table 2: Effect of Vibration of Shaker Device (Vortex) on *Klebsiella pneumonia.*

Concentration	10^2	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	19	**CN**	32	**CN**	28	**CN**	32	**CN**	30	**CN**	40	**CN**	59	**CN**	25
	10^3	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	91	**CN**	142	**CN**	130	**CN**	96	**CN**	95	**CN**	105	**CN**	165	**CN**	87
	10^4	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	500	**CN**	495	**CN**	470	**CN**	483	**CN**	475	**CN**	470	**CN**	495	**CN**	450
	10^5	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN
	10^6	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN
	10^7	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN
	10^8	**T**	0	**T**	2	**T**	3	**T**	4	**T**	5	**T**	6	**T**	10	**T**	15
		F	0	**F**	2	**F**	3	**F**	4	**F**	5	**F**	6	**F**	6	**F**	6
		CN	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN	**CN**	IN

T: Time of Period **F: Frequency** **CN: Colony Number** **IN: innumerable**

Table 3: Effect of *Gundelia tournefortii, ferulago angulata* and *Rheum ribes* on *Escherichia coli.*

Concentration (Mg/ml)	Diameter of Halo (Mm)
250	-
125	-
100	-
50	-
25	-

Table 4: Effect of *Gundelia tournefortii, ferulago angulata* and *Rheum ribes* on *Klebsiella pneumonia*.

Concentration (Mg/ml)	Diameter of Halo (Mm)
250	-
125	-
100	-
50	-
25	-

The results show that the various concentrations of *Gundelia tournefortii, ferulago angulata* and *Rheum ribes* do not effect on *Klebsiella pneumoniae* bacteria and the inhibition zone is unseen (Table 4).

CONCLUSION

The results showed *Gundelia tournefortii, Ferulago angulata* and *Rheum ribes* extracts did not effect on the gram-negative *Escherichia coli* and *Klebsiella pneumoniae* bacteria. With increscent of time vibration and frequency, no significant change is seen in number of colonies as a result of bacteria cell wall strength.

Since the size of *Escherichia coli* bacteria is larger than *Klebsiella pneumoniae*, they had more claches compared to *Klebsiella*. Consequently, their cell wall is weaker than gram-positive bacteria like *Staphylococcus Aureus* because of low layer of peptidoglycans. Thus, concentration shows the most impact on the mentioned bacteria as a result of Escherichia coli bacteria clashes to each other as well as the destruction of their cell wall.

E. coli and *Klebsiella pneumoniae* bacteria both are belonged to the family of intestinal gram-negative bacteria and also they are similar to each other in terms of structural features (except in cases where the *Klebsiella* is like a capsule), the same results obtained through studying the time and frequency in various concentrations without seeing any significant effect. Compounds are found in plants that affect the bacteria. The mentioned compounds are disappeared by drying plants and therefore do not affect the growth of bacteria ((Barfar, 2014).

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