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Article

The Inhibitory Effective Of The Aqueous And Ethanolic Extract Of The Pomegrannte Punicagrantum Fruit Covernd Fenugreek Trigonellfoenum-Greum Seeds Against Some Pathogenic Bacterial Species

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Abstract: The pomegranate, Punicagranatum, is considered as amagical, exclusive fruit grew on a colonisttree culturedall over the Mediterranean area. Pomegranate is useful in some medications1,2. In fact, P. garantumhad antimicrobialaction against a variety of Gram+ve and Gram-vebacteria. The constructions of Pomegranate is comprisingferrous salts.3. Fenugreek plant (the scientific name isTrigonellafoenum-graecum)which is used in variousmedications. The Fenugreek seeds extract is used to determine the antibacterial actionagainst: a variety of Gram+ve and Gram-ve bacteria.4. The fresh pomegranate extractwas prepared by methanol and distilled water (D.W) according to standard method. The pomegranateand bacterial specimens were kept in fridge at 4°C. The antibacterial action of all extracts was examined using the disc diffusion technique. The currentresearchdisplayedthat water and methanol pomegranate extracts hadantibacterial activity on the bacteria. Additionally, The current study established that there was substantialaction on the growth of a someGram+ve and Gram-ve bacteria. a great advantage of pomegranate extract in foods preserving. The antimicrobial activity of pomegranate extract is effective against dental bacteria giving chance to pharmaceutical usage in the herbal remedy.The phytocompounds in pomegranate extract comprising phenol, tannin and flavonoid as main effective compounds in the inhibition of bacterial growth. Additional studies are necessary to separate the active antimicrobial compounds in pomegranate and studying the mechanism of action.The aim of this research is to examine the antibacterial action of aqueous and methanolic extracts of thepomegranate extract and the Fenugreek extract against many pathogenic bacteria comparing with antibiotics Cloramphenicol (C), Erythromycin (E), Trimethoprim (TM), Ampicillin (AM), ofloxacin (OFX), Gentamicin (CN), Cefepime (FEP) and Nalidixic acid (NA)

Keywords: Pomegranate, Bacteria,Punicagranatum.

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1. Introduction

In the previoustwenty years; medical plants have an enormously attentionby the usage of herbal crops as natural medication for their biological action. the WHO showed that> 80% of the population depend on herbal treatments for the primary health care 5. Currently the antioxidants are having animprovedconcernuniversallyto be usedin food industries and medicines 6.P. granatum, which isgenerallycalled pomegranate,it is one of the hoariest fruits. It has been used in folkloric treatment,

displaying antibacterial and antifungal action 1,2,7. This fruit is active in dysentery, cure acidosis, helminthiasis, diarrhea, respiratory pathologies, h+emorrhage, as well as antiviral activity, cancer prevention, treatment of diabetes, cardiovascular disease, male infertility, bacterial infections, Alzheimer's disease, dental conditions, obesity and arthritis 8.Thepomegranate contents are veryeffective antioxidant 9,10.Fenugreekis belong to the family leguminosae. It is cultivated in south Asia.13,14Fenugreek is considered to be the greatest promising pharmaceutical herb.15It has the ability to decrease blood cholesterol and glucose ratiosand so it used fordiabetes and coronary heart diseases therapy, anti-fertility effects, antibacterial activity, anti-helminthic, and antinociceptiveaction 14,15.

2. Materials and Methods

2-1 aqueous extractPreparation

1- A weight of 100 gm of the fruitpowder wasadded to 500 ml of sterilized D.W18. in beakerand kept at room temperature for 24 hrs, still mixing for good extraction.

2- Filter the extract using sterilized filter, then the extract should be concentrated by water evaporation using rotary evaporator in oven at 40 °C to avoid compounds denaturation.

3- Keep the extract in refrigerator at 4°Cuntil use.19

2-2 alcoholic extract Preparation

The same procedure as in aqueous extract Preparation but use ethanol 95% instead of D.W.19

2-3Chemical detection of active group in herbal extract:

2-3-1 detection of tanins:

Amount of 10 gm of plant powder with 50 ml of D.W. was boiled, then the solution was filtered and let it to cool. Then, divide the solution to two parts. Add 1% $Pb(C_2H_3O_2)$tothe first part, the tanins can be detected by the presence of gel precipitation. Add 1% Fe $Cl_3$to the second part, the tanins can be detected by the presence of blue green colour.20

2-3-2 detection of resins:

Amount of 5 gm of plant powder with 50 ml of ethanol 95% in water bath at 100°C for 1 min, then the solution was filtered and 100 ml of acidic water 4% HCl was added, the resins can be detected by the presence of turbidity.20

2-3-3 detection of saponins:

A volume of 1-3 ml of HgCl was added to 5 ml of plant extract. The saponins can be detected by the presence of white precipitation.20

2-3-4 detection of flavonoids:

Two solution were prepared:

The first solution: 10 gm of alcoholic plant extract was dissolved in 5 ml of ethanol 95%, then the first solution was filtered.

The second solution:50% of ethanol was added to 50% KOH.

An equal amounts of both solutions were mixed. The flavonoids can be detected by the presence of yellow colour. 20

2-3-5 detection of alkaloids:

Amount of 10 gm of plant powder was boiled with 50 ml of acidic D.W 4% HCl, then the solution was filtered after cooling at and 30°C. Amount of 0.5 ml of this extract was examined for detection of the following reagents of alkaloids:

Mayer reagent: white precipitation.

dragendroffreagent: orange precipitation

Picric acid: yellow precipitation. 20

2-3-6 detection of glycosides:

Many drops of diluted HCl was added to 5 ml of the aqueous extract and placed in water bath at 100 °C for 20 min., the acidity was neutralized by addition of NaOH. The glycosides can be detected by the presence of red precipitation after addition of Fehling's reagent. 20

2-3-7 detection of coumarin:

Amount of 0.5 mg of plant extract with 1 ml of ethanol in test tube, then cover the tube by filter paper soaked with diluted NaOH and place it in water bath at 100°C for few min. then put the filter paperin UV detector.The coumarincan be detected by the presence of green-yellow colour on the filter paper. 20

2-3-8 detection of phenols:

filter paper was soaked with the plant extract, then a few drops of both $FeCl_3$ and folin reagent, followed by NH_3 evaporation. The phenols can be detected by the presence of blue colour. 20

2-3-9 preparation of stock solution:

This solution is used for further tests by dissolving 1 gm of the dry extract in 10 ml of D.W. with continuing mixing for complete dissolving. All the processes were done under sterile condition to obtain concentration 100 mg/$cm^3$21.

2-3-10 Bacterial cultures

Overnight cultures of the testedGram^{+ve}bacteria*streptococcus mitis,* and *streptococcus salivarius*,and the tested Gram^{-ve}bacteria*citrobacter sp*.and *Yersinia pestis*.All isolates were suspended to give a turbidity equal to (0.5) McFarland (1.5×10^8 CFU/ml). Then,100 µlof the suspension wascultured on MuellerHinton agar plates. Then, paper diskssoaking with the extracts. The discs were put on the inoculated agar surface and incubated at 37 ºC for 24 hrs.22

2-3-11detection of antibacterial activity of plant extract:

The antimicrobial activity of both Fenugreek extract and pomegranate extract wasassessed by agar diffusion method by using wells that has been done by cork borer. Both alcoholic and the aqueous extract in the concentrations (10, 25, 50, 75, 100, 200) mg/ml were tested. Three wells were done on the surface of Muller Hinton agar with triplicate. By using spreader, 0.2 µl of bacterial suspension with 1.5×10^8 cell/mlwas spread on the plate surface. Then, 50 µl was placed into each well and left for 1 hrs, then the plates were incubated at 37 ºC for 24 hrs.The antibacterial action was assessed by diameter measuring of the inhibition zone around theextracts of both Fenugreek and pomegranate.23

2-5 Statistical analysis

Theexperiments were achieved in triplicate. All results were stated as standard and mean deviation. The statistical importance was designedusing ANOVA test (LSD). The mean variance wasimportant at 0.05 level 24.

3. Results and Discussions

In fact, pomegranate is considered as a main source of antioxidant and phenols 25, gallic and ellagic acids 26as well as vitamin C 8.

The current study established that the antibacterial activity of thepomegranate extract againstS. mitis and S.salivarius. Triplet for each bacterial concentration has been done.

3-1 Pomegranate alcoholic and aqueous extracts effect on G+ve bacteria

Pomegranate aqueous extractshowed variation in the inhibition zone diameter values as in (Table-1). Low inhibition activity in the lowest concentration against S. mitis, so the inhibition zone diameter mean was ranging from (12-14) mm in the concentration 10%. While the inhibition zone was (18-20) in the highest concentration 200%.No obviousactivity of this extract against S. salivarius, so the inhibition zone was (12) mm in the concentrations (10%, 25%,50%). While the maximum inhibition activity was (14-18) in the highest concentration 200%.

On the other hand, Pomegranatealcoholicextract showed low inhibition activity in the lowest concentration against S. mitis, it produced inhibition zone (12-14) mm in the concentration (10%,25%,50%). While the inhibition zone diameter mean was ranging from (20-24) in the highest concentration (100%-200%). No obvious activity of this extract against S. salivarius, so the inhibition zone was (12) mm in the concentrations (10%, 25%,50%). While the maximum inhibition activity was (14-18) in the highest concentration 200%.

(Table-1) pomegranate alcoholic and aqueous extracts effect on G+ve bacteria

pomegranate alcoholic extract						pomegranate aqueous extract						bacteria concentration
200	100	75	50	25	10	200	100	75	50	25	10	
20	22	18	12	12	12	18	18	20	18	16	14	*S.mitis*
20	24	16	12	12	12	20	20	16	16	14	12	
22	22	20	14	14	12	20	18	20	16	16	12	
16	14	14	18	18	14	18	16	16	12	12	12	*S.salivarius*
18	16	16	18	18	12	16	14	14	12	12	12	
20	20	18	20	16	14	14	16	16	12	12	12	

3-2 Pomegranate alcoholic and aqueous extracts effect on G-ve bacteria

Pomegranate aqueous extract showed low inhibition activity in the lowest concentration against citrobacter sp., so it produced inhibition zone (12-14) mm in the concentration (10%,25%,50%). While the inhibition zone was (16-20) in the highest concentration 200%. The activity of this extract against Yersinia pestis was as follows: the inhibition zone was (14-16) mm in the concentrations (10%, 25%). While the maximum inhibition activity was (14-20)mm in the highest concentration 200%.

On the other hand, Pomegranate alcoholicextract showed low inhibition activity in the lowest concentration against citrobacter sp., it produced inhibition zone was (14)

pomegranatealcoholic extract (standard error±mean)						pomegranate aqueous extract (standard error±mean)						Bacteria / concentration
200	100	75	50	25	10	200	100	75	50	25	10	
20.67 ± 0.67 ^{Ba}	22.67 ± 0.67 ^{Ca}	18.0 ± 1.16 ^{Ba}	12.67 ± 0.67 ^{Aa}	12.67 ± 0.67 ^{Aa}	12.0 ± 0.0 $^{A}_{a}$	19.33 ± 0.67 $^{A}_{a}$	18.67 ± 0.67 $^{A}_{a}$	18.67 ± 1.33 $^{AB}_{a}$	16.67 ± 0.67 $^{A}_{a}$	15.33 ± 0.67 $^{AB}_{a}$	12.67 ± 0.67 $^{B}_{a}$	*S.mitis*
18.0 ± 1.16 $^{A}_{a}$	16.67 ± 1.76 $^{AB}_{a}$	16.0 ± 1.16 $^{AB}_{a}$	18.67 ± 0.67 $^{AB}_{a}$	17.33 ± 0.67 $^{A}_{a}$	13.33 ± 0.67 $^{B}_{a}$	16.0 ± 1.16 $^{A}_{a}$	15.33 ± 0.67 $^{A}_{a}$	15.33 ± 0.67 $^{A}_{a}$	12.0 ± 0.0 $^{A}_{b}$	12.0 ± 0.0 $^{A}_{b}$	12.0 ± 0.0 $^{A}_{a}$	*S. salivarius*
23.33 ± 0.67 $^{A}_{a}$	22.67 ± 0.67 $^{A}_{a}$	20.67 ± 0.67 $^{A}_{a}$	14.67 ± 0.67 $^{B}_{a}$	15.33 ± 1.33 $^{B}_{a}$	14.0 ± 0.0 $^{B}_{a}$	8.0 ± 1.16 $^{B}_{b}$	16.0 ± 1.16 $^{AB}_{a}$	13.33 ± 1.33 $^{AB}_{b}$	12.0 ± 0.0 $^{A}_{a}$	12.0 ± 0.0 $^{A}_{a}$	12.67 ± 0.67 $^{A}_{a}$	*citrobacter sp.*
20.0 ± 0.0 $^{A}_{a}$	19.33 ± 0.67 $^{A}_{a}$	18.67 ± 0.67 $^{A}_{a}$	18.0 ± 0.0 $^{A}_{a}$	16.0 ± 0.16 $^{A}_{a}$	16.0 ± 0.0 $^{A}_{a}$	18.0 ± 2.0 $^{A}_{a}$	16.0 ± 1.16 $^{A}_{a}$	14.67 ± 0.67 $^{A}_{a}$	16.67 ± 0.67 $^{A}_{a}$	15.3 ± 0.67 $^{A}_{a}$	14.67 ± 0.67 $^{A}_{a}$	*Yersinia pestis*

mm in the concentration (10%). While the inhibition zone was (22-24)mm in the highest concentration (200%). The activity of this extract against Yersinia pestis was as follows: the inhibition zone was (16)mm in the concentrations (10%). While the maximum inhibition activity was (20)mm in the highest concentration 200% as in (Table-2), (Table-3).

(Table-2) pomegranate alcoholic and aqueous extracts effect on G-ve bacteria

pomegranate alcoholic extract						pomegranate aqueous extract						Bacteria concentration
200	100	75	50	25	10	200	100	75	50	25	10	
24	24	20	14	18	14	16	14	12	12	12	12	*citrobacter sp.*
24	22	22	14	14	14	18	16	16	12	12	14	
22	22	20	16	14	14	20	18	12	12	12	12	
20	20	18	18	16	16	20	16	14	16	16	14	*Yersinia pestis*
20	20	20	18	18	16	14	14	16	16	16	14	
20	18	18	18	14	16	20	18	14	18	14	16	

Table-3) The statistical analysis of pomegranate alcoholic and aqueous extracts effect on G+veand G-vebacteri

The superscript capital letters for the vertical comparison, the different letters referred to a significant difference (P≤0.05). While the subscript small letters for the same concentration of the two group , the different small letters referred to a significant difference (P≤0.05).

The inhibition zone diameter around are showed in (Table-1,2,3). The current study revealed that there were substantial changes in the antibacterial activity of both pomegranate and Fenugreek extracts (aqueous and alcoholic extract), and these result agree with a previous study showed that aqueous and alcoholic pomegranate extracts had effect on Streptococcus sp. growth. The present study also agree with another study which

presented that pomegranate aqueous and alcoholic extract were active and effective against 21.

The antibacterial effect of pomegranate extractis more effective on the growth of G-ve bacteria than G+ve bacteria. This is might be because of the cell walls structures of these bacteria 27.The current study showed that no obvious variations between aqueous and alcoholic pomegranate extract.

A previous study showed that alcoholic pomegranate extract is a strong inhibitor for L. monocytogenes and Yersinia sp. 8. The peel extract of pomegranate contains phytocompounds which can inhibit bacterial growth 28. Additionally, peel extract of pomegranate contains wide spectrum antibiotic compounds or metabolic toxins that affect bacterial growth 3. The pomegranate peel is more effective than the entire fruit, since the peel contains effective compounds for example (tannin, anthocyanin, flavone andvitamin C 29.

A previous study revealed that the alcoholic extract of pomegranate is effective against all tested bacteria in their research 30. The results of the current study is also stated that alcoholic extracts have antimicrobial activity against all tested bacterial isolates 31. A previous study showed that alcoholic extract are more effective than aqueous extract. Correspondingly, another study presented that water extract of pomegranate has lower influence than alcoholic extract. 32

3-3 Fenugreek alcoholic and aqueous extracts effect on G+ve bacteria

Fenugreekaqueous extract showed low inhibition activity in the lowest concentration against S. mitis, so the inhibition zone diameter mean was ranging from (4) mm in the concentration 10%. While the inhibition zone was (10)mm in the highest concentration 200%.The activity of this extract against S. salivarius, so the inhibition zone was (4-6) mm in the concentrations (10%), While the maximum inhibition activity was (10-14) in the highest concentration 200%.

On the other hand, Fenugreek alcoholicextract showed low inhibition activity in the lowest concentration against S. mitis, it produced inhibition zone (0-4) mm in the concentration (10%). While the inhibition zone diameter mean was ranging from (10)mm in the highest concentration (200%). No obvious activity of this extract against S. salivarius, so the inhibition zone was (4) mm in the concentrations (10%, 25%). While the maximum inhibition activity was (10)mm in the higher concentrations (75%, 50%, 200%). (Table-4)

(Table-4) Fenugreekalcoholic and aqueous extracts effect on G+ve bacteria

Fenugreekalcoholic extract						Fenugreekaqueous extract						Bacteria / concentration
200	100	75	50	25	10	200	100	75	50	25	10	
10	10	10	6	4	4	10	10	6	10	8	4	*S. mitis*
10	10	10	8	6	2	10	10	4	8	8	4	
10	10	10	6	6	0	10	10	4	8	6	4	
10	10	10	6	4	4	10	12	10	10	8	6	*S. salivarius*
10	10	10	6	4	4	10	12	10	10	8	4	
10	10	10	6	4	4	14	10	10	10	10	4	

3-4 Fenugreekalcoholic and aqueous extracts effect on G-ve bacteria

Fenugreekaqueous extract showed low inhibition activity in the lowest concentration against citrobacter sp., so it produced inhibition zone (3-6) mm in the concentration (10%), While the inhibition zone was (14)mm in the highest concentration 200%. No obvious activity of this extract in the lowest concentration against Yersinia pestis so the inhibition zone was (0) mm. While the maximum inhibition activity was (12)mm in the highest concentration 200%.

On the other hand, Fenugreekalcoholicextract showed no obvious activity of this extract in the lowest concentration against citrobacter sp., so it produced inhibition zone (0) mm in the concentration (10%). While the inhibition zone was (10)mm in the highest concentration (100%, 200%). The activity of this extract against Yersinia pestis was as follows: the inhibition zone was (2-4)mm in the concentrations (10%). While the maximum inhibition activity was (10)mm in the higher concentrations (100%, 200%) as in (Table-5), (Table-6).

(Table-5) Fenugreekalcoholic and aqueous extracts effect on G-ve bacteria

Fenugreekalcoholic extract						Fenugreekaqueous extract						Bacteria concentration
200	100	75	50	25	10	200	100	75	50	25	10	
10	10	8	6	4	0	14	12	10	10	10	3	*citrobacter sp.*
10	10	8	6	4	0	14	12	10	10	10	6	
10	10	8	6	4	0	14	12	10	10	8	6	
10	10	8	8	6	2	12	12	10	10	4	0	*Yersinia pestis*
10	10	8	8	6	4	12	10	10	10	4	0	
10	10	8	8	6	4	12	10	10	10	4	0	

(Table-6) The statistical analysis of Fenugreekalcoholic and aqueous extracts effect on G+ve and G-ve bacteria

Fenugreekalcoholic extract						Fenugreekaqueous extract						Bacteria / concentration
200	100	75	50	25	10	200	100	75	50	25	10	
10.0 ± 0.0 A_a	10.0 ± 0.0 A_a	10.0 ± 0.0 A_a	6.67 ± 0.67 B_a	5.33 ± 0.67 B_a	2.0 ± 1.16 C_a	10.0 ± 0.0 A_a	10.0 ± 0.0 A_a	4.67 ± 0.67 DC_b	8.67 ± 0.67 B_a	7.33 ± 0.67 AD_a	4.0 ± 0.0 C_a	*S. mitis*
10.0 ± 0.0 A_a	10.0 ± 0.0 A_a	10.0 ± 0.0 A_a	6.0 ± 0.0 B_b	4.0 ± 0.0 B_b	4.0 ± 0.0 B_a	11.33 ± 1.33 A_a	11.33 ± 0.67 Aa	10.0 ± 0.0 A_a	10.0 ± 0.0 A_a	8.67 ± 0.67 A_a	4.67 ± 0.67 B_a	*S. salivarius*
10.0 ± 0.0 A_b	10.0 ± 0.0 A_b	8.0 ± 0.0 B_b	6.0 ± 0.0 B_b	4.0 ± 0.0 C_b	0.0 ± 0.0 D_b	14.0 ± 0.0 A_a	12.0 ± 0.0 B_a	10.0 ± 0.0 C_a	10.0 ± 0.0 C_a	9.33 ± 0.67 C_a	5.0 ± 1.0 D_a	*citrobacter sp.*
10.0 ± 0.0 A_b	10.0 ± 0.0 A_a	8.0 ± 0.0 B_b	8.0 ± 0.0 B_b	6.0 ± 0.0 C_a	3.33 ± 0.67 D_a	12.0 ± 0.0 A_a	10.67 ± 0.67 AB_a	10.0 ± 0.0 B_a	10.0 ± 0.0 B_a	4.0 ± 0.0 C_b	0.0 ± 0.0 D_b	*Yersinia pestis*

The superscript capital letters for the vertical comparison, the different letters referred to a significant difference (P≤0.05). While the subscript small letters for the

vertical comparison between the same concentration of the two group, the different small letters referred to a significant difference (P≤0.05).

The current study revealed that the fenugreek seeds methanolic extract results have an antimicrobial action. A previous study showed thatStreptococcus spp. displayed sensitivity to the fenugreek seed methanolic extract at the concentrations (1000, 250 mg/ml). the seed of Fenugreek is rich of galactomannan, polysaccharide, yamogenin, disogenin and gitogenin, in addition to volatile oils and alkaloids for example choline and trigonelline that can be considered as a natural antimicrobial agents33. The extraction of secondary metabolitie is greatly depending on the usage of additional procedures depending on the chemical characteristics of these metabolitie. Water soluble metabolitie could be extracted in the H2O while water insoluble metabolitie could be extracted by organic solvent 34.

3-5 Antibiotic sensitivity test on G+ve bacteria

Antibiotic sensitivity test was done by using the following antibiotics: Cloramphenicol (C), Erythromycin (E), Trimethoprim (TM), Ampicillin (AM), ofloxacin (OFX), Gentamicin (CN), Cefepime (FEP) and Nalidixic acid (NA). The current study revealed thatCefepime (FEP), trimethoprim (TM) and ampicillin (AM) have the lowestinhibition activity against S. mitis, producing inhibition zone (6)mm. While ofloxacin (OFX) has the highest inhibition activity with inhibition zone was (26-28)mm. Trimethoprim (TM) and ampicillin (AM) have the lowestinhibition activity against S. salivarius, producing inhibition zone (6)mm. While ofloxacin(OFX) has the highest inhibition activity with inhibition zone was (36-38)mm, (Table-7).

(Table-7) antibiotic sensitivity test on G+ve bacteria

NA	FEP	OFX	CN	TM	AM	E	C	Bacteria / Antibiotic
14	6	26	18	6	6	16	20	*S. mitis*
14	6	28	18	6	6	18	20	
14	6	28	18	6	6	18	20	
16	16	36	24	6	6	6	18	*S. salivarius*
16	16	38	26	6	6	12	20	
16	16	38	26	6	6	12	20	

3-6 Antibiotic sensitivity test on G-ve bacteria

The antibiotic sensitivity test showed that trimethoprim (TM) has the lowestinhibition activity against citrobacter sp., producing inhibition zone (6)mm. While ofloxacin (OFX) has the highest inhibition activity with inhibition zone was (30-40)mm. Trimethoprim (TM) and Cefepime (FEP) have the lowestinhibition activity against Yersinia pestis, producing inhibition zone (6)mm. While Cloramphenicol (C) has the highest inhibition activity with inhibition zone was (26)mm, (Table-8), (Table-9).

(Table-8) Antibiotic sensitivity test on G-ve bacteria

NA	FEP	OFX	CN	TM	AM	E	C	Bacteria / Antibiotic

24	18	30	26	6	14	14	24	*citrobacter sp.*
26	18	40	30	6	18	16	30	
26	18	40	30	6	18	16	30	
16	6	22	18	6	8	24	26	*Yersinia pestis*
16	6	24	20	6	8	24	26	
16	6	24	20	6	8	24	26	

(Table-9) The statistical analysis of Antibiotic sensitivity test on G+ve and G-ve bacteria

NA	FEP	OFX	CN	TM	AM	E	C	Bacteria / antibiotic
14.0 ± 0.0^{D_c}	6.0 ± 0.0^{E_c}	27.33 ± 0.67^{A_b}	18.0 ± 0.0^{C_b}	6.0 ± 0.0^{E_a}	6.0 ± 0.0^{E_c}	17.33 ± 0.67^{C_b}	20.0 ± 0.0^{B_c}	*S. mitis*
16.0 ± 0.0^{D_b}	16.0 ± 0.0^{D_b}	37.33 ± 0.67^{A_a}	25.33 ± 0.67^{B_a}	6.0 ± 0.0^{F_a}	6.0 ± 0.0^{F_c}	10.0 ± 2.0^{E_c}	19.33 ± 0.67^{C_c}	*S. salivarius*
25.33 ± 0.67^{C_a}	18.0 ± 0.0^{D_a}	36.67 ± 3.33^{A_a}	28.67 ± 1.33^{B_a}	6.0 ± 0.0^{F_a}	16.67 ± 1.33^{E_a}	15.33 ± 0.67^{E_b}	28.0 ± 2.0^{B_a}	*citrobacter sp.*
16.0 ± 0.0^{D_b}	6.0 ± 0.0^{F_c}	23.33 ± 0.67^{B_c}	19.33 ± 0.67^{C_b}	6.0 ± 0.0^{F_a}	8.0 ± 0.0^{F_b}	24.0 ± 0.0^{B_a}	26.0 ± 0.0^{A_b}	*Yersinia pestis*

The superscript capital letters for the vertical comparison, the different letters referred to a significant difference (P≤0.05). While the subscript small letters for the horizontal comparison between the bacterial isolates' antibiotic resistance, the different small letters referred to a significant difference (P≤0.05).

The Gram-ve bacteria contain an outer membrane with the lipopolysaccharide (LPS) making the cell wall resistant to antibacterial agents. Alternatively, The Gram+ve bacteria is sensitive to antibacterial agents because it has peptidoglycan layer only without LPS. Peptidoglycan layer is not vigorous diffusion barrier, so Gram-ve cell wall is more complex than the Gram+ve because they act as a permeability barrier so the bacteria will be less sensitive to antibacterials than Gram+ve. 35,36

3-7 The statistical analysis

The mean and the SE mean are measured via IBM SPSS version "26.0". Moreover, the possibilitytestedvia student -T-test besidesDuncan test in addition to ANOVA table. 22

4. Conclusion

The presentresearchesimprovea greatadvantage of pomegranate extract in foodspreserving. The antimicrobial activity of pomegranate extract is effective against dental bacteria giving chance to pharmaceutical usage in the herbalremedy.The phytocompounds in pomegranate extractcomprising phenol, tannin and flavonoid as maineffectivecompoundsin the inhibition of bacterial growth.Additional studies are necessary to separate the active antimicrobial compounds in pomegranate and studying the mechanism of action.

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