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ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF *PUNICA GRANATUM* AGAINST ANTIBIOTIC-RESISTANT *CLOSTRIDIUM PERFRINGENS* TYPE (D)

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ABSTRACT

The search for new antibiotics and alternative products to solve the increasing number of bacterial resistance to customary antibiotics has become an urgent need. To investigate the effectiveness of the extracts prepared from different parts of Syrian *Punica granatum* Linn (family Punicaceae), against *Clostridium perfringens type (D),* which is resistant against many antibiotics, 684 samples were isolated from intestines and livers of death goats by using blood agar, and a selective agar for growing of *Clostridium perfringens*(SPS agar). The isolated bacteria were typed by using ELISA apparatus. Many parts of *Punica granatum* was extracted with water, absolute alcohol, then ether by using soxhlet apparatus and rotary evaporator. The Antibiotic susceptibility testing of many antibiotics was conducted by using disc diffusion method in anaerobic atmosphere and break points method. The alcoholic extracts prepared from many parts of *punica granatum* (pericarp, leaves, flowers, seeds) showed different antibacterial effect against *Clostridium perfringen*s type(D),whereas the studied antibiotics had not shown any antibacterial effect, except Clindamycin which showed partial effectiveness.

KEY WORDS: *Punica granatum, Clostridium perfringens*, resistant bacteria, anaerobic bacteria.

INTRODUCTION

Clostridium perfringens is a Gram-positive, rod-shaped, anaerobic, spore-forming bacterium of the genus *Clostridium*. *C. perfringens*is pervasive in nature and can be found as a normal constituent of decompose vegetation, the intestinal tract of humans, and soil. In meat their spores are resistant to temperature at 100 °C for more than 1hr[1]. *Clostridium perfringens* strains are classified into five types (A–E) on the basis of their ability to produce major lethal toxins,alpha (α), beta (β), epsilon (ε), and iota (ι), which are responsible of their pathogenicity[2]. Toxins are absorbed into the general circulation with usually distressing effects on the host[3],and are responsible for severe diseases in humans and animals including gangrene, skin and soft tissue infections, enterotoxemias, food toxinosis, septicemia after parturition and abortion, wound infection, pneumonia and empyema, meningitis, mionecrosis and cystitis[1].

***Punicagranatum* Linn** (*pomegranate*) belonging to family *punicaceae*, is native to the Mediterranean region and has been extensively used in the folk medicine.The medicinal parts are the root, the bark, the fruits, the peel of the fruit and the flowers [4],which have been used in several medicinal purposes [4, 5]. The fruit is good for dysentery, diarrhea and stomachache[6].It has also known as an anti-diarrhea, antiparasitic agent, ulcers, diuretic, and an antibacterial activity[5]. Although many studies have reported the antibacterial activity of pomegranate[7, 8], but it did not reveal enough studies about its effect on bacterial resistance, and did not determine the most effective part of the plant in dealing with bacteria, whether peeled fruit, leaves, flowers, or seeds of the pomegranate.

Nowadays, uncontrolled and frequent use of antibiotics may cause emergence of microbial resistance among pathogenic agents. Therefore, the use of new synthetic and natural antimicrobial compounds are necessary for antibiotic resistant bacteria [9]. So we tried in our investigation to discover if the plant has the ability to deal with these bacteria. We believe that, **this is the first study** describing the antibacterial activity of*P. Granatum* extracts against *Clostridium perfringens*, and we hope that its results will be a starting point in administering the plant extracts on infected animals.

MATERIALS AND METHODS:

Collection of plant material:

Leaves and the flowers of *pomegranate* were collected in the early morning hours during the period from March to April, while the ripe fruits were collected during the period from July to September, from Damascus rural area,which were identified by Prof. Dr. Anwaral khatib of Damascus University. The peel were separated from the fruits, washed with cold water, distilled water, then dried with hot air at a temperature not exceeding60°C in shadow. Then they were crushed properly by metal mortar in order to obtain fine homogeneous powder, kept in paper bags with free humidity conditions, ready to prepare extract[10].

Preparing plant extracts:

Plant parts were extracted separately by continuous extraction device (Soxhlet apparatus), adopted method described by Wang [11] for preparing plant extracts by organic solvents. 50 gof plant powder were placed byan electricmortar, inside the thimble-holder of Soxhlet apparatus, with 500 mlofeachorganic solvent (rate: 1:10weight: volume). Threedifferentpolarsolventshavebeen selectedto extract the components of the plants, which are respectively: water, absoluteethanol, petroleum ether. Extractionperiodwas4 hours, until the used solventcomes out of thimble colorless. Then to concentrate the extracts; the ethanol, and petroleum ether extracts were driedusing rotary vacuum evaporatorat a temperaturenot exceeding40°C, while the aqueous extract was dried usingfreeze dryer. The thicklayerof thebottom was stored insterilebottles at 4°C for further experiments. All extracts were filter-sterilized using a 0.45 µm membrane filters (whatman,Co.,UK) [10].

Cultured and identification Methods of pathological sample:

668 Samples of dead calves (liver or intestines) were submitted daily to the morgue of the Central Laboratory of Veterinary. First the samples were planted in the thioglycolate liquid, then the tubes were put in a water bath of 80 ° C for 20 minutes, then incubated in anaerobic incubator at 37 °C for 24 hours. Secondly, part of it was transferred to the blood agar, the second part to the selective medium SPS Agar (Sulfite Polymyxin Sulfadiazine),and the third to meat and liver medium (VF).The medium were incubated at 35-37°C for 40-48 hours at anaerobic culture incubator [12] .

***Identification Method of the bacteria**:*

*Clostridium perfringens*were selected after the following steps:

The bacteria was identified culturally, morphologically and biochemically:
14 standard strains were cultured on the same time and were compared with the tested milk samples. These strains were sensitive to clindamycin.

Microscopic examination:

Microscopic examination was carried out after 48 hours of incubation on SPS agar plates using the Gram- stain, light microscope.

Biochemical tests:

All of the following biochemical tests were conducted [13, 14] : Methyl red test, Voguesproskauer test, Indole test.In addition to Glucose, lactose, Maltose, Sucrose fermentation, oxidase, catalase, and testing of gelatin liquification.This technique was performed according to the manufacturer's instructions Bio Merieux, France.

Testing Enzyme-linked immunosorbent assay (ELISA) to type *Clostridium perfringens* :
According to the manufacturer's instructions EuorcloneSpA (Life Sciences Division, Italy) of this test, *Clostridium perfringens* type was identified. All the kit components and samples were put at room temperature at least half an hour before use. Wash Solution concentrate was diluted with distilled water (1:20). Buffer solution concentrate was diluted with distilled water (1:5). Sample (small part of intestine) was diluted with the diluent buffer solution to get bacterial suspension. 100 µl bacterial suspension was added to each microplate well. Wells were incubated at room temperature for one hour. Wells were rinsed three times with diluent washing solution, taking care not to form bubbles. Conjugate solution concentrate was diluted with diluent buffer (1:50), this solution is specific conjugate solution for each type of Clostridium perfringens. 100 µl of diluent conjugate solution was added per well. Wells were incubated at room temperature for one hour. Wells were washed three times with diluent wash liquid. 12 points of chromogen solution concentrate with 9.5 mL of the accompanying solution to get diluent chromogen solution. 100 µl of diluent chromogen solution was added to each well on the plate. Wells were incubated at room temperature for 10 minutes. After this incubation, reaction was stopped by adding 50µl of stop solution. Same steps of sample preparation were performed with positive control, and negative control for each type of Clostridium perfringens, but without adding the bacterial suspension of the sample. Finally, resulting optical density at 450 nm can be recorded using a plate reader. Readings were compared with references accompanying the stander solutions. The same steps for the type of microbial (A, B , C, D, E, F) was Performed.

Antibiogram study:

Clostridium perfringens type (D) according to[3, 15] was selected for testing sensitivity to antibiotics by following methods:
7-1- Antibiotic susceptibility using disc diffusion method [15]:

5mm diameter standard discs contain certain concentrations of many antibiotics (Bioanalyse),were as follows: amikacin (30µg), ampicillin (10 µg), cephalexin (30µg), cephalothin (30µg), doxycycline (30 µg), cefadroxil (30µg), ciprofloxacin (5µg), clindamycin (2µg), chloramphenicol (30 µg), erythromycin (15µg), gentamicin (10µg), norfloxacin (10µg), oxytetracycline (30 µg), pefloxacin (5 µg), oxacillin (1µg), enrofloxacin (5 µg), tetracycline (30 µg) and amoxicillin (25µg). the resistant breakpoints were those defined by the national committee for Clinical Laboratory Standards (NCCLS, 2000) for Gram-negative bacteria [16].

The sterile antibiotic disks were fixed in an empty sterile petri dish by drops of agar, then meat and liver medium (VF) (the third part of culture) were poured over. The dish was cover upside down. After the anaerobic incubation in the incubator for 18 hours, the plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zones of inhibition was measured and the antibacterial activity expressed in terms of the average diameter of the zone inhibition in millimeters.

Antibiotic susceptibility using concentration breakpoint test for antibiotics[17]:

These steps were follow to conduct this test:
Preparation of antibiotic solutions:
Co-amoxiclav: a concentration of 100 mg /L was attended (Glaxo Smith Kline company). Gentamicin solution: a solution of a concentration of 10 mg/L was used (Hospiracompany). Cefazolin solution: a concentration of 100 mg/L was attended (Glaxo Smith Kline company). Ciprofoloxacin : a concentration of 2 mg/L was attended (Ortin Laboratories). Clindamicin solution: a concentration of 50 mg /L was attended (BioMerieux, Pariscompany). Chloramphnicol solution: a concentration of 100 mg/L was attended (BioMerieux, ParisSmith Kline company).

Erythromycin solution: a concentration of 150 mg/L was attended (BioMerieux, Paris Smith Kline company). Then to get the desired concentration of the antibiotic amount was calculated to add to the meat and liver VF medium. For Co-amoxiclav and ciprofoloxacin: the supreme value concentration required is Cmax = 32 mcg/ml and the minimum value concentration required is 1/4 Cmax = 8 mcg/mL. For clindamaicin and Erythromycin: the supreme value concentration required is Cmax = 256 mcg/ml and the minimum value concentration required is 1/4 Cmax = 64 mcg/mL. For chloramphnicol: the supreme value concentration required is Cmax = 64 mcg/ml and the minimum value concentration required is 1/4 Cmax = 16 mcg/mL.

Plant extracts study:

Clostridium perfringens type (D) according to [3, 15]was selected for testing sensitivity to plant extracts by the following methods:

a bacterial growth inhibition test of plant extracts by the disk diffusion method:

Sterile filter paper discs (5 mm) were soaked with 5mcl of the diluted extracts (66 mg/ml) of pericarp, leaves, flowers ,seeds inethanol,water ,and petroleum ether, so that each disc was impregnated with 0.33 mg / tablet.Control disks also prepared with absolute ethanol, Water, and petroleum ether. The Disks were placed inPetri dishes containingMuellerHinton agarandincubatedfor 16 hours at 37 ˚C.After incubation, all dishes were observed for zones of growth inhibition, and the diameter of these zones were measured in millimeters with a ruler. Results were expressed as the percentage of inhibition of bacterial growth, determined by comparing it with control disks, and standard susceptibility disks [15]. Aftercompleting thework the petri dishes were eliminated through theautoclave.

Antibiotic susceptibility using concentration breakpoint test for plants[17] :

The dried extracts was re-suspended in water as follows:

Aqueous extract: an aqueous solution was attended at a concentration 60 g /L, as well as the negative control. Alcoholic extract: Alcoholic solution was attended with the addition of dimethylsulfoxide (6%) at a concentration of 66 mg / ml, and the negative control. Ether extract: Ether solution was attended with the addition of dimethylsulfoxide (9%) at a concentration of 66 mg/ml, and the negative control. To get the desired concentration of the plant extract amount was calculated to add to the meat and liver VF medium: 0.33 mg/ml for the peel, seeds, leaves, and flowers as a minimum concentration and 1.32 mg/ml as a supreme value concentration were used.

Statistical Study: We have a statistical study of the results we have obtained, by calculating the arithmetic mean μ and standard deviation σ .

RESULTS :

Identification of the bacteria:

Microscopic examination:

Large Gram- positive, straight parallel rods, anaerobic, spore-forming rod, were observed under microscopes after Gram-staining, compatible with reference[1, 15].

Culture on blood agar added 5% sheep's blood:

The isolated *C. perfringens*produced hemolytic colony on blood agar,β- haemolytic colonies with double zone of haemolysis was observed, compatible with reference[1, 15].

Culture on selective medium SPS Agar:

This organism produced black colonies due to the sulfur dioxide formation, compatible with reference[18].
1-4-Biochemical tests: all the results are tabulated (Table1), These results complied with[13, 14].

Different biochemical tests	**Result**
Indol	-
VogesProskaure	-
Methyl Red	+

Table 1: Biochemical tests
According to the previous tests the percentageof samples determined to be positive for *C. perfringens*was 170(25.36%)out of the total number of samples.

ELISA test:

According to the manufacturer's instructions (EuroClone) of this test, *Clostridium perfringens* type was identified. Based on the ELISA results toxin types α and ε toxins were found to be positive, compatible with reference[19].

Toxin	Major toxin produced
Alpha	+
Beta	-
Epsilon	++
Iota	-

Table 2. Major toxins produced by Clostridium perfringens
Samples were examined for all toxins; accordingly,68 of the 668 samples were positive for *Clostridium perfringens*type(D)toxins.

Total number of samples	668
Number of samples has *C.perfringens*	170
the percentage of samples has *C. perfringens*	25.45%
Number of samples has *C.perfringens type(D)*	68
the percentage of samples has *C. perfringens type(D) of the total number of samples*	10.18%

Table 3. Show the samples has *Clostridium perfringens*

Antimicrobial sensitivity tests:
Antibiotic susceptibility using disc diffusion method [15]:
Growth of *Clostridium perfringens* type (D) was observed, in spite of various concentrationsagainst all antibiotics studied.*Clostridium perfringens* type (D) was resistant to the studied antibiotics, where all the diameter zones of inhibition were lower than the required values of eachantibiotics,based on the criteria of NCCLS2000[16,] and to the standard's leaflet of antibiotic discs from the manufacturer, except Clindamycin.

Antibiotic	**diameters zones of inhibition (mm)± Standard Deviation**	**Antimicrobial susceptibility results**	**Percentage of** Resistant**bacteria %**
Oxytetracycline(T)	7,6±0,8	Resistant	97.8
Amoxicillin(AX)	8,5±1,2	Resistant	98
Oxacillin(OX)	8,5±0,9	Resistant	97.5
Cefadroxil(CER)	7±1,1	Resistant	99.4
Pefloxacin(PEF)	8.25±0.8	Resistant	96.5
Amikacin(AK)	9.4±0.95	Resistant	97.5
Tetracyclin(TE)	6.4±1.8	Resistant	94.7
Ciprofloxacin(CIP)	8.5±1.3	Resistant	98.7
Norfloxacin(NOR)	6,5±0.7	Resistant	96.56
Gentamycin(CN)	7.2±0.4	Resistant	95.67
Chloramphenicol(C)	7.8±0.78	Resistant	93.89
Enrofloxacin(ENR)	8±0.99	Resistant	89.78
Doxycyclin(DO)	8.5±1.09	Resistant	93.98
Cephalexin(CL)	7.5±0.67	Resistant	98.76
Cephalotin(KF)	6.6±0.65	Resistant	88.98
Clindamycin(DA)	**10.47±0.45**	**Intermediate**	**98.76**
Ampicillin(AM)	7.8±0.87	Resistant	93.87
Erythromycin(E)	7.5±1.06	Resistant	98.76

Table (4): diameters of bacterial growth inhibition zones using antibiotics

The results of Antibacterial Efficacy of plant extracts*using disc diffusion method*:

As shown in Table 2, the ethanol extracts from different parts of the plant studied (pericarp, leaves, flowers, seeds) showed antibacterial activity against *Clostridium perfringens* type (D), with the diameters of inhibition zone ranging between 11 and 16 mm. Of the parts studied, the most active extracts were those obtained from the Pericarp of *punicagranatum*(98,76%).

The organic solvent petroleum Ether, and water extract from all parts of the plants were not active against *Clostridium perfringens* type (D) (diameters of zone of inhibition were zero).

Punica granatum	**Inhibitionzone diameter(mm)ofplantextractsconcentrationof5mcl of the diluted extracts 66mg/ml (0.33 mg/tablet)** **(mean ± standard deviation)**	**Percentage of sensitive bacteria %**
Control/5 µl	0	100
Pericarp	15.56± 0.45	98.76
Leaves	12.09±0.88	98
Flowers	11.47±0.57	96.8
Seeds	10.59±0.61	97.58

Table 5: Antibacterial activity of different extracts of studied plants against *Clostridium perfringens* type (D)

Antibiotic susceptibility using concentration breakpoint test:

At the minimum andmaximum concentrationsfor all studied antibiotics,growth of bacteria *Clostridium perfringens* was discovered, except with clindamycin which hadbacterial growth just at minimum concentration.

Antibacterial Efficacy of plant extracts using concentration breakpoint test:

Growth of *Clostridium perfringens* type (D) was observed, in spite of various concentrations, against water, and petroleum Ether from all parts of the plants; pericarp, and leaves ethanol extracts, were not active (diameters of zone of inhibition were zero). However, at higher concentration offlowers and seedsin ethanol extracts, no growth of *Clostridium perfringens*type (D) was observed, while at minimum concentration bacterial growth was discovered.

The results of the statistical analysis:

The results were shown in Tables (6-10).

classes	x_i	f_i	$f_i . x_i$	$(« - x_i)^2$	$f_i (« - x_i)^2$
- 9	9.5	4	38	$(0.97)^2$	3.67
- 10	10.5	27	238.5	$(-0.03)^2$	0.02
11-12	11.5	3	34.5	$(- 1.03)^2$	3.18
Total		34	356		6.96
Mean $« = 10.47$			Stander Division $± = 0.45$		

Table 6: Clindamycin *susceptibility results against Clostridium perfringens type(D)*

classes	x_i	f_i	$f_i . x_i$	$(« - x_i)^2$	$f_i (« - x_i)^2$
- 14	14.5	4	58	$(1.06)^2$	4.49
- 15	15.5	24	372	$(0.06)^2$	0.09
16-17	16.5	6	99	$(-0.94)^2$	5.30
Total		34	529		9.88
Mean $« = 15.56$			Stander Division $± = 0.54$		

Table 7: Antibacterial activity of pericarp ethanol extract against *Clostridium perfringens type(D)*

classes	x_i	f_i	$f_i . x_i$	$(« - x_i)^2$	$f_i (« - x_i)^2$
- 10	10.5	4	42	$(1.59)^2$	10.11
- 11	11.5	11	126.5	$(0.59)^2$	3.83
- 12	12.5	14	175	$(-0.41)^2$	2.35
13- 14	13.5	5	67.5	$(- 1.41)^2$	9.94
Total		34	411		26.23
Mean $« = 12.09$			Stander Division $± = 0.88$		

Table 8: Antibacterial activity of leaves ethanol extract against *Clostridium perfringens type(D)*

Classes	x_i	f_i	$f_i \cdot x_i$	(« − x_i)^2	f_i (« − x_i)^2
- 10	10.5	6	63	$(0.97)^2$	5.65
- 11	11.5	23	264.5	$(-0.03)^2$	0.02
12-13	12.5	5	62.5	$(-1.03)^2$	5.30
Total		34	390		10.97
Mean « = 11.47			Stander Division ± = 0.57		

Table 9: Antibacterial activity of flowers ethanol extract against *Clostridium perfringens type(D)*

classes	x_i	f_i	$f_i \cdot x_i$	(« − x_i)^2	f_i (« − x_i)^2
- 9	9.5	5	47.	$(1.09)^2$	5.94
- 10	10.5	21	220.5	$(0.09)^2$	0.17
11 -12	11.5	8	92	$(-1.09)^2$	6.62
Total		34	360		12.73
Mean « = 10.59			Stander Division ± = 0.61		

Table 10:Antibacterial activity of seeds ethanol extract against *Clostridium perfringens type(D)*

DISCUSSION:

The bacterial toxins are a major cause of diseases since they are accountable for the majority of symptoms and lesions during infection. The clarification of the cellular mechanism of bacterial toxins remains a complex problem, but they appear to share a common mechanism of action such as (i) binding to specific receptors on the plasma membranes of the sensitive cells, (ii) preformation,(iii) internalization or translocation across the membrane barrier and (iv) direct secretion[1].
The present study showed that the incidence ratio of *Clostridium perfringens* was 170(25.45%),and *Clostridium perfringens* type(D) was 10.18%out ofintestinal and liver samples of death goats, nearly to the infection rates 16% in Miah's study [13]. While Hadimli's study revealed that *C. perfringens*was isolated from 8.66% out of 150 intestinal samples of lambs, and according to the ELISA results, 28.125% were *C. perfringens*type D[20].

Regarding the efficiency of the antibiotics, *Clostridium perfringens* type (D) was resistant to 18 tested antibiotics. Clindamycin was the only agent to which no resistance was observed, it was intermediate susceptible with inhibition zone diameter 9-12mm. As well as, bacteria's growth at minimum and maximum concentration of antibiotics by using concentration breakpoint test, except clindamycin has growth at 64 mcg/ml,and no growth at 256 mcg/ml.

Clostridium perfringens strains were resistant to tetracycline, erythromycin, clindamycin, lincomycin, kanamycin, and streptomycin, according to rood's study[9], and chloramphenicol, gentamicin, amoxycillin, enrofloxacin, azithromycin and neomycin in Rahaman's study[14], which confirms our study.But in contrast, which we haven't observed in our study,Alexander's study exhibited that all antimicrobial agents tested including penicillin G, metronidazole, clindamycin, cefoxitin, cefotetan, imipenem, meropenem, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin,tazobactam, and vancomycinwere susceptible to *C. perfringens* strains[21], and to ciprofloxacin, levofloxacin and penicillin[14].Stevens revealed that in gangrene caused by *Clostridium perfringens*, clindamycin, metronidazole, rifampin, and tetracycline were all more effective than penicillin[22].

Many herb and spice extracts contained high levels of phenolic and exhibit antibacterial activity against bacteria. Gram-positive bacteria are generally more sensitive to the tested extracts than Gram-negative ones. Phenolic compounds can denature enzymes but they can also bind to substrates such as minerals, vitamins and carbohydrates making them unobtainable for microorganisms. Furthermore, phenols can be absorbed to the cell wall, resulting in a disruption of the membrane structure and function.
The results showed that *Punicagranatum* possesses strong antibacterial activity directed against a Gram--positive anaerobic bacterium *Clostridium perfringens type (D), and* this is reported for the first time. Previous studies[5] showed that the methanol and water extracts of the *Punicagranatum*leaf, peel have the antibacterial activity, but our study showed an opposite result for water extract. Both aqueous and ether petroleum extracts from different parts of the plant studied (pericarp, leaves, flowers,seeds) did not have antibacterial effect, inhibition zone diameter was zero, and no growth of bacteria at minimum and maximum concentration. While ethanol extracts produced disparate zones of inhibition against resistant *Clostridium perfringenstype(D)*, with the inhibition zone diameter ranging between 11 to 16 mm. Also, in the method of studying susceptible test by using concentration breakpoint test, no growth of bacteria at minimum and maximum concentration of suspended *pomegranate*peel, as well as of suspended *pomegranate* leaves. However, for suspended flowers and seed there was no growth at maximum concentration, in contrast of minimum concentration.
In the present study, the ethanol extracts of the seeds and flowers exhibited modest effect twhen compared with the influence of alcoholic extracts of pericarp and leaves. Where the greatest zone of inhibition induced by the action of *pomegranate* peel extracts was 16mm, and the smallest zone of inhibition 11mm was induced by seeds. This result was in agreement with the findings of Iranian's study in *pomegranate* peel extract13 mm[23].

In comparing the ethanol extracts with antibiotic susceptible, the activity of seeds alcoholic extract was similar to clindamycin.Although the global studies pointed out the impacts of flowers, in decreasing the blood glucose[24], and reducing the cholesterol or triglycerides[25]; but it did not show the antibacterial effect, while our study proved it in the alcoholic extract which had produced 11mm diameter zones of inhibition.

Pomegranate fruit peel composites tannins, piperidine alkaloids and pelletierin triggers like strychnine, a raised stimulant reflex, which can escalate to tetanus and is effective against diverse tapeworms, ring worms and nematodes. The tannins in the drug make it useful as an astringent for sore throats, diarrhea and dysentery, but the drug which contains tannins and alkaloids, is anthelminticand amoeboid[26]. Tannins present in many plants including *pomegranate* (*Punicagranatum L.*) fruit pericarp (peels). They are water-soluble polyphenolic polymers, and have capacity to form complexes mainly with proteins, and carbohydrates due to the presence of a large number of phenolic hydroxyl groups[27].The peels also have multiplicity of phytochemical compounds, e.g., gallotannins, ellagic acid, gallagica cid, punicalins, punicalagins[28].

This fruit is found to be a rich source of polyphenolic compounds. The antibacterial effect may be due to phenoliccompounds which could increase due to the presence of organic acids; or to the presence of some secondary metabolites[29].

CONCLUSION: The ethanol extracts of the Punica Granatum revealed antibacterial activity against Clostridium perfringenstype(D) and further studies are required to understand the compounds responsible for this mechanism of action.

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