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In-vitro Antibacterial Activity of Algerian Pomegranate (*Punica granatum linn*) Peels on Some Antibiotic Resistant Gram-Negative and Positive Bacterial Strains

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Abstract: The infections treatment failures and their costs were mainly caused by resistant bacteria required to find alternative care, recently, natural products have proved to be an alternative to synthetic chemical substances, for that the present study was initiated to evaluate the antibacterial activity of aqueous decocted and methanolic pomegranate peels extract against two pathogenic multidrug resistant Gram-negative and positive bacteria (*Salmonella typhi, Enterobacter cloacae Staphylococcus aureus ATCC 43300, Bacillus subtilis)* by disc diffusion method; the aqueous decocted extract showed strong antibacterial activity against *Staphylococcus aureus ATCC 43300* and *Bacillus subtilis* compared to methanolic extract, otherwise the inhibition zones produced at 50 mg/ml were respectively 28±0 and 24±0 mm for aqueous decocted extract and 26.76±1.36, 22±0mm for methanolic extract ; for Gram-negative bacteria,it has been observed a decrease in the activity of the same extracts compared to Gram positive bacteria, the diameter of inhibition zone at 50 mg/ml of *Enterobacter cloacae* and *Salmonella typhi* tested with methanolic extract was respectively 14±0 and 9.76±1.36 mm, while the inhibition zone produced tested with aqueous decocted extract were respectively 20.5±0.5 and 10.5±0.5mm.

Key words: Antibacterial · Methanolic and water extracts · Aqueous decocted extract · Pomegranate · *Bacillus subtilis* · *Salmonella typhi*

INTRODUCTION

Punica granatum L. (Punicaceae) is a native shrub of occidental Asia and Mediterranean Europe, popularly referred to in English as pomegranate [1]. For centuries the bark, leaves, fiowers and fruit of this plant have been used to ameliorate diseases ranging from conjunctivitis to hematuria [2], where its several parts have been used as an astringent, haemostatic, as a remedy for diabetes, as an anthelmintic specifically against tapeworms and for diarrhoea and dysentery [3].

The antimicrobial activity of *Punica granatum Linn* has been widely investigated [4-5].The findings of several studies suggest that the phytotherapeutic use of this plant might be a viable option in controlling different microbial species. The largest components of the fruit extract *Punica granatum L.* are tannin and polyphenolics [6].The tannin-rich peels are byproducts of food industry and are only used in animal feeds in many developed countries such as the U.S.A. In fact, the antimicrobial activity of peels has been demonstrated against pathogenic bacteria [7-14]. For this, our study aims to

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evaluate the *in -vitro* antibacterial activity of the methanolic and aqueous decocted extract of *Punica granatum* peels using various pathogenic bacterial strains.

MATERIALS AND METHODS

Our plant material was consisted of pomegranate peels (*Punica granatum Linn*.) ;to get this fruit part, pomegranate was collected during November 2008, in Sidi Ali Benyoub (25 km south of Sidi-Bel-Abbés town: 450 km west of Algiers); fruits and leaves have been authenticated, a voucher specimen of the plant was sent to plant taxonomy laboratory (Department of Environment, Faculty of Natural Sciences and Life of the university Djillali Liabes Sidi-Bel-Abbes Algeria) for the identification and the confirmation, the samples were removed and coded under No. PG P 2008. The fruit was washed with water; then, the peels were dried in the shade for 01 month on a wooden plate, away from light and at room temperature. After drying, the peels were crushed in a mortar, then sprayed with traditional manual grinder until obtaining a powder, which was stored in a refrigerator at +4°C in hermetically closed tubes; this powder will be subsequently used in the preparation of the two studied extracts [15].

Maceration of *Punica Granatum* Peels in Methanol: 53.2 g of Peel powder were mixed in 250 ml of pure methanol extra 99% supplied by Acros Organics, the mixture was placed under magnetic stirring (1250 rpm stirrer Heildoph by Type MR 3001) for 48 hours at room temperature with protection from light, magnetic stirring is favored by using a magnet rod, after 48 hours a settling of the sample was performed, the supernatant was filtered under vacuum by Laurent Prat-Dumas filter No. 02 and the marc separated was subjected to a second extraction,for that 80 ml of pure methanol at 99% was mixed with the marc humidified under the same conditions, several extractions were performed to obtain in the end a lower yields compared with previous extractions, the collected supernatants were concentrated by rotary evaporator (Buchi rotavapor R125 coupled with vacuum pump controller Buchi V 700-855), at a pressure of 270 mbar, a rotation of 95 to 100 rpm, a temperature of the bath container of 41°C and with a vapor temperature of 36 °C to 39°C, the obtained extract was labeled (MEPP) [15].

Aqueous Decoction of *Punica Granatum* Peels (Hot- Water Extract): The peels powder (40g) was put into 100 ml of distilled water and boiled under reflux for 6 hours at a temperature of 200°C (Fig. 1).

Fig. 1: Aqueous decoction under reflux of pomegranate peels

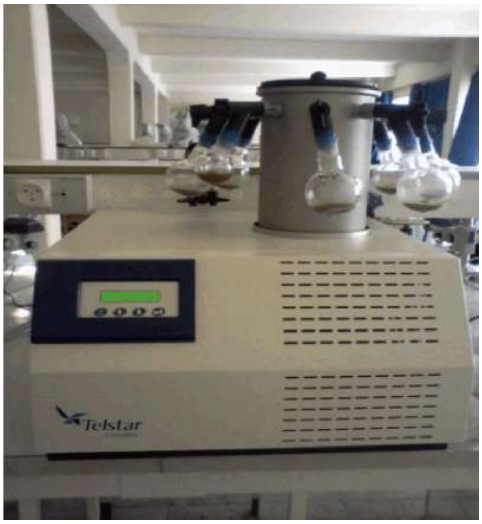

Fig. 2: Lyophilizer used in our work

After stirring, the cooled decoction was filtered under reduced pressure through Whatman paper (No. 3), the separate marc was subjected to a second extraction using 50 ml of distilled water mixed with 90 ml of marc moistened under the same experimental conditions, many extractions were performed to obtain a lower yields compared with previous extractions, the collected supernatants were concentrated by a Tel Star lyophilizer at a temperature of -45°C and pressure of 4.10^{-2} mbar, the obtained lyophilized extract was marked DAPP (Fig. 2).

The extracts yields (per g) relative to the plant mass treated were calculated using the formula: Y = m.100 / m °, Y: Yield of the crude extract as a percentage (%), m : the mass of the crude extract obtained after extraction (g), m ° :the plant mass (g) [16],the extracts obtained were kept and stored in tubes covered with aluminum paper in a refrigerator at +4 °C and protected from light until use for bioassay. The lyophilzed extracts were dissolved in 10% of aqueous DMSO, knowing that this concentration is not toxic to bacteria [17]; a final concentration of 50 mg / ml was filtered through a millipore filter sterile 0.45 µm of diameter. The concentrations range of the plant extract was prepared in test tubes by a dilution method in a geometric progression of 2, from 50 mg / ml to 0.195 mg / ml [18].

Table 1 : Various antibiotics used in this study

Abbreviation	Antibiotic name	Concentrations	Gram type focused
AK 30	Amikacin	30 µg	Gram positive & negative
AML	Amoxicillin	25 µg	Gram positive & negative
AMP 10	Ampicillin	10 µg	Gram negative
CIP 5	Ciprofloxacine	5 µg	Gram negative
DO	Doxycyclin	30 µg	Gram positive & negative
AF	Fusidic Acid	10 µg	Gram positive
GEN	Gentamicin	10 µg	Gram negative
P	Penicillin	6 µg	Gram positive
PT	Pristinamycin	15 µg	Gram positive
RA 30	Rifampicin	30 µg	Gram positive
SP 100	Spiramycin	100 µg	Gram positive
SXT	Sulfamethoxazole	25 µg	Gram negative
VA 30	Vancomycin	30 µg	Gram positive & negative
OX 1	Oxacillin	1 µg	Gram positive
E	Erythromycin	15 µg	Gram positive
L	Lincomycin	15 µg	Gram positive

Used Microorganisms: *Enterobacter cloacae* was obtained from the medical analysis laboratory of Sidi-Bel-Abbés University Hospital Center, *Bacillus subtilis* from Medical Analysis Laboratory of Tiaret University Hospital Center (Algeria), *Salmonella typhi* from Sidi-Bel-Abbés Hygiene Laboratory and *Staphylococcus aureus ATCC 43300* from Laboratory for Research on Bioactive Product and Valorization of Biomass (Kouba High Normal School in Algiers).

Preparation of Bacterial Strains Inoculum: The bacterial strains were inoculated by streaking in nutrient agar (NA) and incubated at 37 ° C for 24 h. From this culture, we prepared a suspension in 0.9%NaCl saline solution, the bacterial suspension was well homogenized and its opacity should be aproximatively equivalent to 0.5 McFarland (10^6 CFU/ ml colony -forming unit) at a wavelength 625nm [19].

Susceptibility Testing (Agar Diffusion Method: Disc Method): The inoculation was carried out by using 5 ml of tested bacterial solutions on Petri plates containing 10 ml of Muller Hinton agar with a thickness of 4 mm, the plates were left open 10 min for drying [20-21]. Sterile paper discs Whatman No. 1 of 5 mm of diameter were impregnated with 10 µl (500 mcg / disc) of each extract [22] at different concentrations from the stock solution prepared in DMSO (10%) [23], then deposited on the agar surface [11], the discs impregnated with DMSO (10 µl) used as negative control were also deposited on the inoculated agar surface, each test was repeated 3 times [20].The bacterial strains were incubated at 37 ° C for 24 h [24],the biological activity was manifested by the appearance of a clear inhibition zone of the bacterial growth around disc containing the tested extract, the reading is done by measuring the inhibition diameter observed, which was expressed in mm [25-26].

The susceptibility of bacterial strains tested (Antibiotic susceptibility testing): Susceptibility testing was performed following the same steps as described above (method of agar diffusion); the antibiotics used were provided by Medical Analysis Laboratory of Sidi-Bel-Abbés University Hospital Center (presented in Table 1), the test is repeated 3 times and serves as a positive test.

Statistical Analysis: The statistical analysis was performed by using the statistical program. Statview 5.0 SAS Institute. We compared the mean by the use of Student test followed by the covariance calculate. The test is considered significant for values of $p < 0.05$. The mean diameter of 3 inhibition tests for each extract concentration and antibiotic was calculated from all samples of each strain.

Determination of Antibacterial Parameters by Macro-Dilution Techniques: The plant extract concentration range was prepared in test tubes by the dilution method [27] according to a geometric progression of 2 [28]. Basing on the results obtained after susceptibility t esting and to clarify the MICs values, a dilution series of each extract was made in sterile distilled water for all extracts tested against all strains studied.

Microbial Strains Inoculation: In a series of test tubes numbered Tt1, Tt2, Tt3..... ect, we introduced 1 ml of nutrient broth inoculated with the tested bacterial

strains,then we added in these tubes 1 ml of plant extract according to well-known range of prepared concentration; in the last tube, the extract was substituted by 1 ml of sterile distilled water which was considered as a growth control, the first tubes are called test tubes (Tt) and the last tube is noted: growth control (Tc), these inoculated tubes are incubated at 37°C for 24 h, this step was performed after reading the values of their initial turbidity with a spectrophotometer UV -visible. After incubation the germ counting was made directly in the liquid medium (nutrient broth) by measuring the turbidity, the enumeration was performed by the difference between the OD (optical density) value found before and after the incubation for each tube [29].

Determination of Minimum Inhibitory Concentration (MIC): The evaluation of the MIC is to determine the lowest concentration of an antimicrobial agent required to inhibit completely the bacterial growth [22-5], it was found by the use of a dilution range of the cultures series of added agent,its determination was performed from the measurement of the turbidity induced by studied microorganisms growth [29], the inhibition is due to a lack of visible culture growth [30], so it is the first tube which di value is equal to df (df = di) [di: the OD value of the test tube before incubation and df: the OD value of the test tube after incubation],the minimum inhibitory concentration was expressed in mg / ml,and the MIC was indicated in the dilution tube in which no bacterial growth was observed, thus no turbidity was observed in the medium [29].

Determination of Minimum Bactericidal Concentration (MBC): Minimum bactericidal concentration (MBC) is the lowest antimicrobial concentration, that lets at maximum 0.01% of surviving germs; for its determination, the control tube was diluted to 10^{-4}, this dilution represented 0.01% of survival; it was subcultured by streak of 5 cm on a Mueller Hinton agar and incubated at 37 ° C for 24 h. The number of bacteria obtained on streak of dilution 10^{-4} was compared to that of each experimental tube also transferred by streak of 5cm, thus, the first test tube in which the number of germs in the streak was less than or equal to 10^{-4} dilution corresponds to the MBC[29].

RESULTS AND DISCUSSION

The extracts obtained have different colors and aspects (Table 2 and Fig. 3) and a plant characteristic odor; the yield was determined from the material plant dry mass of the concentrate in the form of powder or paste; the results were expressed in percentage (%), the different yields depend on the method and the conditions under which the extraction was performed, these results could be explained by the difference in solubility of the components of crude plant extracts which vary according the solvent used and the manner in which they are prepared. Our results show that the yield of aqueous decocted extract was higher than the methanol extract (46.08 ±0.15% and 34.92 ±0.33% respectively) which could be explained by the high water miscibility and polarity; water seems to be the best solvent to extract the majority of chemical constituents from the fruit bark responsible of different biological activities.

These results agree with findings of [24], it can be concluded that the majority of chemical groups present in the fruit peel such as tannins, flavonoids, punicalagins, the saponins, coumarins, mucilage and alkaloids are water extractable [24]; however, the activity of a plant substance depends on several factors, including the used plant part (leaves, stems, roots, peels, whole plants), the extraction method and concentration of active ingredients [31-32]. It is impossible to compare these results with those of the bibliographic literature because these variations are relative to geographical situation, place and time of harvest, climatic conditions, soil type, diseases caused by exogenous agents and the difference of composition and chemical structures contained in the natural product [33].

The extracts antibacterial activity study requires the use of an approved test called aromatogram based on agar diffusion technique of natural substance, the antibacterial activity of the two *Punica granatum* peels extracts, obtained by applying the diffusion method on agar showed that almost the both possess inhibitory activity on all the strains tested, this antibacterial property was confirmed by the presence of inhibition zones around the discs; the inhibition zones diameters measured in different samples were shown respectively in (Table 3, Fig. 4,5).

Table 2: Characteristics of peels Punica granatum extracts obtained

Extract	Texture and color
Peels methanolic extract (MEPP)	Hard paste with very deep brown color
Peels decocted water extract (DAPP)	Powder with very deep brown color

Table 3: Inhibition zones (mm) with various concentrations of two studied extracts of peels pomegranate

		The zones of inhibition produced in (mm) with various concentrations of extracts (mg / ml), the diameters are in mm ± SD					
Strains tested	Extract §	3.125 mg/ml	6 .25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	Covariance
Bacillus subtilis*	MEPP	12.5±0.5(I)	15.5±0.5(I)	19.83±1.04(I)	20±0(I)	22±0(I)	48.67
	DAPP	18±0(I)	20±0.5(I)	21.5±0.5(I)	22±0(I)	24±0(I)	31.19
Staphylococcus*	MEPP	22±0(I)	24.5±0.5(I)	25±0(I)	25.33±1.26(I)	26.76±1.36(S)	22.21
aureus ATCC 43300	DAPP	23.5±0.5(I)	24±0(I)	25.33±1.26(I)	26±0(I)	28±0(S)	26.55
Enterobacter cloacae	MEPP	9.5±0.5(I)	10.83±1.04(I)	11±1(I)	13±1(I)	14±0(I)	25.95
	DAPP	15.5±0.5(I)	16±0(I)	18.83±1.04(I)	19±0(I)	20.5±0.5(I)	28.67
Salmonella typhi*	MEPP	0±0(R)	3±0(I)	6±0(I)	8.76±1.36(I)	9.76±1.36(I)	53.51
	DAPP	0±0(R)	0±0(R)	6.83±1.04(I)	8.66±0.29(I)	10.5±0.5(I)	64.66

±SD (Standard deviation) * $p<0.05$ (significative difference between tested strains),§ $p>0.05$ (without significative difference between tested extracts)

Table 4 : Inhibition zones (mm) with various concentrations of different antibiotics tested

Antibiotics tested	Bacillus subtilis*	Enterobacter cloacae	Salmonella typhi*	Staphylococcus aureus ATCC 43300*
AK 30*	34.33±0.8(S)	9±0(S)	25.33±0.68(S)	25±0(S)
AML	0±0(R)	14±0(R)	22.21±0.5(R)	40±0(R)
AMP10	NT	12±0(S)	5±0(I)	NT
CIP5*	NT	19.33±0.67(R)	30±0(S)	NT
DO	10.33±2.64(I)	2±0(R)	3.5±0.45(R)	26.33±1.2(S)
AF	0±0(R)	NT	NT	40±0(S)
GEN	NT	11±0(R)	30±0(S)	NT
P	0±0(R)	NT	NT	9±0(S)
PT	9.1±0.5(R)	NT	NT	41.66±0 .33(S)
RA30	20.33±1.1(R)	NT	NT	45±0(S)
SP100	10.33±1.3(S)	NT	NT	0±0(R)
SXT*	NT	15.66±2.52(R)	22.21±1.1(S)	NT
VA30	0±0(R)	21 .45±0.8(S)	22 ±0(I)	21.33±0 .5(I)
OX 1*	0±0(R)	NT	NT	0±0(R)
E*	0±0(R)	NT	NT	0±0(R)
L*	0±0(R)	NT	NT	0±0(R)

NT :not tested, ±SD (Standard deviation), * $p<0.05$ (significative difference between strains tested), I : Intermediate, S : Sensitive, R: Resistant

Table 5: MIC (mg/ml) and MBC (mg/ml) of two peels extract of pomegranate

Tested bacteria	Extracts	MIC mg/ml
Bacillus subtilis	MEPP	$1.56 \geq MIC \geq 0.78$
	DAPP	$0.78 \geq MIC \geq 0.39$
Enterobacter cloacae	MEPP	$3.125 \geq MIC \geq 1.56$
	DAPP	$1.56 \geq MIC \geq 0.78$
Salmonella typhi	MEPP	$12.5 \geq MIC \geq 6.25$
	DAPP	$6.25 \geq MIC \geq 3.125$
Staphylococcus aureus	MEPP	$0.39 \geq MIC \geq 0.195$
ATCC 43300	DAPP	$0.39 \geq MIC \geq 0.195$

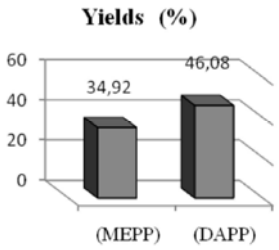

Fig. 3: Representation schedules of the MEPP and DAPP yields

Determination of Antibacterial Activity by the Disc Diffusion Method: The antibacterial effectiveness of DAPP and MEPP against the 4 strains was evaluated via determination of the surrounding inhibition zones. Table 3 shows the mean values of inhibition zones produced varying from 9. 5±0,5to 28±0 mm, which increase in a dose-dependent manner,the DAPP demonstrated the highest inhibitory activity against the 4 resistant strains to the 16 antibiotics tested (Table 4 and Fig. 6) specially gram positive bacteria with 24±0 mm and 28±0 mm respectively for *Bacillus subtilis* and *Staphylococcus aureus ATCC 43300* at 50 mg/ml of extract with no significative difference observed at all concentrations tested of the two extracts ($p>0.05$), on the other side there is a significative difference between strains tested with $p<0.05$. more the concentration of the two extracts is higher more the inhibition zone diameters increase, this is confirmed by covariance calculate, this last one of 4 bacteria is superior than 0,thus it means that there is a positive correlation.Less activity was observed with the gram negative bacteria specially

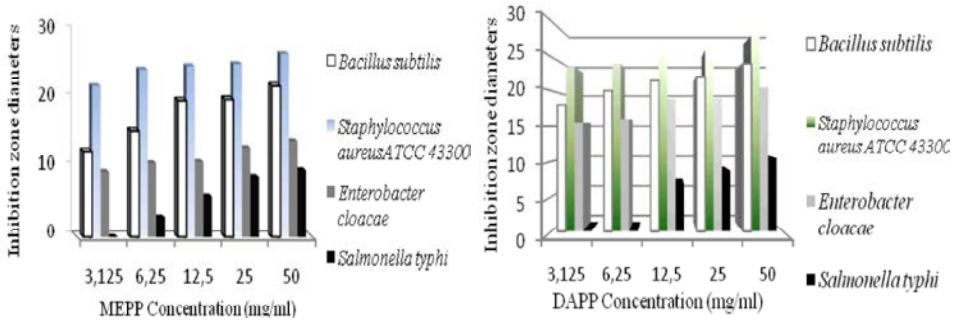

Fig. 4: Representation schedules of inhibition zones of MEPP (right) and DAPP (left) on 4 tested bacterial strains.

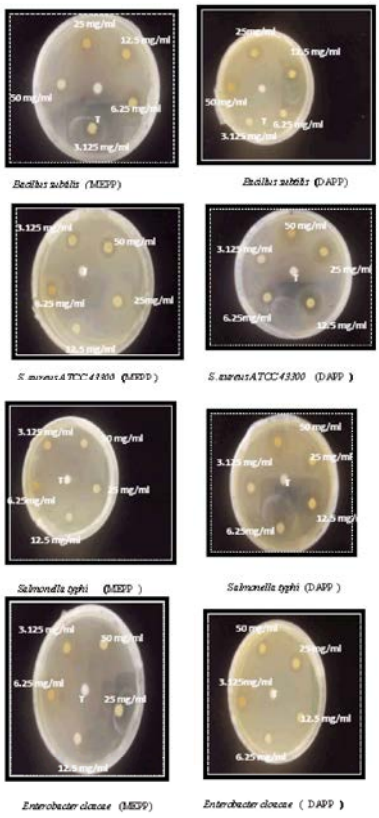

Fig. 5: Inhibition zones obtained with the two extract of peels pomegranate on four bacteria tested

Salmonella typhi which was resistant (0±0 mm) starting from 6.25mg/ml when it was tested with DAPP and from 3.125mg/ml when it was tested with MEPP, this resistance is probably due to their thick murein layer preventing the inhibitors entry [34].

Determination of MICs and MBCs: The MICs of the DAPP and MEPP against the 4 strains tested are shown in (Table 5). The MICs values determined using the broth dilution method confirmed the results

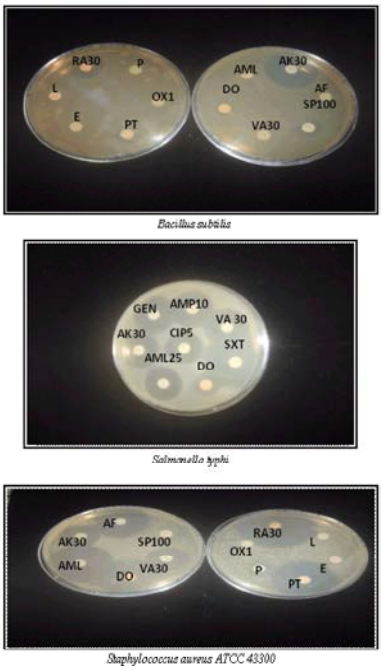

Fig. 6: Diameters (mm) of inhibition zones of some strains tested with different antibiotics

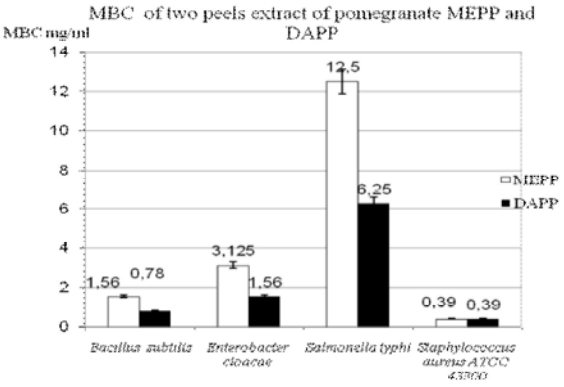

Fig. 7: MBC of two peels extract of pomegranate MEPP and DAPP

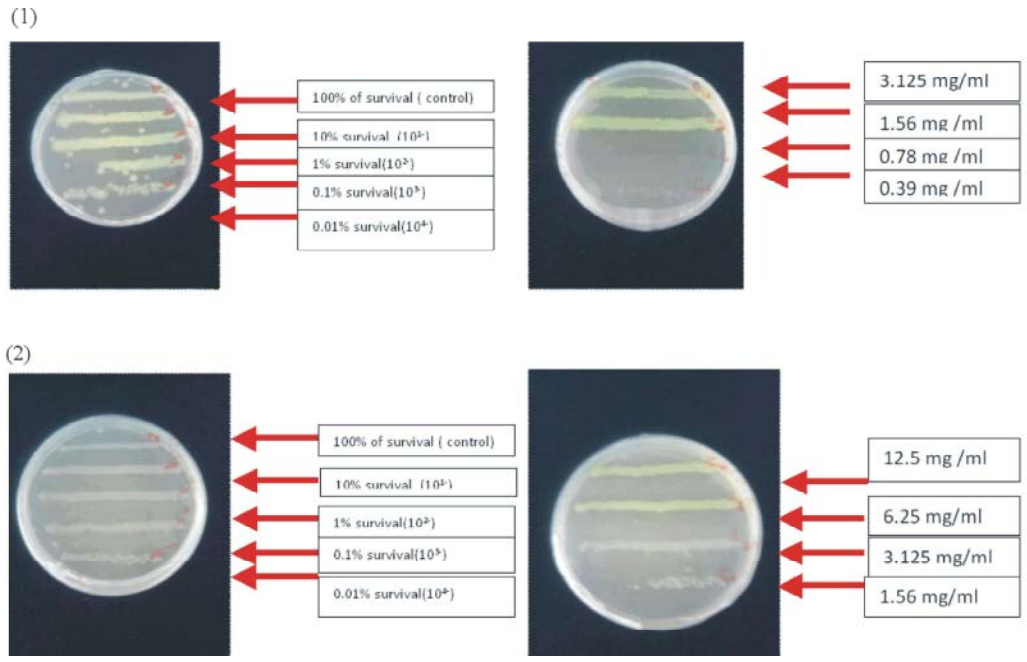

Fig. 8: Determination of minimal bactericidal concentrations of two extracts studied of peels pomegranate (example of S.aureus ATCC 43300 tested with MEPP (1) ,and Bacillus subtilis tested with MEPP (2)

obtained by the disc difiusion method, these values were ranged from 0.39 to 12.5 mg/m1; the DAPP extract tested on both *Bacillus subtilis* and *Staphylococcus aureus ATCC 43300* (Table 6 and Fig. 7,8) showed that it is a very good inhibitor with low MIC and MBC values: (0.78 mg/ml $\geq$ MIC $\geq$ 0.39 mg/ml and MBC $\geq$ 0.78 mg/ml, for *Bacillus subtilis*; 0.39 m g/ml $\geq$ MIC $\geq$ 0.195 mg/ml, MBC $\geq$ 0.39 mg/ml for *Staphylococcus aureus ATCC 43300.*

It was reported that the *P. granatum* peels extracts showed antibacterial activity against *Escherichia coli O157* and methicillin resistant *Staphylococcus aureus* [11 -35]. Our results were confirmed by those obtained by Prashanth *et al*. Holetz *et al.* and Machado *et al*. [8- 36 -9],which have shown that *Punica granatum* is effective at inhibiting Gram-positive bacterial growth, specifically *Staphylococcus aureu*s.

The water and methnaol remain a good extraction solvents of secondary metabolites, furthermore the phenolic compounds solubility is affected by the solvent polarity used which makes it difficult to establish all plant phenolic compounds extracting process. In our study the DAPP was more effective than the MEPP, this results are not correlated with those found by Negi and Jayaprakasha [37], who observed that the higher antibacterial activity are represented by acetone extract with the complete growth inhibition of *Bacillus subtilis* at 150 ppm compared with methanolic and water extract of pomegranate peels ; the effectivness of DAPP on all strains tested supports the traditional use of pomegranate peels according to this form ; these findings are similar to the data presented by El-Mahmood [38], which showed that the antibacterial activity of *Euphorbia hirta* is enhanced at elevated temperatures. The traditional practitioners usually boil the plants before dispensing out to patients.

These differences in the pomegranate peel extracts antibacterial activity through studies could be partially explained by variations in extraction methods, fruits freshness, variations in the season and region of growth, strains sensitivity and antibacterial procedures adopted in tests [26].Our results are also comparables to those obtained by Machado *et al*. [35],who reported that the antibacterial activity for punicalagin (250 µg) by disc afforded a clear inhibition zone of 20 mm for all bacteria tested, in the same work the minimum inhibitory concentration was 61.5 µg /ml. Furthermore this authors show that the ellagitannin (punicalagin) is the substance responsible of the pomegranate antimicrobial activity.

The MIC value of two extracts (DAPP,MEPP) tested on *Staphylococcus aureus ATCC 43300* was less than obtained by Endo *et al*. [39], which found that MIC value of pomegranate crude extracts tested on

Staphylococcus aureus ATCC 25923, was of 125 µg/ml. The minimal inhibitory concentrations for DAPP and MEPP tested on *Salmonella typhi* are respectively between 6.25 mg/ml = MIC =3.125 mg/ml and 12.5 mg/ml = MIC =6.25 mg/ml,our results are similar to those achieved by Klervi [40] which found that the minimal inhibitory concentrations of the Korean peels *Punica granatum* were in the range of 62,5-1000µg/ml. The effectiveness of an extract depends on its concentration, the plant from which it is derived and the strain tested [40 -41-42]; this biological activity of *P. granatum* peels extract could be due to the presence of certain other substances in this two extracts which had an synergistic effect of the bioactive coumpounds and might be related to the action of its antibiotic compounds or to the presence of metabolic toxins. This suggests that these components may also provide antibacterial activity against all strains tested and provide a plausible explanation for the higher antibacterial activity of the DAPP extract. This activity could be due to the presence of a / or more bioactive constituents as the phenols, phenolic acid, tannins, quinones, coumarins and flavonoids [43] ; the ellagitanins, punicalagins, punicalins have been previously shown to possess antibacterial activity and anti-*Candida* activity, they have shown that they inhibit the growth of *Staphylococous aureus*, *Pseudomonas aeruginosa*, *Salmonella, Escherichia coli, Mycobacterium tuberculosis* and *Vibrio* species [11-9-44]. Furthermore, gallic acid, the main monomer in the composition of the hydrolyzable tannins also provides antibacterial and antifungal activity by inhibition of *Corynebacteria, Staphylococci, Streptococci, Bacillus subtilis, Shigella, Salmonella, Vibrio cholerae* and *E. coli* [35].

The diameters of the inhibition zones measured in various antibiotics were illustrated in (Table 4) and were specific to gram positive and gram negative bacteria.

The antibiogram results also show that the diameters measured around the discs can be evaluated by comparing the antimicrobial power of the previews extracts. The results of the susceptibility (antibiotic and extracts) show that according to the diameters measured around the discs, can be classified bacterial strains into three categories: sensitive strains(S), intermediate strains (I) and resistant strains(R), this types are determined by the obtained diameters and by the formula: $D \geq S$, $R \leq d \leq d I \leq D$ (D, d are respectively critical values of higher and lower diameters of the inhibition test) [45] (Table 3 and 4).

The higher diameter measured around the antibiotic disc was represented by Rifampicin (30 µg) tested on *Staphylococcus aureus ATCC 43300* with 45±0 mm followed by Pristinamycin (15 µg) with 41.66±0 .33 mm ; furthermore for Gram negative bacteria like *Salmonella typhi*, the higher diameter was represented by Amikacin (30 µg) with 25.33±0.68 mm,this sensitivity difference was significative between strains and antibiotics tested with $p<0.05$. The findings results are encouraging, because it exist antibiotic-resistant strains but sensitive to our extracts mainly *Bacillus subtilis* which was resistant (0±0mm) to Amoxicillin (25 µg), Lincomycin (15µg), Oxacillin (1 µg), Erythromycin (15 µg), Fusidic Acid (10 µg), Penicillin (6 µg) and Vancomycin (30 µg); and *Salmonella typhi* which was resistant (3.5±0.45mm) to Doxycyclin (30 µg), but intermediate at the concentrations ranged from 50 mg/ml to 6.25 mg/ml of MEPP and from 50 mg/ml to 12.5 mg/ml of DAPP.

CONCLUSION AND PERSPECTIVES

Antibiotic resistance is a public health problem due to the treatments difficulty related to bacterial multidrug resistance and the lack of innovative drugs, the accumulation of resistance genes has been demonstrated in both industrialized and in under-developing countries; for this purpose the pomegranate peels have been selected in this work for its antibacterial effects, we can conclude that all the bacterial strains are sensitive to our extracts. This sensitivity is different depending on the strains (highly sensitive or medium sensitive) and on the extraction solvent, both methanolic and decocted aqueous extracts have an inhibitory effect against *S.aureus ATCC 43300*, *Bacillus subtilis, Enterobacter cloacae* and *Salmonella typhi*, furthermore the DAPP showed that it's the most active against the two Gram positive strains (*S.aureus ATCC 43300* and *Bacillus subtilis*) according to the dose-response relationship, thus decreasing the MIC and MBC values ; these results obtained *in -vitro* are only the first step in the search for biologically active substances from natural sources. Further testing will be required and must be able to confirm these preliminary results. In addition, identification of compounds with more efficient methods studies is needed.

To better evaluate the antibacterial activity, other *in-vitro* and *in-vivo* studies are recommended and it would be desirable to study the toxicological field to make available active populations plant with specific doses.

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