In vitro evaluation of pomegranate (*Punica granatum* L.) peels antibacterial activity against bacteria responsible of diabetic foot infections

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Abstract:
Diabetic foot infections are common complications of diabetes. Antibioresistant bacteria are associated with a higher incidence of lower-limb amputation. *Punica granatum* L. is one of the plants used by many civilisations in the treatment of infectious maladies. Our work consists of evaluating *in vitro* the antibacterial activity of three extracts of pomegranate peels on Gram positive and Gram negative bacterial strains isolated from diabetic foots pus. The active molecules were obtained by maceration of pomegranate peel powder in ethanol/water (30/70) and fractionation using three solvents: dichloromethane (EDCM), ethyl acetate (EEA) and the n-Butanol (En-BuOH). The bacterial susceptibility to the extracts was determined using the disks diffusion test and the Minimal Inhibitory Concentration (MIC) by dilution in liquid medium using sterile microplates. The three extracts were active against the tested strains with maximum activity of the extract (En-BuOH) against *Staphylococcus epidermidis* (35.3 ±0.3 mm). *Enterobacter aerogenes* was less sensitive to the extracts (EDCM) and (EEA) (9±0.9 and 9.6±0.6 mm respectively). The weakest MIC (0.12 mg/ml) was recorded within *Morganilla morganii* with the three fractions and within *Proteus mirabilis* with the fraction ethyl acetate. Furthermore, the highest MIC (> 250 mg/ml) was fond within *Staphylococcus aureus* and *Enterobacter aerugenes* with dichloromethane, *Escherichia coli* and *Pseudomonas aeruginosa* with different fractions. More of that, the extracts were characterised by an antibacterial activity better than some used antibiotics to whom the strains were multiresistant.

Key words: Antibacterial activity, diabetic foot, minimal inhibitory concentration, *Punica granatum* L.

Introduction:

Diabetes has become an increasingly prevalent and severe public health issue in Algeria. The national evidence suggests that the prevalence of diabetes in Algeria has increased from 6.8% in 1990 to 12.29% in 2005 (Lamri et *al*., 2014). Algeria is particularly impacted by the epidemic to the point of appearing in the "top 10" 2016 of countries with very high incidence of T1D (Touhami et *al*., 2019). Foot problems in diabetes are common and costly, and people with diabetes make up about half of all hospital admissions for amputations (Boulton et *al*., 2018). Among patients with diabetes presenting with a foot wound, about half have clinical evidence of infection (Lipsky, 2016). For people with diabetes, DFIs are the most common diabetes-related reason for hospitalizations and for lower extremity amputations (Boulton et *al*., 2018). This is due to the disruption of the protective skin. The deformed foot becomes colonised with infectious bacteria (Fisher et *al*., 2010) . Gram cocci bacteria and specially *Staphylococcus aureus* are the most isolated germs from DFI infections (Lipsky et *al*.,2012; Citron et *al*., 2007; Roberts and Simon, 2012). Antibiotics are one of the mainstays of treating diabetic foot infections. Drug-resistant organisms are over-represented in samples obtained from diabetic foot ulcers (Powlson and Coll , 2010) and are associated with a higher incidence of lower-limb amputation (Richard, 2008)**.** Plants are prospective source of antimicrobial agents in different countries (Alviano DS and Alviano CS, 2009). *Punica granatum* L. (pomegranate) is one of the plants arousing interest for its virtues medicinal. Their Peel have been commonly employed as a crude drug in traditional medicine for the treatment of diarrhea as well as for use as an astringent, antihelminthic, asphrodisacs, laxative, diuretic, stomachic, cardiotonic and refrigerant (Pradeep et *al*., 2008). Therefore, our objective is firstly to confirm *In Vitro* the antibacterial activity of the pomegranate peel extracts (En-BuOH, EDCM and EEA) against multiresistant bacteria isolated from diabetic foot ulcers and secondly to ameliorate the costs of diabetic foot infections treatment by substituting the classical antibiotics by the tested extracts or conjugating the two treatments and this to limit the rate of lower-limb amputation.

Material and methods:

Our work consists of three parts; extraction of active molecules from grenade peel, isolation of bacterial strains from infected diabetic foot and testing the effect of the extracted active molecules on the isolated bacterial strains.

Sampling and macroscopic exams

The method of sampling depends on the infection level, superficial or deep. When samples are quite abundant, macroscopic exams may provide interesting information: a bad smell pus

for the anaerobic bacteria, granular aspect for streptococci and creamy aspect for staphylococci or pneumococci.

Cytologic exam:

It consists of counting the number of polynuclear and neutrophil cells as well as the presence and absence of germs by the methods of Hanging-drop. The methods of simple coloration using methylene blue and Gram coloration determine the form , the size and the regrouping mode of the bacterial cells. They are indicative exams for the choice of the culture media.

Bacteriological exams:

The isolation of bacterial strains was done by culturing pus directly in three media: Chapman, Hektoen and Blood agar. In case of negative culture the previously enriched pus in brain heart infusion broth (BHIB) or in glucose buffered broth medium was used. Enterobacteria were identified by the biochemical miniaturised kits (API 20E). Staphylococci strains were identified according to the presence and absence of the catalase and coagulase enzymes. Two reference strains were also used, *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC29213 .

Preparation of the three extracts :

The pomegranate fruit (*Punica granatum* L.) was obtained from Ramdan Djamel town , the state of Skikda during the month of October 2015. The fruit peel was then isolated and dried at the temperature room, in the darkness and grinded. The obtained powder was used to prepare the different extracts.

Maceration

The method of Merghem et *al*.(1995) was used with modification of the used solvents. The pomegranate peel powder (200g) was added to ethanol/water (30/70) v/v. The mixture was well shacked during 72h with renewal of the mixture each 24h.

Extraction liquid/liquid:

The macerates were reunited and filtered by filter paper. The recovered filtrate was then evaporated using a rotary evaporator. The dry residue was recuperated in 200ml boiling distilled water . The extract was then decanted during a night and the limpid phase underwent fractionation using three solvents with increasing polarity: dichloromethane (EDCM), ethyl acetate (EAA) and the n-butanol (En-BuOH) . The aqueous phase and the solvent were well agitated and left to rest for 30 minutes. The aqueous phase in the bottom of the separating

funnel and the phase charged with active molecules were recovered separately. The active phase charged with polyphenols was dried and the following equation was used for the determination of the extraction yield (Harborne, 1998): Yield= (weight of the extract / weight of the vegetal material) *100.

Determination of antibacterial activity of pomegranate peel extracts

Antibacterial susceptibility to pomegranate peel extracts was tested using agar disks diffusion technique . Stock solutions of the three tested extracts were prepare by diluting each dried extract in dimethyl sulfoxide (DMSO) to obtain a final concentration of 250mg/ml. Muller Hinton medium was aseptically poured into sterile Petri plates. The bacterial inoculums (300Ul) adjusted to 0.5 Mac Farland (10^8 Cells/ml) (CA-SFM, 2012) and diluted to 1/10 (10^7 cells/ml) (CA-SFM, 2010) was swabbed on Muller-Hinton medium. 6 mm filter paper disks loaded into 10ul of the tested extract were placed on the plates at equivalent interval. A pure DMSO loaded disk was added as a negative control. The cultures were left 15 minutes for a pre-diffusion before they were incubated at 37°C for 24 hours. The experiment was realised in triplicate and mean value of zone inhibitions was calculated. The bacterial sensitivity toward the extracts was classified according to Moreira et *al*. (2005): 8≤mm: Not sensitive (-), 9-14 mm: Sensitive (+), 15-19 mm: Very sensitive (++), ≥20 mm: Extremely sensitive(+++)

Evaluation of minimal inhibitory concentration (MIC) in liquid medium:

The minimal inhibitory concentration was determined using sterile microplates (8x12 wells).0.1 ml of nutrient broth were added to the wells of the same line, then 0.1 of the tested extract (250mg/ml) were added to the first well and well homogenized with nutrient broth. 0.1 ml of the mixture were transferred from well to well to obtain dilutions at a factor of 1/2. Finally, 0.1 ml of the inoculums previously diluted to 1/100 (10^6 cells/ml) were added to each well (EUCAST, 2003)**.** Thereby**,** the concentration 250 mg/ml was diluted successively to 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/156, 1/512, 1/2048 and 1/1024 . The plates were incubated at 37°C for 24 hours. The MIC was determined by the concentration where no trouble was observed.

Antibiogram:

Antibacterial susceptibility to the tested antibiotics was determined based on the directives of CA-SFM (2010). A bacterial suspension of 0.5 Mac Farland was previously prepared. Muller Hinton medium poured into Petri plates was seeded by swabbing the swab onto its surface to achieve tight steaks. The antibiotics disks were placed on the surface of the medium and the plates were incubated at 37°C for 16-24 hours (CA-SMF, 2010). The obtained inhibition

zones around the antibiotics were measured and the bacterial strains were classified as sensitive (S) or resistant (R) (Ca-SFM, 2012).

Statistical analysis:

Inhibition zones were done in triplicate sets and the results were expressed in mean value± SD (Steel et *al*., 1995)

Results and discussion:

Bacterial strains:

Eight bacterial strains were isolated from diabetic foots: *Proteus mirabilis, Pseudomonas aeruginosa, Morganella morganii, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Staphylococcus epidermidis and Staphylococcus aureus*. Two reference strains *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC29213 provided by Pasteur institute of Algeria were also tested. Enterobacteria strains were identified using their macroscopic, microscopic and biochemical characters (Table1). *Staphylococcus aureus* was Catalase and Coagulase positive however *Staphylococcus epidermidis* was Catalase positive and Coagulase negative.

Table 1: Biochemical characters of selected Enterobacteria

		P. mirabilis	*P. aeruginosa*	*M. morganii*	*E. aerogenes*	*E. cloacae*	*E. coli*
Biochemical characters	Glucose	+	+	+	+	+	+
	Lactose	-	-	-	+	-	+
	Saccharose	+	-	-	+	+	+
	H_2S	+	-	-	-	-	-
	Gaz	+	-	-	+	+	+
	Simons Citrate	+	+	-	+	+	-
	Mannitol	-	-	-	+	+	+
	Mobilité	+	+	+	+	+	+
	Urease	+	+	+	-	-	-
	Indole	-	-	+	-	-	+
	L .D.C	-	-	-	+	-	+
	O .D.C	+	-	+	+	+	+
	A.D.H	+	+	-	-	+	-

+: Positive , - :Negative

Yield of polyphenols extraction:

Three fractions were obtained , dichloromethane fraction (EDCM), n-butanol fraction (En-BuOH) and ethyl acetate fraction (EEA). The best yield of extraction was obtained with En-BuOH (3.27%) followed by EEA (0.25%) and finally EDCM (0.20%). Sajjad and his

collaborators (2015) showed that the yield of *Punica granatum* L. ethyl acetate extract was 6% where as the ethanolic extract yield was 3%. Our yields were less than that of the methanolic extract tested by Sultana and his collaborators (2008) (16.4%). These variations depend on the phytochemistry of different pomegranate extracts (El-Falleh et *al.*, 2012). The composition of pomegranate peel depends on many environmental factors, processing, cultivar and post harvesting (Houston, 2005)

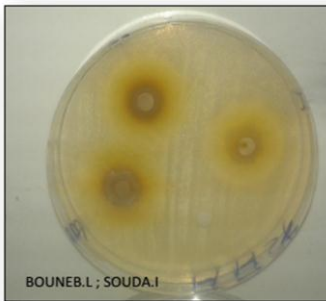
Antibacterial activity of the three extracts:

The obtained results (Table 2 and Figure 1) showed that the three fractions of pomegranate peel were active against the eight strains. This activity indicates the presence of toxic secondary metabolites towards the tested strains (Voravuthikunchai, 2004). Pomegranate is known as a rich source of pharmacological properties which have been evaluated due to antiparasitic, antibacterial, antifungal antiproliferative, apoptotic and anti-cancer effects (Reddy et *al*., 2007; Kim et *al*., 2002; Naz et *al*., 2007). The inhibition zones vary according to the used extract and the tested strain. The comparison of the average of three inhibition zones replicates revealed that the highest antibacterial activity was recorded against *Staphylococcus aureus* and *Staphylococcus epidermidis* as well as *Staphylococcus aureus* ATCC29213 with the three extracts (29.3±0.3-35.3±0.3 mm) and against *Morganella morganii* and *Proteus mirabilis* with En-BuOH and EEA (21±0.6-22.6±0.6 mm). Moderate inhibition zones were observed against *Escherichia coli* ATCC 25922 within the three extracts (14.6±0.3-17±07mm) as well as *Pseudomonas aeuginosa*, *Enterobacter cloacae* and *Morganella morganii* within En-BuOH, EEA and EDCM respectively (14.6±0.2-18.3±0.3mm). *Escherichia coli* and *Enterobacter aerugenes* were less sensitive to EEA and EDCM fractions (9±0.9-12±0.2 mm) as well as *Proteus mirabilis* and *Pseudomonas aeruginosa* with EDCM (11.6±0.6-13.3±0.3 mm). Gram negative bacteria were less sensitive in comparison with Gram positive bacteria. The outer membrane of Gram negative bacteria high in polysaccharides prevents certain antibacterial biomolecules from entering into the cell (Bagamboula et *al*., 2004). Our results corroborate those of Reddy et *al*.(2007) and those of Parimala Celia and Jenifer (2018) who demonstrated that the pomegranate peel extracts constitute a very strong inhibitor of *Staphylococcus aureus* growth (Inhibition zones> 20mm). Hence, the antibacterial activity of *Punica granatum* L. may be related to polyphenol structures (Rathia et *al*., 2014; Barathikannan et *al*., 2016) because polyphenols may affect the bacterial cell wall, inhibit enzymes by oxidized agents, interact with proteins and disturb co-aggregation of microorganisms (Naz et al., 2007; Vasconcelos et *al*., 2003).

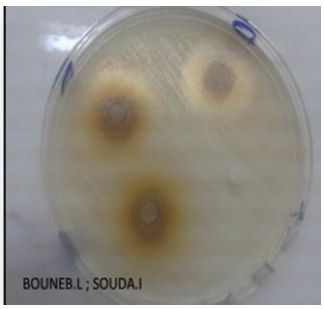
Table 2: Bacterial strains sensitivity towards the three pomegranate peel extracts

Bacterial strains / **Inhibition zones(mm)**	*S.aureus* ATCC29213	*S.aureus*	*Staphylococcus epidermidis*	*E.coli* ATCC25922	*E.coli*	*Enterobacter aerogenes*	*Enterobacter Cloacae*	*Morganella Morganii*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
n- butanol	32.3±0.3	32.6±0.6	35.3±0.3	17±07	11.6±0.6	14.6±0.6	12.3±0.6	21.6±00	17±0.7	21±0.6
Ethyl acetate	31.6±0.2	29.3±0.3	33.6±0.2	15.5±0.2	10±0.2	9.6±0.6	15.6±0.2	22.6±0.6	14.6±0.2	21.3±0.3
Dicloromethane	30.3±0.2	29.6±0.3	33.6±0.2	14.6±0.3	12±0.2	9±0.9	10.3±0.3	18.3±0.3	11±0.7	13.3±0.3

8≤mm: Not sensitive (-), 9-14 mm: Sensitive (+), 15-19 mm: Very sensitive (++), ≥20 mm: Extremely sensitive(+++)



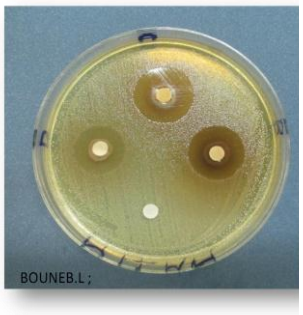
Staphylococcus epidermidis



Staphylococcus aureus

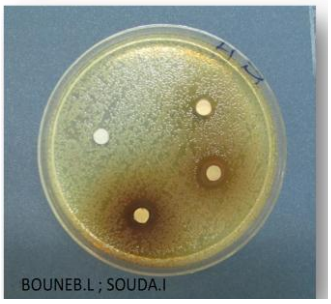
Escherichia coli

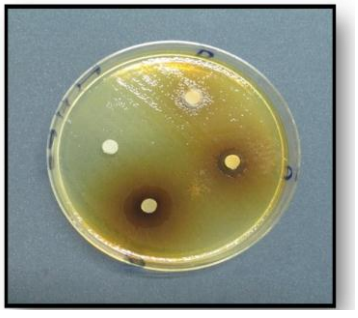
Proteus mirabilis



Morganella morganii

Enterobacter cloacae





Enterobacter aerogenes *Pseudomonas aeruginosa*

Figure 1: Inhibition zones of the three extracts En-BuOH, EAA and EDCM **against the tested bacterial strains.**

Comparison of antibacterial activity of antibiotics and pomegranate peel extracts:

Enterobacteria strains: *Escherichia coli*, *Morganella morganii* and *Enterobacter aerugenes* are multiresistant bacteria (Table 3). They were resistant to three antibiotics families (B-Lactamines, Polymixines and Aminoglygosides). They were on the other hand sensitive to the three tested extracts with inhibition zones varying from 9±0.9-21.6±0.0mm (Table2). Pradeep et *al*. (2008) and Khan and Hanee (2011) demonstrated that the tested *E.coli* was more sensitive to Tetracyclin, Viprofloxacin and Oflaxacin in comparison with the tested extracts. *Proteus mirabilis* and *Escherichia coli* ATCC259 were however sensitive to the tested antibiotics as well as the tested extracts (13.3±0.3-21. 3±0.3).

Pseudomonas aeruginosa which is a multiresistant strain to more than three antibiotics families (B-Lactamines, Quinolones, Aminosides, Tetracyclines and Fosfomycines) (Table4) was very sensitive to the extract En-BuOH (17±0.7mm) and sensitive to EEA (14.6±0.2 mm) and EDCM (11±0.7mm). These values are superior of that obtained by Belaidi (2012) and Pradeep et *al*. (2008) as well as those of Sajjad et *al*. (2015) with ethyl acetate extract. They were on the other hand inferior of that obtained by Khan and Hanee (2011)**.** This is due to the differences in the solubility of active molecules in each used solvent.

The tested Gram positive bacteria, *Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus aureus* ATCC29213 were highly sensitive to the tested extracts (29.3±0.3-35.3±0.3mm) (Table 2). *S.epidermidis* showed however high resistance levels to the tested antibiotics (Fosfomycines, Acides fucidiques, Quinolones, Glycoproteines, Macrolides, Rifamycine and B-Lactamines) (Table 5). The advantage of natural antibiotics (plants extract), is that they don't push the microbes to develop resistance against them. They are composed of many different molecules so that the microbe needs to synthesise many enzymes to be able to neutralise all of these (Candan et *al*., 2003)

Table 3: Antibiogram results of the tested Enterobacteria

Bacterial strains **Antibiotics**	***Morganella morganii***	***Escherichia coli* ATCC25922**	***Escherichia coli***	***Enterobacter coloacae***	***Enterobacter aerogenes***	***Proteus mirabilis***
AM	R	S	R	R	R	S
AMC	S	S	R	R	R	S

AMX	R	S	R	R	/	R
CIP	S	S	R	R	S	S
CRO	S	S	R	R	S	S
CTX	R	S	R	R	S	R
IPM	S	S	S	R	S	S
TIC	S	S	R	R	R	S
CZ	R	S	R	R	R	S
AK	S	S	S	R	/	S
GEN	S	S	R	R	R	S
FOS	S	S	/	R	S	S
CT	R	S	S	R	R	S
TMP	/	/	/	S	/	/
En-BuOH	+++	++	+	+	++	+++
EEA	+++	++	+	++	+	+++
EDCM	++	++	+	+	+	+

S: sensitive, R: resistant (Co-SFM, 2012) ; + : sensitive (9 - 14 mm), ++ : very sensitive (15 -19 mm), +++ : extremely sensitive (>20 mm) (Moreira et *al*., 2005).

Table 4: Antibiogram results of *Pseudomonas aeruginosa*

Antibiotics	***Pseudomonas aeruginosa***
ATM	S
CAZ	S
PIP	R
IMP	S
TIC	R
TCC	R
FA	R
AK	S
GN	R
TOB	R
CIP	R
LVX	R
CT	S
FOS	R
DO	R
En-BuOH	++
EEA	+
EDCM	+

S: sensitive, R: resistant (Co-SFM, 2012); + : sensitive (9 - 14 mm), ++ : very sensitive (15 -19 mm) (Moreira et *al*., 2005).

Table5: Antibiogram results of *Staphylococcus* strains

Antibiotics	*Staphylococcus aureus* ATCC*29213*	*Staphylococcus aureus*	*Staphylococcus epidermidis*
FOX	**S**	**R**	**R**
OX1	**S**	**R**	**R**
P	**R**	**R**	**R**
TET	**S**	**S**	**S**
E	**S**	**R**	**R**
L	**S**	**S**	**S**
VA	**S**	**S**	**S**
SP	**S**	**S**	**S**
TEC	**S**	**S**	**S**
GEN	**S**	**S**	**R**
OFX	**S**	**S**	**R**
C	**S**	**S**	**S**
FA	**S**	**S**	**R**
FOS	**S**	**S**	**R**
En-BuOH	+++	+++	+++
EEA	+++	+++	+++
EDCM	+++	+++	+++

S: sensitive, R: resistant (Co-SFM, 2012); +++ : extremely sensitive (>20 mm) (Moreira et *al*., 2005).

Minimal inhibitory concentration(MIC):

The method of dilution in liquid medium was used to confirm quantitatively the previously obtained results. It was found that En-BuOH inhibited the growth of *Morganilla morganii*, *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC29213 with a low concentration (0.12mg/ml), followed by *Staphylococcus aureus* (7.8 mg/ml) and *Enterobacter colacae* (15.6 mg/ml) (Table 6). It inhibited however the growth of *Staphylococcus epidermidis* with a high concentration (125 mg/ml). The ethyl acetate extract (EAE) was characterised by a weak inhibitory concentration against *Proteus mirabilis* and *Morganilla*

morganii (0.12 mg/ml for each) followed by *Enterobacter cloacae* (0.98 mg/ml) and *Staphylococus epidermidis* (3.9mg/ml). The lowest minimal inhibitory concentration of the Dichloromethan extract (EDCM) was recorded with *Morganilla morganii* (0.12 mg/ml) and *Staphylococcus epidermidis* (3.9mg/ml). High inhibitory concentrations were recorded with *Enterobacter aerogenes* (62.5 mg/ml) , *Escherichia coli, Staphylococcus aureus*, and *Enterobacter aerogenes* (>250 mg/ml). Our results are partially in contradictory with those of Lairini et *al*.(2014) who reported weak inhibitory concentration of his aqueous extract against *Escherichia coli* (0.31 mg/ml). The minimal inhibitory concentration of the three extracts are superior than that of the methanolic extract used by Belaidi (2012). The minimal inhibitory concentration of En-BuOH and EDCM against *Pseudomonas aeruginosa* was less than that recorded by Naziri et *al*.(2012). The inhibition of *Staphyloccus aureus* needs high concentrations in comparison with the aqueous extract of Lairini and his collaborators (2014) as well as of the methanolic extract of Naziri et *al*. (2012). The level of sensitivity of the tested strains to different Pomegranate peel extracts differs according to the type of active compounds in each extract and the type of strain (Ozçelik et *al*., 2011; Su et *al*., 2014). It depends on the capacity of liposoluble molecules to intercalate in the bacterial membranes and to damage them (Candan et *al*., 2003; El amri et *al*., 2014).

Table 6: Minimal inhibitory concentration of the three extracts

Tested strains / **Extracts**	*Proteus mireabilis*	*Pseudomonas aeruginosa*	*Morganilla moraanii*	*Enterobacter aeroaenes*	*Enterobacter cloacae*	*E. coli*	*E. coli* ATCC25922	*S.aureus* ATCC29213	*Staphylococcus aureus*	*Staphylococcus epidermidis*
n-butanol(mg/ml)	15.6	31.25	0.12	62.5	15.6	125	0.12	0.12	7.8	125
Ethyl acetate (mg/ml)	0.12	250	0.12	62.5	0.98	250	15.6	7.8	7.8	3.9
dicloromethane (mg/ml)	15.6	15.6	0.12	>250	62.5	>250	15.6	15.6	250	3.9

Conclusion:

This study is an approach which aims to discover new antibacterial agents made of *Punica granatum* L. peel extracts in order to face the problem of diabetic foot infections and to minimise the rate of infected foots amputations. The three tested extracts showed antibacterial activity against the eight isolated strains. More of that, the extracts were characterised by an antibacterial activity better than some used antibiotics to whom the strains were even multiresistant. The extracts and mainly the n-Butanol extract demonstrated high

activity against Gram positive bacteria in comparison with Gram negative bacteria. Our *in vitro* research confirms the possibility of exploiting pomegranate peels as an alternative or a conjugating treatment of diabetic foot infections, but more experiments are necessary to determine the concentration and the nature of the active molecules in the pomegranate peel. The pomegranate peel is a promising source of diabetic foot infections drugs which may help in limiting lower-limb amputation.

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Z. CHEKROUD supervised the work, wrote the manuscript, discussed the results and corrected the references.

L. Bouneb: carried out the identification of the bacterial strains, the extraction of polyphenols and tested the antibacterial activity of the extracts by the method of diffusion on solid medium.

I. SOUDA carried out the identification of the bacterial strains and determined the minimal inhibitory concentration of the extracts by the method of dilution on solid medium

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COVER LETTER FOR SUBMISSION OF NEW MANUSCRIPTS

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I am enclosing here with a manuscript entitled "**Pomegranate (*Punica granatum* L.*)* peel extracts: a new approach for the treatment of diabetic foot infections**" submitted to "**Malaysian Journal of Pathology**" for possible evaluation.

With the submission of this manuscript I would like to undertake that the above mentioned manuscript is an **original Article.** It has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere. The manuscript has been read and approved by all the authors of this submission. The project was partially financed by the Directorate General for Scientific Research and Technological Development. No potential conflict of interest was reported by the authors. **The obtained results** of this research are unique because the tested extracts were active against all the tested pathogenic strains Gram positive and Gram negative isolated from diabetic foots and showed important inhibition zones. The research gives proposals for new natural drugs which may be used in complementary with antibiotics to overcome the problem of multiresistant bacteria responsible of diabetic foot infections which may help in limiting lower-limb amputation.