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In Vitro Antimicrobial Activity of Phenolic Extracts of the Pomegranate (*Punica granatum*)

Athmen Reguieg Yssaad, Kheira Hammadi*

Laboratory of Pharmacogonosy Api Phytotherapy, University Abdelhamid Ibn Badis of Mostaganem, Algeria

Email address

kyrabiology@yahoo.fr (K. Hammadi)

*Corresponding author

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Abstract

In this study, we evaluated the antimicrobial properties of phenolic extracts of the fruit of *Punica granatum* on microbial strains associated with several human pathologies. The extracts also underwent a phytochemical study. The evaluation of the antimicrobial properties was carried out by the agar diffusion method for the determination of the diameters of the zones of inhibition and by the micro dilution method in liquid medium for the determination of the minimum inhibitory concentrations MIC and MBC. At the end of the antimicrobial tests, we found that this extract has a very high activity with diameters of zones of inhibition varying between 07 and 22 mm. However, regardless of the germ, methanol extract was found to be the most active with Inhibitory Minimum Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of 0.03 and 0.07 mg/ml. According to our results, the antimicrobial activity of the phenolic extracts of *Punica granatum* highlighted in this study may justify the therapeutic use of this plant in traditional medicine in the treatment of a large number of microbial infections.

1. Introduction

Grenadier or *Punica granatum* is a medicinal species. It is a species belonging to the family of *Punicaceae*. It is a bushy tree or shrub 2 to 5 m high, slightly thorny, with deciduous foliage and tortuous trunk. It grows predominantly throughout the Mediterranean region, either spontaneously or cultivated [1]. The fruit of grenadine is rich in phenolic compounds. This composition has attributed to him several properties as well in the medical field as in the agro alimentary field; In fact studies have confirmed that the pomegranate has anti-microbial and anti-carcinogenic antidiabetic properties. Other studies have shown that the pomegranate has antioxidant and antimicrobial properties against the deterioration of food products. This justifies its use as a natural preservative because at present there is a tendency to substitute chemical and synthetic agents having antimicrobial activity by natural agents present in fruits, vegetables and aromatic herbs [2].

To this end, our study encompasses two aspects, the first of which is phytochemical based mainly on the extraction, screening and quantification of phenolic compounds. The second aspect devoted to in vitro evaluation and the antimicrobial activity of these phenolic extracts

2. Material and Methods

2.1. Plant Material

Pomegranate fruits *(Punica granatum)* are harvested at maturity in the month of October (2015), in the Mascara region. The epicarp of these fruits is isolated, dried in the open air and protected from light, and then ground with a mortar until a fine powder is obtained to prepare the fruit. Different extracts.

2.2. Preparation of Phenolic Extracts

10 g of the plant powder of *punica granatum* are macerated for 24 hours at room temperature, in a solvent-water mixture (70:30 V / V) or in 100 ml distilled water, the whole is then filtered on Whatman paper, and the extraction is repeated several times with renewal of the solvent. The solvent is removed from the filtrate by rotary evaporation in a Rota vapor (BÜCHI).

The extraction series makes it possible to obtain three extracts; Aqueous, ethanolic and methanolic extract. The extracts are dried and stored until later use

2.3. Determination of Total Polyphenols

The total polyphenols were assayed using the Folin-Ciocalteu colorimetric reagent according to the method cited by [3].

2.4. Sensitivity of Strains to Antibiotics

The aim of carrying out an antibiogram is to predict the sensitivity of a germ to one or more antibiotics in an essentially therapeutic perspective.

2.5. Antibacterial Activity by Diffusion Technique in Solid Medium

The antibacterial activity of the extracts was determined by the diffusion method in agar medium standardized by (NCLLS) cited by [4]. NCCLS: (National committee for clinical laboratory standards)

2.5.1. Preparation of Concentrations

The extracts were taken up with sterile distilled water. Serial dilutions of 1/2 to 1/8 were then carried out to obtain concentrations of 100 to 12.5 mg/ml.

2.5.2. Insemination

Within 15 minutes following the turbidity adjustment of the inoculum suspension (0.5 Mc Farland), a swab was dipped into the suspension and the entire surface of the agar (Mueller Hinton Agar) was plated three times. The sterile discs impregnated with increasing concentrations of extracts at a rate of 10 µl per disc were deposited sterile using a forceps on the surface of the agar. The dishes were incubated for 24 h at 37°C. in a normal atmosphere. The antibacterial activity was determined by measuring with a rule the diameter of the inhibition zone, determined by the different concentrations of the various extracts around the disc.

2.6. Study of Growth Kinetics and MIC by Micro plate

MICs are determined by the standardized method of micro-dilution in liquid medium. The study is carried out in a micro plate of plastic material comprising 96 then (08 rows and 12 then numbered from 01 to 12) in Muller Hinton broth. With a final density inoculum, the micro plates are incubated from 18 h to 24 h. The dilutions of the sample were cascaded in the wells of the highest concentration (10 mg/ml) to the lowest (0.004 mg/ml), the dilutions of the extracts were carried out in DMSO. The MIC corresponds to the first dilution where growth is negative (no visible culture**)** [5]. The kinetics are evaluated by measuring the optic density of the micro plates at 620 nm at t0 and after 4h, 8h, 4 pm and 24 pm.

2.7. Statistical Study

All tests were performed in duplicate or triplicate. Results are presented as mean ± standard deviation of two or three independent determinations. All statistical analyses were carried out by Graph pad prism 5 using analysis of variance (ANOVA) and differences among the means were determined for significance at $p \leq 0.05$ using least significant.

3. Results and Discussion

3.1. Extraction Yield

The extraction method must allow the complete extraction of the compounds of interest and must avoid their chemical modification. Water, aqueous mixtures of ethanol are generally used for extraction [6].

The solubility of the phenolic compounds depends on their degree of polymerization, the interaction with the other constituents and the type of solvent used. Methanol has been recommended and frequently used for the extraction of phenolic compounds [7]. 70% aqueous methanol is twice as effective as pure methanol for the extraction of phenolic compounds [8]. The extraction efficiency of reading shows that the yield obtained by the methanol extract is important with a value of 28.5%

3.2. Determination of Total Polyphenols

Phenolic compounds such as phenolic acids, flavonoids and tannins are considered major contributors to the antioxidant capacity of plants [9]. These compounds also possess various biological activities such as anti-inflammatory, antibacterial, antiviral, antiallergic, antithrombotic and vasodilating activities which can be linked to their antioxidant activity [10]. This is why the dosages of total polyphenols of *punica granatum.*

According to the results illustrated above, it can be seen that the polyphenol content depends on the solvent polarity.

Our results show that the extracts are rich in polyphenols with total polyphenol values ranging from 194.96 µg EAG / mg to 336.14 µg EAG / mg. Generally, all plants of the family *Punicaceae* are known for their phenolic compounds [11]. This is in accordance with our results.

The extracts are mixtures of several compounds, with different functional groups, polarities and chemical behaviors. This chemical complexity of the extracts could lead to scattered results according to the test used. [12].

3.3. Sensitivity of Strains to Different Antibiotics

The interpretation of the susceptibility test (sensitive, intermediate and resistant) was performed in accordance with the recommendations of the French company.

Table 1. *Diameter of the antibiotic inhibition zone.*

Bacterial strains	Diameter of the inhibition zone*(mm)				
	Gentamycin	Tétracyclin	Colistin	Aztreonam	PénicillinG
Enterobacter agglomerans	12±0,05	14±0,02	19±0,05	21±0,08	16±0,06
Citrobacter amalonaticus	11±0,02	12±0,03	22±0,06	20±0,05	21±0,04
Proteus vulgaris	11±0,02	14±0,06	16±0,06	16±0,03	15±0,02
Lactobacillus	14±0,02	18±0,05	25±0,06	25±0,08	19±0,09
Clostridium sulfito-réducteur	13±0,07	15±0,08	18±0,05	21±0,06	22±0,01

3.4. Antimicrobial Activity of Phenolic Extracts

The antimicrobial activity of the phenolic extracts of *punica granatum* is evaluated on 5 bacterial reference strains. This activity is evaluated by the aromatogram method; the antimicrobial power of the phenolic extracts is obtained by measuring the diameter of the inhibition zone in mm.

The diameter of the inhibition zone differs from one bacterium to another and from one extract to another. It appears that all microbial strains tested are inhibited by at least one of the extracts, confirming the broad spectrum of the antimicrobial activity of this fruit. As has been reported in the literature, we considered that an extract has a bacteriostatic action if its inhibition diameter is greater than 12 mm [13]. According to our results, the extracts have a very diversified and variable activity, they attack the strains tested with a different intensity according to the concentration, the type of extract and the microbial strains it is for these reasons that they are presented in the form of tables as follows:

Table 2. *Diameter of the inhibition zone of the aqueous extract.*

Bacterial strains	Diameter of the inhibition zone *(mm)			
	concentrations of the Aqueous extract mg/ml			
	12.5	25	50	100
Enterobacter agglomerans	11±0,05	12±0,002	14±0,09	17±0,01
Citrobacter amalonaticus	11±0,07	13±0,02	15±0,04	16±0,07
Lactobacillus	13±0,002	16±0,03	17±0,05	19±0,02
Clostridium sulfito-réducteur	12±0,002	15±0,07	17±0,05	18±0,04
Proteus vulgaris	07±0,03	07±0,03	08±0,03	10±0,05

Table 3. *Diameter of the inhibition zone of the ethanol extract.*

Bacterial strains	Diameter of the inhibition zone*(mm)			
	concentrationsoftheethanolextractmg/ml			
	12.5	25	50	100
Enterobacter agglomerans	12±0,05	13±0,02	17±0,08	17±0,09
Citrobacter amalonaticus	13±0,06	16±0,001	17±0,03	18±0,06
Lactobacillus	14±0,002	18±0,06	20±0,06	21±0,03
Clostridium sulfito-réducteur	14±0,06	16±0,03	17±0,02	20±0,01
Proteus vulgaris	07±0,04	08±0,01	10±0,001	11±0,03

Table 4. *Diameter of the inhibition zone of the methanol extract.*

Bacterial strains	Diameter of the inhibition zone*(mm)			
	Concentrations of the methanol extract mg/ml			
	12.5	25	50	100
Enterobacter agglomerans	12±0,05	14±0,04	16±0,06	19±0,05
Citrobacter amalonaticus	14±0,001	15±0,03	17±0,001	20±0,07
Lactobacillus	12±0,02	16±0,02	19±0,02	22±0,01
Clostridium sulfito-réducteur	14±0,03	19±0,06	25±0,07	21±0,001
Proteus vulgaris	09±0,04	09±0,006	11±0,03	12±0,05

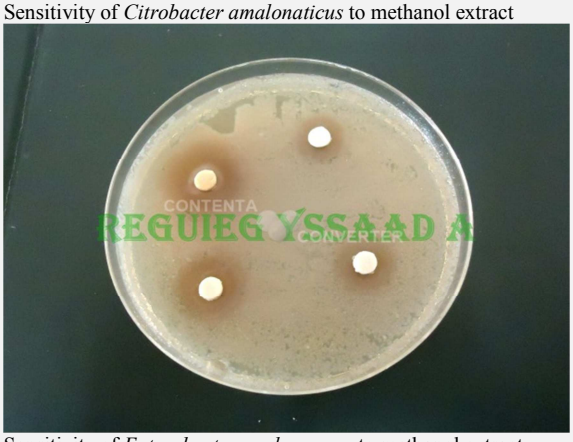
(*) Diameter of the inhibition zone produced around the discs by the addition of 15 µl of extract (the diameter of the disc is included) the values represent the average of 3 measurements ± SD.

Table 5. *Sensitivity of the tested strains to phenolic extracts.*

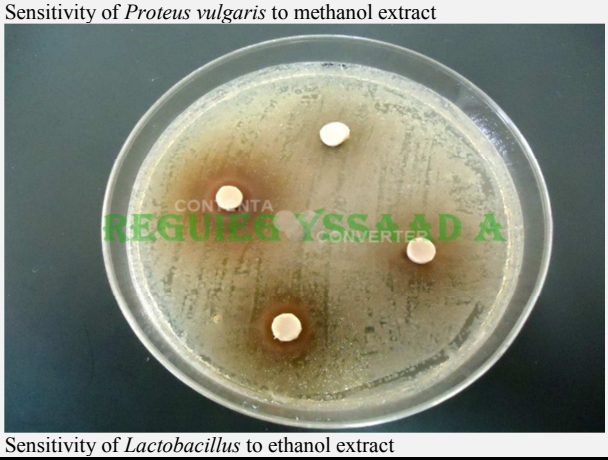
Sensitivity of *Lactobacillus* to methanol extract

Sensitivity of *Citrobacter amalonaticus* to methanol extract

Sensitivity of *Proteus vulgaris* to methanol extract



Sensitivity of *Enterobacter agglomerans* to methanol extract



Sensitivity of *Lactobacillus* to ethanol extract

Sensitivity of *Lactobacillus* to methanol extract

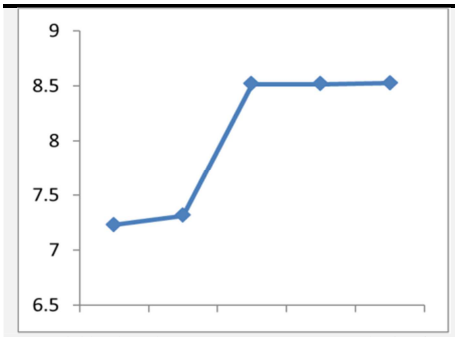
The evaluation of the antimicrobial activity showed great heterogeneity in the results. Methanolic extract of *punica granatum* showed the best effect in this method with the largest zone of inhibition recorded against *Lactobacillus* of 22 ± 0.01 mm, it is greater than the diameter given by the action of the antibiotics on the other hand Strain Proteus vulgaris shows resistance to these extracts.

This is proved by the study by Naz et al (2007) [14] on ecotype of *punica granatum* where the methanol extract had an interesting inhibitory activity with a diameter of 20 mm. These results corroborate those of [15], which demonstrated that pomegranate extracts exhibit significant antimicrobial activity against the strains tested, those of Al-Zoreky (2009) [16]. Which showed that extracts of the pomegranate bark constitute A potent inhibitor of microbial growth and Choi et al. (2009) who studied the in vivo and in vitro effect of applying various concentrations of pomegranate bark extracts to inhibit Salmonella growth. [17].

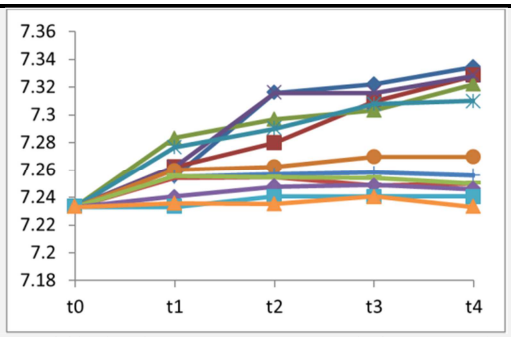
This antimicrobial activity of this extract is due, at least partially, to the presence of the polyphenols. This is confirmed by other research which has attributed the antimicrobial activity to the presence of polyphenols.

According to the results of the antibacterial tests, it is found that the bacterial strains exhibit a high sensitivity. And the latter is related to the composition of the bacteria's membrane (Gram-positive and Gram-negative) and the major component of the extract.

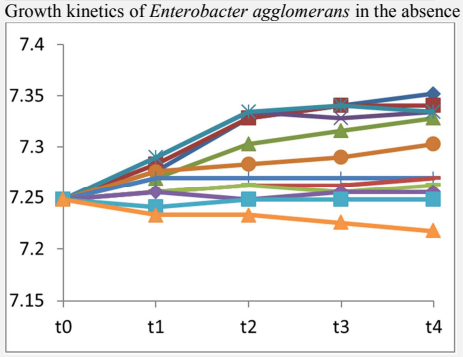
Table 6. *Growth kinetics of the strains tested in the presence and absence of the phenolic extracts.*



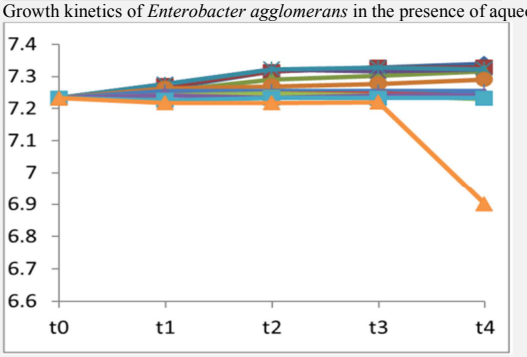
Growth kinetics of *Enterobacter agglomerans* in the absence of the phenolic extract



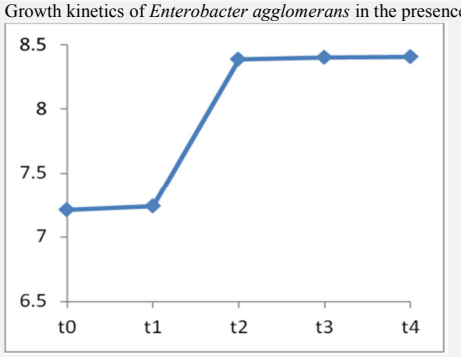
Growth kinetics of *Enterobacter agglomerans* in the presence of aqueous extract



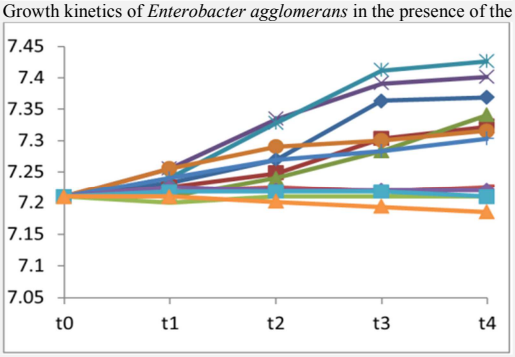
Growth kinetics of *Enterobacter agglomerans* in the presence of the ethanol extract



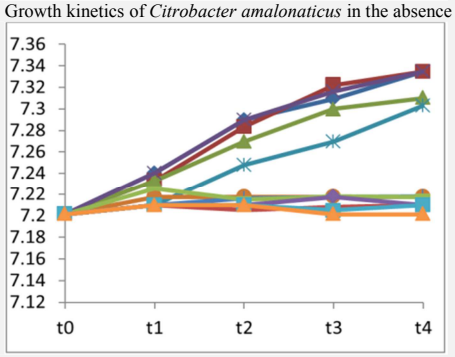
Growth kinetics of *Enterobacter agglomerans* in the presence of the methanol extract



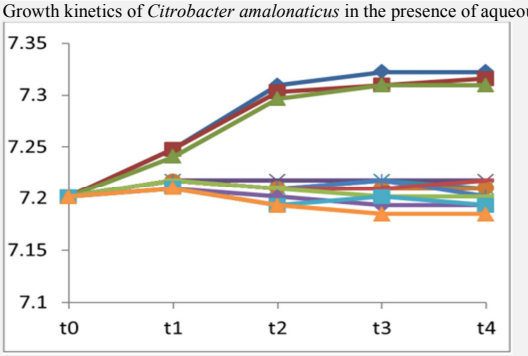
Growth kinetics of *Citrobacter amalonaticus* in the absence of the phenolic extract



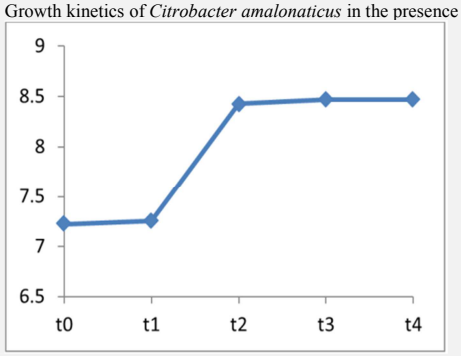
Growth kinetics of *Citrobacter amalonaticus* in the presence of aqueous extract



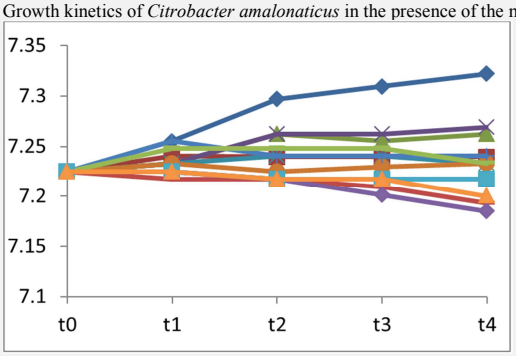
Growth kinetics of *Citrobacter amalonaticus* in the presence of the ethanol extract



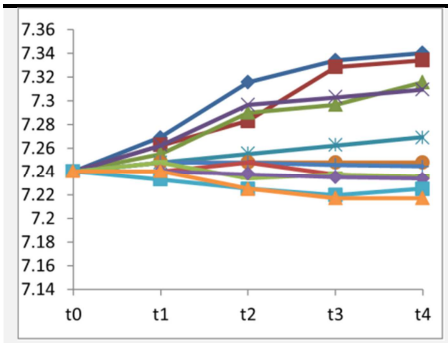
Growth kinetics of *Citrobacter amalonaticus* in the presence of the methanol extract



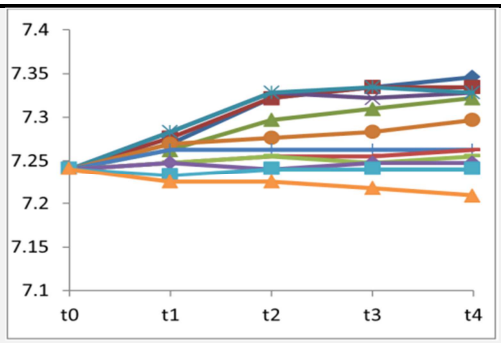
Growth kinetics of *Lactobacillus* in the absence of the phenolic extract



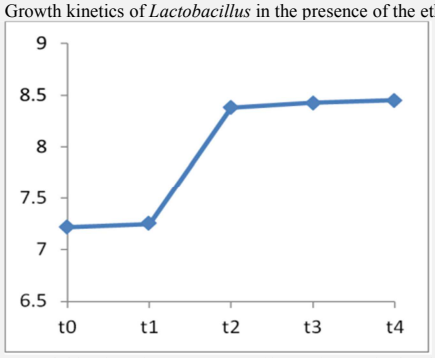
Growth kinetics of *Lactobacillus* in the presence of aqueous extract



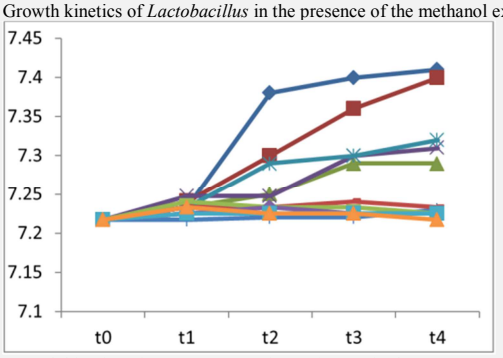
Growth kinetics of *Lactobacillus* in the presence of the ethanol extract



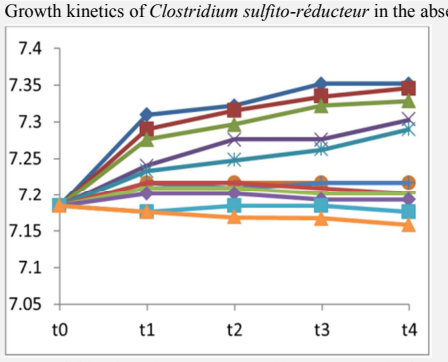
Growth kinetics of *Lactobacillus* in the presence of the methanol extract



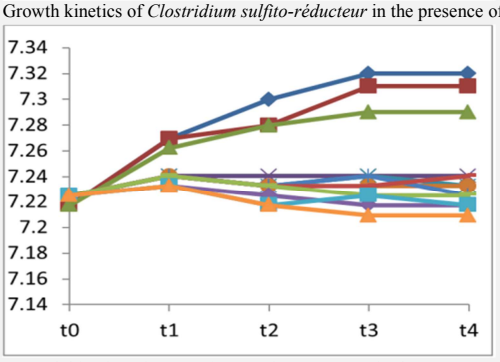
Growth kinetics of *Clostridium sulfito-réducteur* in the absence of the phenolic extract



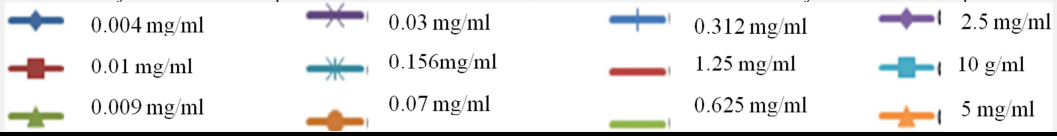
Growth kinetics of *Clostridium sulfito-réducteur* in the presence of aqueous extract



Growth kinetics of *Clostridium sulfito-réducteur* in the presence of the ethanol extract



Growth kinetics of *Clostridium sulfito-réducteur* in the presence of the methanol extract



The results of the evaluation of the bactericidal effects of plant extracts are given in the following tables or are included on the one hand the minimum bactericidal concentrations (mg/ml) of all the extracts and on the other hand the values of the ratio between The MBC and the MIC.

Table 7. *Minimum Bactericidal Concentrations of Aqueous Extract and MBC/MIC Report.*

Bacterial strains	**Aqueous extract in mg/ml**			
	MIC	**MBC**	**MIC/MBC**	**Interpretation**
Enterobacter agglomerans	0.312	2.5	8	ModerateInhibition
Citrobacter amalonaticus	0.625	2.5	4	Strong inhibition
Lactobacillus	0.07	0.156	2	Strong inhibition
Clostridium sulfito-réducteur	0.312	0.625	2	Strong inhibition

Table 8. *Minimum Bactericidal Concentrations of Ethanol Extract and MBC/MIC Report.*

Bacterial strains	**Ethanol extract in mg/ml**			
	MIC	**MBC**	**MIC/MBC**	**Interpretation**
Enterobacter agglomerans	0.312	0.625	2	Stronginhibition
Citrobacter amalonaticus	0.156	0.625	4	Strong inhibition
Lactobacillus	0.07	0.156	2	Strong inhibition
Clostridium sulfito-réducteur	0.156	0.312	2	Strong inhibition

Table 9. *Minimum Bactericidal Concentrations of Methanol Extract and MBC/MIC Report.*

Bacterial strains	Methanol extract in mg/ml			
	MIC	MBC	MIC/MBC	Interpretation
Enterobacter agglomerans	0.312	0.625	2	Stronginhibition
Citrobacter amalonaticus	0.07	0.156	2	Strong inhibition
Lactobacillus	0.03	0.07	2	Strong inhibition
Clostridium sulfito-réducteur	0.07	0.156	2	Strong inhibition

According to the previous tables the MBC / MIC ratio does not exceed 8 for all the tests, we can conclude that the bacteria tested do not exhibit a tolerance to phenolic extracts *punica granatum*. In all strains, a concentration ranging from 0.03 to 2.5 mg/ml is capable of killing more than 90% of the initial bacterial population. For methanolic extract, CMBs range from 0.03mg/ml to Gram + bacteria (enterobacteria) and 2.5mg/ml against Gram-bacteria.

This increased sensitivity is confirmed by the MBC values which are closer to those of the MICs for all extracts. When the MBC / MIC ratio is less than or equal to 4, the antibacterial agent is considered to be bactericidal. We find that the action of the methanolic extract of *punica granatum* is bactericidal. However, for the aqueous extract the action varies according to the germ. [18].

View the qualitative and quantitative phytochemical results of the phenolic extracts of *punica granatum* found previously and the presence of the tannins in these extracts which bind to proteins rich in proline and which can interfere with the synthesis of the proteins of the bacterial walls, this is proposed As a mechanism explaining the antibacterial effect of this extract [19].

Overall, the inhibitory action is noted in Gram + bacteria than Gram – bacteria differences in sensitivity of Gram-negative bacteria and Gram-positive bacteria indicated by the presence of antimicrobial substances seen to be related to the structure and composition of their cell walls. Indeed Gram-positive bacteria have a more permeable outer layer rich in peptidoglycan whereas Gram-negative bacteria have a more rigid outer barrier phospholipids.

The results of the antibacterial activity revealed the efficacy of all the extracts against all the strains tested except for the strain Proteus vulgaris. The antibacterial activity of plant extracts is due to the various chemical agents present in these extracts, including flavonoids and tannins as well as other free phenolic compounds or hydroxyl groups which are classified as highly active antibiotic compounds.

The richness of *punica granatum* in tannins [20] and according to Cowan (1999) suggest that the antimicrobial properties of tannins may be related to their ability to inactivate microbial adhesion, synthesis of certain membrane enzymes and proteins of microorganisms by the complex with polysaccharides, their ability to bind substrates such as mineral salts, vitamins and carbohydrates, which makes them unavailable for microorganisms and their ability to modify the morphology of microorganisms. [21].

On the other hand, since fractions of grenades contain a wide range of flavonoids, particularly anthocyanins, they may exert antibacterial effects since they are potent inhibitors in vitro of DNA Gyrase by two mechanisms, either it binds to the DNA at the sites of insertion of the enzyme thus blocking its activity or it blocks the ATP binding site on the DNA Gyrase. [22].

4. Conclusion

The plant kingdom is an inexhaustible source of new molecules that can be used directly as an active principle or that can serve as a guide molecule for the development of new therapeutic agents. The search for new drugs of natural origin with antimicrobial action is an important research focus at the global level.

In this work, we investigated the antimicrobial effects of the various extracts of the fruit of *Punica granatum*, a plant widely used in traditional medicine throughout the world.

However, *Punica granatum* reveals an immense richness of phenolic compounds especially total polyphenols with values ranging between 194.96 µg EAG / mg and 336.14 µg EAG / mg. Moreover, according to the results of the antibioaromatogram, all our phenolic extracts have proved an antimicrobial effect on all the strains tested with a strong inhibiting power like antibiotics or more times.

Given that *Punica granatum* is characterized by a fairly large reservoir of secondary metabolites with particular therapeutic and pharmacological characteristics that need to be exploited by subsequent research.

References

[1] Malik, A., Afaq, F., Sarfaraz, S., Adhami, VM., Syed, DN., Mukhtar, H. Proc Natl Acad Sci USA. 102 (2005), 14813–1418.

[2] Navarro, P., Nicolas, TS., Gabaldon, JA., Mercader-Ros, MT., Calín-Sánchez, Á., Carbonell-Barrachina, ÁA y., Pérez-López, AJ. Food Sci 76 (5) (2011) 319-32.

[3] Wang, L.,. Waller, C. L., 2006. Recent advances in extraction of nutraceuticals from plants, Trends in Food Science & Technology. p 300 – 312.2485.

[4] Celiktas, O. Y., Hames Kocabas, E. E., Bedir, E., Vardar Sukan, F., Ozek, T., Baser, K. H. C., 2007 Antimicrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. Food Chem. 100: 553-559.

[5] Kahlmeter G., Brown D. F. J., Goldstein F., Macgowan A. P., Mouton J. W., Osterlund A., Rodloff A., Steinbakk M., Urbaskova P. and Vatopoulos A., 2003. European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J. Antimicrob. Chemother., 52, 145-148.

[6] Turkmen, N., Velioglu, Y. S, Sari, F., Polat, G., 2007. Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. Molecules, 12: 484-496.

[7] Falleh, H., Ksouri, R., Chaieb, K., Karry-Bouraoui, N., Trabelesi, N., Boulaaba, M., Abdelly, C., 2008, Phenolic composition of cynara cardunculus L. organe, and their biological activites. C. R. BIOLOGIES. 331: 372-379

[8] Vuorela, S. (2005) Analysis, isolation, and bioactivities of rapeseed phenolics. Helsinki

[9] Li Y., Wens S., Kota P. B., Peng G., Li G. Q., Yamahara J., Roufogalis B. D., 2005. Punica granatum flower extract, a potent alpha-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic Fatty rats. Journal of Ethnopharmacology, 99: 239-244.

[10] Aehle, E., Raynaud-Le Grandic, S., Ralainirina, R., Baltora-Rosset, S., Mesnard, F., Prouillet, C., Mazière, J.-C., Fliniaux, M.-A., 2004. Development and evaluation of an enriched natural antioxidant preparation obtained from aqueous spinach (Spinacia oleracea) extracts by an adsorption procedure. Food Chemistry, 86, 579-585.

[11] Wald Elodie., 2009. le grenadier (punica granatum): plante historique et évolution thérapeutiques recentes. Université Heneri Poincare-Nancy 1, diplome d'état de docteur en Pharmacie, 158: 22-41.

[12] Ozturk, M., Aydogmus-Ozturk, F., Duru, M-E., Topcu, G. 2007. Antioxidant activity of stem and root extracts of Rhubarb (Rheum ribes): An edible medicinal plant. Food Chem. 103: 623-630.

[13] SFM. Société Française de Microbiologie. 2010, comité de l'antibiogramme de la société française de microbiologie, recommandation 2010, édition de janvier 2010.

[14] Naz S., Siddiqi R., Ahmad S., Rasool S., Sayeed S. Antibacterial activity directed isolation of compounds from Punica granatum. Journal of Food Sciences, 72, 341–345, 2007.

[15] Reddy MK, Gupta SK, Jacob MR, Khan SI, Ferreira D., 2007 Antioxydant, activités 1089. Antioxidant activity of tannic acid. Arabian Journal of Chemistry. 3: 43-53. antipaludiques et antimicrobiennes de fractions riches en tanins, acides phénoliques et ellagitannins de Punica granatum L. Planta Med, 73: 461-467.

[16] Al-Zoreky, N. S., 2009, International Journal of Food Microbiology; 134, 244–248.

[17] Choi, JG., Kang, OH., Lee, YS., Chae, HS., Oh, YC., Brice, OO., Kim, MS., Sohn, DH., Kim, HS., Park, H., Shin, DW., Rho, JR y., Kwon, DY., 2009 Evid Based Compl Alter Med 17, 1–8.

[18] SFM. Société Française de Microbiologie. 2010, comité de l'antibiogramme de la société française de microbiologie, recommandation 2010, édition de janvier 2010.

[19] Shimada, 2003. Nat. Med. 57 (2003) 464.

[20] Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA., 2000, «Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing.», J Agric Food Chem., vol. 48, no 10, p. 4581-9

[21] Cowan M-M., 1999. Plant Products As Antimicrobial Agents. Clinical Microbiology Reviews. 12 (4): 564 582.

[22] Seeram, N. P., Henning, S. M., Zhang, Y., Suchard, M., Li, Z. & Heber, D. 2006, Journal of Nutrition, 136 (10) 2481-2485.