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Evaluation of the antibacterial activity of oily extracts of the grains of two varieties of red onion from Morocco

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Abstract

The aim of the present work is to evaluate the antibacterial activity of the oils extracted from the seeds of two varieties of red onion Doukkala and Amposta using hexane and ethanol. The ethanol extracts are more active than hexane extracts vis-a-vis bacteria gram+ tested (Staphylococcus aureus CIP 53154, Enterococcus faecalis CIP 103214, Bacillus cereus and Streptococcus A IPL141 IPT 5-84) and gram- (Escherichia coli CIP 54127, Entérobactersp, Pseudomonas aeruginosa CIP A 22 and Salmonella typhi CIP 5535). The MICs determined by the well-diffusion method for each extract show that the ethanolic oil extracts are bactericidal towards Staphylococcus aureus (SASM) and Staphylococcus aureus (SARM) strains. This could be explained by the fact that the ethanolic extracts are richer in polyphenols (98.7 ± 0.2 mg EGA / g extract) for Doukkala ethanol extract (EED) and (85.7 ± 0.2 in mg of EGA / g of extract) for the ethanolic extract of Tetouan (EET) (Amposta) which is also bactericidal with respect to the Bacillus cereus strain and this for a MIC of 0.125 mg / mL. The strain Enterobacter is sensitive in all the extracts while the 6-aminopenicillanic acid antibiotic 6-APA is not sensitive.

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1.Introduction

The discovery of antibiotics in the last century has radically changed the course of infectious diseases of bacterial origin [1]. The microbial resistance to antibiotics is now a global public health problem, concerning the hospital doctor as the doctor of city [2]. This resistance concerns both Gram-negative bacteria (especially Enterobacteriaceae, Pseudomonas and Acinetobacter) [3] and Gram-positive bacteria (mainly Streptococcus, Enterococcus, Staphylococcus) [4]. In order to help solve the problem of bacterial multiresitency, work has been carried out to replace synthetic antibiotics with plant extracts known traditionally by their antibacterial power [5] and thus to isolate molecules having demonstrated significant antibacterial activity at MICs very low on pathogenic bacteria which have developed resistance against several antibiotics [6]. In order to contribute to the development of extracts possessing significant antibacterial activity, we extracted oils from the seeds of two varieties of red onion from Doukkala and Amposta of Morocco [7] using hexane and ethanol.

II. Material and methods

II.1. Plant material

The red onion seeds of Doukkala were harvested in 2014 in the region of Chaouia in the west of Morocco and the seeds of the red onion of Amposta were harvested in 2014 in the region of Tetouan located north of Morocco. The seeds are cleaned, dried and then finely ground [8].

II.2. Preparation of oily extracts

The extraction of the oils from the grains of two varieties of red onionwas carried out by the extraction process which we have developed from previous work [9].

II.3. Identification and dosage of total phenols

II.3.1. Identification of some polyphenols by HPLC

a-Apparatus and conditions for the analysis of polyphenols

The qualitative analysis of the phenolics contained in the extracts of the seeds of the red onion of the two varieties was carried out using a phase chromatograph High Performance Liquid (HPLC) with a Diode Array UV / Screw Detector and Azur Version 4.0 Data Processing Software.

Conditions of analysis:

- Column: C18 (1.7 µm 2.1x150 mm)
- Wavelength: 285 nm
- Flow: 1mL / min, eluent: water / acetonitrile (88/12).

b-Preparation of the standard solution

The polyphenol reference solution was prepared by dissolving 5 mg of each polyphenol (catechin, trolox, rutin, vanillin, gallic acid, vanillic acid, caffeic acid, syringic acid and ferulic acid) in 1 mL of the acetonitrile / water mixture (88/12) and then the solution obtained is then filtered on a Whatman nylon membrane.

II.3.2. Determination of Polyphenols by HPLC

The determination of the total phenols was carried out according to the Folin-Ciocalteau colorimetric method [10]. The reagent used consists of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). This reaction is carried out under basic conditions (pH= 10) which is achieved by the addition of a

sodium carbonate solution. The dissociation of a phenolic proton leads to the formation of a phenolate ion able of reducing the Folin-Ciocalteau reagentfollowing the chemical reaction:

$$Mo(VI) + e(From\ polyphenols\ or\ reducing\ agents) \rightarrow Mo(V)$$
$$blue\ color$$

The blue coloration produced has a maximum absorption at 760 nm. It is proportional to the concentration of the phenolic compounds. The tests were carried out three times to ensure the reproducibility of the results. The total phenolic content was expressed as mg Equivalent of Gallic Acid per gram of sample.

II.3.3. Dosage of total flavonoids

The total flavonoids were assayed according to the method reported by M. Donzo [11]. 1 ml of the solution of the extracts prepared at different concentrations (0.1 to 0.5 µg / ml) was added to 1 ml of the aluminum chloride methanol solution ($AlCl_3$, 2%). After incubation for 60 min at room temperature, the absorbance is determined at 420 nm. The content of the total flavonoids is calculated in relation to the standard made with different concentrations of Quercetin. The results are expressed as Quercetin equivalent (QE) per gram of dry plant material. Three trials were conducted for each sample. The calibration curve obtained is shown in the following figure 1.

II.4. Antibacterial activity of seeds of two varieties of red onion in Morocco

II.4.1. Microorganisms

The microorganisms used in this work are Gram-positive bacteria (Staphylococcus aureus CIP 53154, Enterococcus faecalis CIP 103214, Bacillus cereus IPL141 and Streptococcus A IPT 5-84) and gram negative (Escherichia coli CIP 54127, Pseudomonas aeruginosa CIP A 22 and Salmonella typhi CIP 5535). All these microorganisms were provided to us by the microbiology laboratory of the Pasteur Institute of Casablanca.

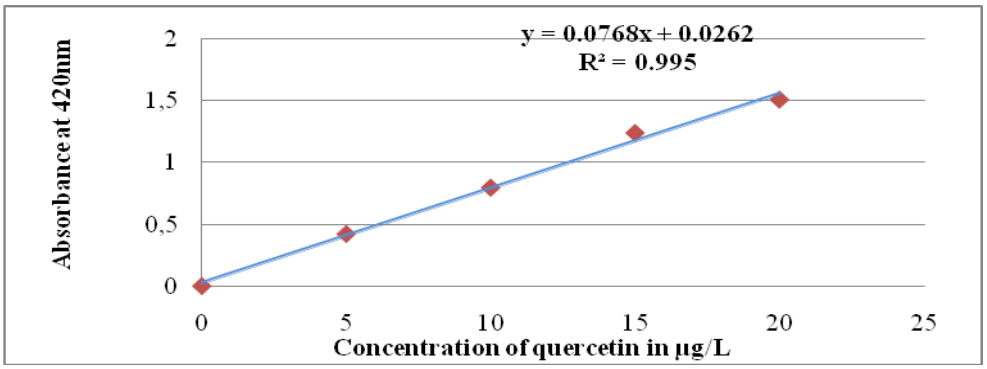

Figure 1: Quercetin Calibration Curve

II.4.2. Method of studying antibacterial activity

For the evaluation of the antibacterial activity of the oil extracted from the red onion seeds, we used the method of diffusion per well. In this method each bacterium was subcultured in nutrient broth at 37 ° C. for 24 h. 100 µL (about 106 cfu.mL^{-1}, standardized by Mac-Farland 0.5) of each test bacterium was spread on a sterile Muller-Hinton plate in order to achieve confluent growth [12]. The plates were allowed to dry and a 5.0 mm diameter sterile cork was used for the wellbore in the agar plates. Subsequently, a volume of 50 µL of the extract was introduced into the wells. The plates are then incubated for 24 h at 37 ° C. The antibacterial activity is determined by measuring the diameter of the inhibition zone around each well [13]. Sterile distilled water, hexane and ethanol were used as negative controls, while

the 6-aminopenicillanic acid antibiotic (6-APA) was used as a positive control. Each experiment was carried out in duplicate. A classification of the extracted oils in relation to their spectrum of antimicrobial activity can be established depending on the extent of inhibition. A scale for measuring the antimicrobial activity [14, 15] of the extracted oils was emitted by:

- Strongly inhibitory when: Ø = 28 mm of the inhibition zone;
- Moderately inhibitory when: 16 mm <Ø <28 mm of the inhibition zone;
- Slightly inhibitory when: 10 mm <Ø <16 mm of the inhibition zone;
- Non-inhibitory when: Ø <10 mm of the inhibition zone.

II.4.3.Determination of the MIC and the MBC

MICs and MBCs were determined by liquid dilution, which medium was represented by Mueller-Hinton broth (OXOID, Basingstoke, UK, pH: 7.4 ± 0.2) (MHB), or Half-skimmed UHT milk (1.5% fat, pH: 6.6). The extracts were used after dilution with MHB of a stock solution containing 1 mg of active product per mL. Each dilution was placed in contact with a bacterial inoculum in exponential growth phase in the culture tubes. A control culture without extract was also carried out. The initial bacterial concentration was adjusted to 5x105cfu.mL^{-1} after 24 hours of incubation at 37 ° C. For a better comparison of the results obtained, the counts before and after incubation were carried out in MHB. The MIC is defined as the lowest concentration of a range of extract dilutions from half to half, which results in inhibition of any visible bacterial growth, we defined the MIC in our study as the lowest concentration of extract for which a value below 5x106cfu.mL^{-1}of culture is displayed after 24 h of incubation. We considered a value greater than a multiplication of the initial bacterial population likely to lead to a visible disorder of the culture medium.The MBC was defined as the lowest antibiotic concentration destroying 99.9% of the inoculum, which in our study corresponded to a bacterial count of less than 103 cfu.mL^{-1} after 24 h incubation [16].

III. Results and Discussions

III.1. Identification and Determination of Total Phenols and Total Flavonoids

III.1.1. Identification of some polyphenols

The ethanolic extracts of the red onion seeds of the two regions (Doukkala, and Tetouan) were analyzed by HPLC after alkaline hydrolysis. The phenolic compounds Present in the oil extracts of the Doukkala red onion seeds and Amposta red onion seeds are identified by comparing the retention times obtained with respect to the retention times of the standards whose peaks are represented in figure 2 below :

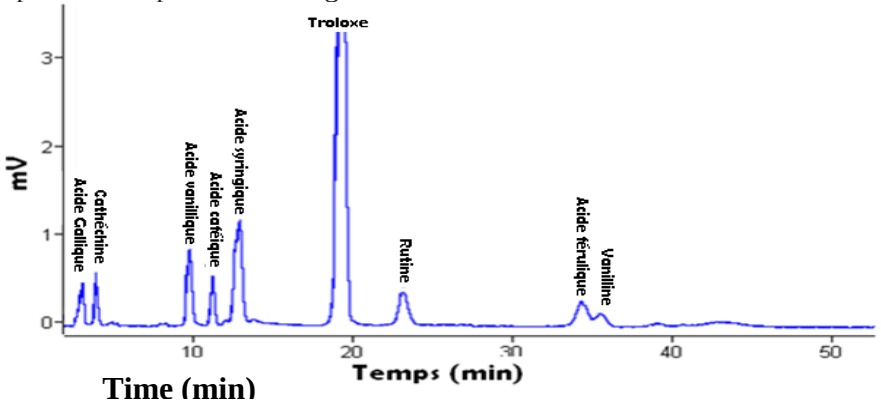

Figure 2: Chromatogram of nine standard phenol compounds

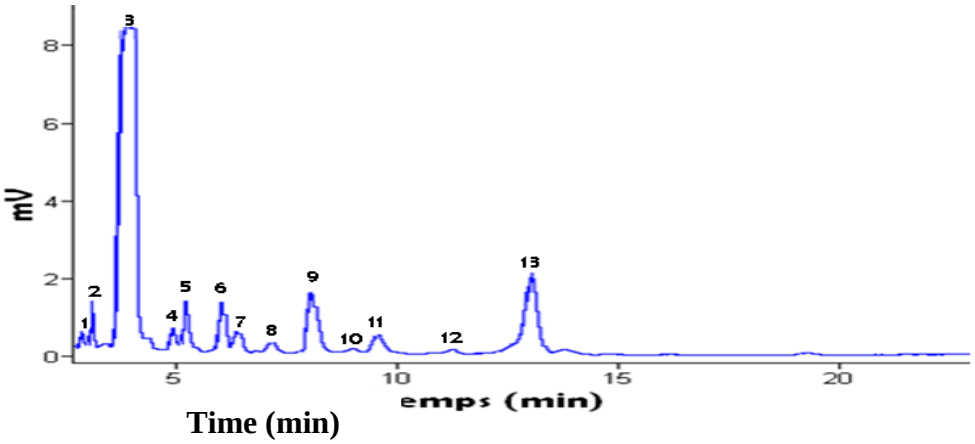

Figure 3: Chromatogram of the ethanol extract of Doukkala red onion seeds (EED)

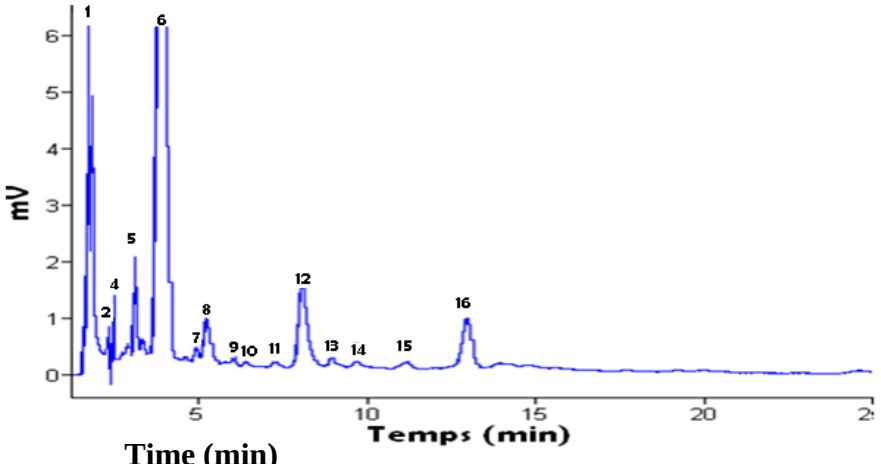

Figure 4: Chromatogram of the ethanol extract of the seeds of Amposta red onion EET

Table 1: identification of the polyphenols contained in the EED and EET extracts

Standards	Retention time reference	EED extract : Peak number of the polyphenols	EED extract : Retention time of the polyphenols	EET extract : Peak number of the polyphenols	EET extract : Retention time of the polyphenols
Gallic acid	1.30	2	1.25	5	1.20
Catechin	2.40	3	2.66	6	2.40
Vanillic acid	10	11	9.68	14	9.66
Cafeic acid	11.42	12	11.30	15	11.20
Syringic acid	13.57	13	13.22	16	13.03
Trolox	19.64	AB	-	AB	-
Rutine	23.21	AB	-	AB	-
Ferulic acid	34.29	AB	-	AB	-
Vanillin	35.36	AB	-	AB	-

From this analysis of comparison of the standard chromatogram with the chromatogram of the ethanol extract of the seeds of the red onion of the two varieties, it is deduced that this extract contains the phenolic compounds summarized

in the following table. Concerning the oil chromatogram of the Doukkala EED red onion seeds, peaks 2, 3, 11, 12 and 13 were identified respectively as: Gallic acid, catechin, vanillic acid, caffeic acid and syringic acid. The same compounds were identified in the extract of the seeds of the red onion of Amposta EET, their peaks in the chromatogram are respectively 5, 6, 14, 15 and 16. The most important proportion in the two oily extracts is allocated to catechin, which represents more than 50% of the polyphenols present in the extract, whereas there is no rutin.

III.1.2. Determination of total phenols and flavonoids contained in oils of red onion seeds

The determination of the total phenols and flavonoids contents in the red onion extracts of the two varieties was carried out separately using the colorimetric methods (Folin-Ciocalteux andaluminum trichloride (AlCl3).

II.1.2.1. Determination of the total phenols contained in the oils of the red onion seeds

The total phenol content estimated by the Folin-Ciocalteu method for each extract was reported in mg equivalent gallic acid / g dry plant material. The results (Table 2) show that the ethalonic extracts are richer in total phenols than hexane extracts. The largest amount is recorded in the EED and EET extract respectively (98.7 ± 0.2 mg (EGA) / g) and (85.7 ± 0.2 mg (EGA) / g), while the hexane EHD are slightly lower (69.8 ± 0.2 mg (EGA) / g) and EHT (63.7 ± 0.2 mg (EGA) / g).

III.1.2.2. Determination of the flavonoids contained in the oils of the red onion seeds of the two varieties

The total flavonoids were assayed according to the spectrophotometric method reported by Adedapo [15]. The absorbance is determined at 420 nm. The total flavonoid content is calculated by the standard carried out with different concentrations of Quercetin (Figure 5). The results are expressed as Quercetin equivalent (QE) per gram of dry plant material. Three trials were conducted for each sample. The results show that the same ethanolic extracts have moderate flavonoid contents (Table 2).

Table 2: Total phenol and flavonoid content of the hexane and ethanol extracts of red onion

Extract	Red onion seeds	Total phenols contents mg of EGA/g of extract	Flavonoids contents mg of EGA/g of extract
Ethanol	Doukkala	98,7±0.2	1.408±0.075
	Amposta	85,7±0.2	3.257±0.162
Hexane	*Doukkala*	69,8±0.2	0.345±0.046
	Amposta	63,7±0.2	0.701±0.078

The flavonoid contents obtained for the ethanolic extracts EET and EED are respectively 3.257mg / g and 1.408mg / g, while the contents of the hexane extracts EHD and EHT are 0.345mg / g and 0.701mg / g. It can be said that the flavonoid contents of the oil of the seeds of the red onion of Amposta are twice as great as in the oil of the seeds of the red onion of Doukkala. The results are given on average ± SD of three different experiments.

III.2. Evaluation of the antibacterial activity of red onion seeds

The diameters of the zone of inhibition of growth of the bacteria observed in the presence of the oils extracted from the seeds of red onion of Doukkala and Amposta are gathered in Table 3 below. The analysis of the results obtained shows that the oil extracted from the seeds harvested in the region of Doukkala has a significant antibacterial activity

against most of the microorganisms tested. The diameters of the inhibition zone are relatively high, particularly in the case of Staphylococcus aureus (SARM) and Staphylococcus aureus (SASM) microorganisms. The results show that the red onion seed extract has an inhibitory effect on the growth of Staphylococcus aureus (SASM) and (SARM) bacterial strains, and moderate antibacterial activity against Salmonella typhi, Enterococcus faecalis, Pseudomonas aeruginosa , And Bacillus cereus tested. In contrast to the Escherichia coli strain, it revealed insufficient inhibition and no inhibitory activity on the strain Pseudomonas aeruginosa ATCC. The antibacterial activity of the ethanol oil extracts is significantly greater than that of the oily hexane extracts.

Table 3: Antibacterial Activity of Oil Extracted from Red Onion Seeds

Bacterial strains	Inhibition area (mm)							
	EED	**EHD**	**EET**	**EHT**	**6-APA**	Negative control		
						Ethanol	**water**	**Hexane**
Escherichia Coli ATCC	8	6	9	8	30	Na	Na	Na
Staphylococcus aureus ATCC (SASM)	18	14	17	11	42	Na	Na	Na
Staphylococcus aureus ATCC (SARM)	22	18	23	18	45	Na	Na	Na
Salmonella typhi	11	10	12	12	33	Na	Na	Na
Enterococcus faecalis	10	10	11	10	24	Na	Na	Na
Pseudomonas aeruginosa ATCC	Na	Na	Na	Na	Na	Na	Na	Na
Entérobactersp	15	14	14	10	Na	Na	Na	Na
Bacillus cereus	12	9	11	9	18	Na	Na	Na

Na : no active 6-APA : Aminopenicillinic Acid

Table 4 : Diameters of the inhibition zone of the EED extract

Bacterial strains	Diameters of the inhibition zone *(mm) The dilutions of the EED extract			
	½	¼	1/8	1/16
Staphylococcus aureus ATCC (SASM)	16,5	14	12	8
Staphylococcus aureus ATCC (SARM)	19	15	11	8.5
Pseudomonas aeruginosa ATCC	14	12	9	6
Bacillus cereus	11.5	10	7	6

Table 5: Diameters of the EET inhibition zone

Bacterial strains	Diameters of the inhibition zone *(mm) The dilutions of the EED extract			
	½	¼	1/8	1/16
Staphylococcus aureus ATCC (SASM)	16	12	10	6
Staphylococcus aureus ATCC (SARM)	20	19	13	10
PseudomonasaeruginosaATCC	12	10	8	6

Bacillus cereus	10	7	6	-

Table 6 : Diameters of the extract EHD inhibition zone

Bacterial strains	Diameters of the inhibition zone*(mm) The dilutions of the EED extract			
	½	¼	1/8	1/16
Staphylococcus aureus ATCC (SASM)	12	10	6	-
Staphylococcus aureus ATCC (SARM)	15	13	8,5	7
PseudomonasaeruginosaATCC	12	10	7	-
Bacillus cereus	8	6,5	-	-

Table 7 : Diameters of the extract EHT inhibition zone

Bacterial strains	Diameters of the inhibition zone *(mm) The dilutions of the EED extract			
	½	¼	1/8	1/16
Staphylococcus aureus ATCC (SASM)	10	8	6	-
Staphylococcus aureus ATCC (SARM)	16	13.5	7	6
PseudomonasaeruginosaATCC	8	6	-	-
Bacillus cereus	8.5	7	-	-

(*) Diameter of the inhibition zone produced around the disks by the addition of 10 µL of extract. (Disc diameter is included) the values represent the average of 3 measurements.

The results presented in the tables and figures below show that:

- The oil extracted from the red onion seeds by the two solvents was found to be moderately active against most of the strains tested. The extracts of the red onion seeds by ethanol exhibit important activities which extend over the totality of the strains of the collection, of which the oily extract EED is the most active.
- The Pseudomonas aeruginosa and Escherichia coli ATCC strains have a very high resistance potential against the antibacterial action of the 4 extracts (EED, EHD, EET, and EHT) of red onion seeds.
- Some moderate zones of inhibition with the extracts were recorded with the two strains Bacillus cereus and Enterococcus faecalis.

The highest activity with the 4 extracts of red onion seeds, was observed in Staphylococcus aureus ATCC.

The inhibitory effects increase considerably with the concentration of the extracts. The majority of the extracts can retain detectable activity after dilutions. It is found that the two oily extracts EED and EET are active to a dilution of 1/16 (0.0625 $mg.mL^{-1}$), in particular for the two strains Staphylococcus aureus ATCC.

Sensitivity to different oils is classified according to the diameter of the zones of inhibition as follows: non-sensitive (-) for diameter less than 8 mm; Sensitive (+) for a diameter between 9-14 mm; Very sensitive (+ +) for a diameter between 15-19 mm and extremely sensitive (+++) for the diameter more than 20 mm. The results show the sensitivity of the strains tested with respect to the four extracts.

The results reveal variable responses depending on the strains, the concentration, the type of extract tested and the susceptibility or resistance to antibiotics.

An interesting activity of the EED and EET extract on the Staphylococcus aureus ATCC (SARM) strain and an average activity of the EED extract on the strain Enterobacterp while the 4 extracts showed an activity too low on the strain Escherichia coli ATCC and on the strain Pseudomonas aeruginosa ATCC.

Table 8: The sensitivity of the bacterial strains to the four extracts

Bacterial stains	Sensitivity				
	EED	EHD	EET	EHT	6-APA
Escherichia coliATCC	-	-	+	-	+++
Staphylococcus aureus ATCC (SASM)	++	+	++	+	+++
Staphylococcus aureus ATCC (SARM)	+++	++	+++	++	+++
Salmonella typhi	+	+	+	+	+++
Enterococcus faecalis	+	+	+	+	+++
Pseudomonas aeruginosa ATCC	-	-	-	-	-
Entérobactersp	++	+	+	+	-
Bacillus cereus	+	+	+	+	+

III.3. Determination of the minimum inhibition concentration (MIC) and the minimum bactericidal concentration (MBC)

The MIC and MBC values for each of the strains tested are shown in Table 9. The present study shows that the BMH MICs of the EED extract gave a MIC of 0.125 $mg.mL^{-1}$for the Staphylococcus aureus ATCC strain (SASM) And Staphylococcus aureus ATCC (SARM) strain of 0.250 $mg.mL^{-1}$for the Enterobacterp and Bacillus Cereus strain. A comparable effect for the first three strains on the EET extract, which shows a value of 0.125 $mg.mL^{-1}$ for Staphylococcus aureus ATCC (SASM), Staphylococcus aureus ATCC (SARM), and 0.250mg $.mL^{-1}$ for Enterobacter and a value of 0.500 $mg.mL^{-1}$ for the Bacillus Cereus strain. These values must be at most doubled in order to obtain a bactericidal effect for the hexane extracts. The BMH MICs of the EHD extract are 0.250 $mg.mL^{-1}$for strains Staphylococcus aureus ATCC (SASM), Staphylococcus aureus ATCC (SARM) and Enterobacterp, in addition they have a value of 0.500 $mg.mL^{-1}$ for Bacillus Cereus. The EHT extract has the values 0.250 $mg.mL^{-1}$for Staphylococcus aureus ATCC (SARM) and 0.500 $mg.mL^{-1}$for Staphylococcus aureus ATCC (SASM), and for the two other strains Enterobacterp and Bacillus Cereus 1 $mg.mL^{-1}$. The MICs were two-fold (0.250 $mg.mL^{-1}$) for two strains of Staphylococcus aureus ATCC (SASM), Staphylococcus aureus ATCC (SARM), four times (1 $mg.mL^{-1}$) for Enterobacterp and Bacillus Cereus. The two ethanolic extracts EED and EET. In the case of hexane extracts, CMBs for EHD gave a value of 1 $mg.mL^{-1}$for both strains Staphylococcus aureus ATCC (SASM), Staphylococcus aureus ATCC (SARM), and 1 $mg.mL^{-1}$for Enterobacter, and about 8 $mg.mL^{-1}$ for Bacillus Cereus. Finally, the EHT extract gives Staphylococcus aureus ATCC (SARM) and 4 $mg.mL^{-1}$for the Staphylococcus aureus ATCC (SASM) strain and the value $8mg.mL^{-1}$for Entelrobacter and Bacillus Cereus, this shows that the ethanolic extracts EED and EET are considered as bactericides against Staphylococcus aureus (SASM) and Staphylococcus aureus (SARM), as well as the EET extract with respect to Bacillus cereus, whereas the remainder is generally bacteriostatic, therefore probable that they contain compounds which, once purified, have an activity comparable to that of an antibiotic. Finally, they are extracts, containing a large number of different compounds. It is very interesting to exploit this plant for the research of the active ingredients, responsible for these antibacterial activities.

Table 9 : MIC and MBC values of extracts of red onion seeds vis-a-vis the most sensitive bacterial strains

Bacterial stains	MIC and MBC in mg.mL^{-1}							
	EED		EHD		EET		EHT	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus ATCC (SASM)	0.125	0, 250	0,250	1	0.125	0,500	0,250	2
Staphylococcus aureus ATCC (SARM)	0.125	0,250	0,250	1	0.125	0,500	0,500	4
Entérobactersp	0,250	1	0,250	1	0,250	1	1	8
Bacillus cereus	0,250	1	0,500	8	0,500	1	1	8

Table 10: MBC / MIC ratio values of red onion seed extract

Extracts	**MBC /MIC**							
Stains	EED	Interpretation	EHD	Interpretation	EET	Interpretation	EHT	Interpretation
Staphylococcus aureus (SASM)	2	Bactericidal	4	Bactericidal	2	Bactericidal	8	Bacteriostatic
Staphylococcus aureus (SARM)	2	Bactericidal	4	Bactericidal	2	Bactericidal	8	Bacteriostatic
Entérobactersp	4	Bactericidal	4	Bactericidal	4	Bactericidal	8	Bacteriostatic
Bacillus cereus	4	Bactericidal	16	Bacteriostatic	2	Bactericidal	8	Bacteriostatic

IV. Conclusion

This study, which aimed to evaluate the antibacterial activity of oils extracted from red onion seeds by ethanol and hexane, concluded that:

- Analysis of the total phenols of ethanol extracts (EED, EET) and hexaneextracts (EHD, EHT) allowed to identify 5 polyphenols whose catechin represents more than 50% and to determine the total phenol content of each extract which shows that the EED and EET extracts have contents of 98.7 ± 0.2 and 85.7 ± 0.2 mg (EGA)/g respectively, whereas the EHD and EHT extracts contain respectively 69.8 ± 0.2 and 63.7 ± 0.2 mg (EGA) /g. It appears that the oil of the seeds of the red onion of Amposta is distinctly rich in flavonoids as the oil of the seeds of the red onion of Doukkala.
- All the bacterial strains studied are sensitive to our extracts except Escherichia coli ATCC and Pseudomonas aeruginosa ATCC.
- This sensitivity is different according to the strains. There are very sensitive strains, moderately sensitive strains and less sensitive strains. The ethanol extracts EET and EED can be considered as bactericides because their MBC/MIC ratio is equal to 2 for the two bacterial strains Staphylococcus aureus (SASM) and Staphylococcus aureus (SARM). The same is true for the extract EET which is additionally bactericidal with respect to Bacillus cereus.
- While EHT and EHD extracts with a MBC/MIC ratio greater than 2 may be considered as bacteriostatics for all bacterial strains. The bacterial strain tested Pseudomonas aeruginosa ATCC shows resistance against all the extracts and the Bacillus cereus strain is resistant to the antibiotic 6-APA and sensitive to very sensitive to all the extracts. The extract EET is bactericidal with respect to this latter strain whereas the other extracts are

bacterostatic this may be explained by the fact that this extract is twice as rich in flavonoids as the EED extract.

- These activity tests vary according to the bacterial species, the nature and the concentration of the product tested and essentially to the extraction solvent, which allows us to conclude that the ethanolextracts EET and EED appear to be more effective than the hexane extracts, and that this efficacy could be explained by their richness in phenolic compounds.

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