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Promising effects of *Rosa damascena* petal extracts as antioxidant and antibacterial agents

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Abstract: In the present work, the antioxidant properties of methanolic (MeOH), ethyl acetate (EtOAc) and chloroformic ($CHCl_3$) fractions of *Rosa damascena* petals were evaluated. Antioxidant capacity was assessed by free radical scavenging assays (DPPH•) and ferrous ions (Fe^{2+}) chelating activity. Antibacterial activity was evaluated using minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and IC_{50}. Qualitative analysis of chemical composition was carried out by HPLC and showed variability in the chemical constituents with a richness in flavonones and phenolic acids. Acute toxicity study and hemolysis test were also assessed. The MeOH and EtOAc fractions are of real and potential interest by their antioxidant activities. Furthermore, the microbiological study of the fractions showed a high activity of the EtOAc fraction which possesses bactericidal properties, followed by a moderate activity of the methanolic MeOH. The most sensitive strains were *S. aureus* and *B. cereus* while the most resistant were *P. aeruginosa* and *E. coli* (R). On the other side, no cytotoxicity was observed towards erythrocytes isolated from human blood and on a warm-blooded animal model. Therefore, the *R. damascena* petals constitute a promising source of molecules for clinical use without cytoxicity.

Keywords: *Rosa damascena*, antimicrobial activity, cytotoxicity, toxicity, HPLC, antioxidant activity.

INTRODUCTION

In the last years, there is an increase in the prevalence and morbidity of infectious and chronic diseases led researchers to explore new treatment approach (Nayebi *et al*., 2017). Plants are rich in secondary metabolites, such as phenolic and flavonoid compounds are known to have high antioxidant capacity and constitute an important alternative to take over classical drugs (Lesjak *et al*., 2011; Ben Mrid *et al*., 2019, Ramdan *et al.,* 2019).

One of the major concerns of scientists about the antibacterial treatments is related to the loss of effectiveness of the anti-bacterial drugs. In return, just few new classes of these anti-bacterial molecules are released, leading to a serious public health problem. Therefore, finding new antimicrobial agents is of crucial and permanent priority.

Rosa damascena Mill. (*R. damascena*), which belongs to the *Rosaceae* family, is well known as medicinal herb (Tavirani *et al*., 2013). The beneficial effects of this vegetal species have been described by Avicenna more than 1000 years ago (Dalfardi *et al*., 2014). Currently, several studies reported an antioxidant and antimicrobial activities of different *Rosa* species (Nayebi *et al*., 2017). In fact, the ethanol and acetone extracts of *R. damascena* petals showed high antibacterial activity in previous studies conducted on *S. typhimurium, S. aureus*, *B. cereus*, *C. albicans, E. coli and B. subtilis* (Mahboubi, 2016).

In this work, we aimed first to analyze the chemical composition of methanolic, ethyl acetate and chloroformic fractions of *R. damascena* petals. Subsequently, the antibacterial and cytotoxic effects were investigated. On one hand, nine different reference strains and food-borne isolates were used for assessing the *R. damascena* antimicrobial properties; including Gram-positive and Gram-negative bacteria. On the other hand, we investigated the cytotoxic effect of *R. damascena* extracts towards erythrocytes isolated from human blood and on a warm-blooded animal model.

MATERIALS AND METHODS

Chemicals

All reagents (PCA, MH, Agarose, Resazurin, Ethanol, Folin–Ciocalteau reagent, Folin–Denis reagent, sodium carbonate, Gallic acid, potassium acetate, Aluminum trichloride, Quercetin, PBS), unless otherwise stated, were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

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Plant collection and extract preparation

Plants were collected in June 2016 from Kelaa M'Gouna, Morocco and identified. The petals were dried at 40°C for 15 h, then ground into a fine powder and passed through an 80-mesh sieve. Hydro-alcohol extract was obtained by extraction of sample (20 g) with 200 mL of ethanol solution (70%) for 24 h. The extraction was performed three times. After evaporation, the hydro-ethanolic extract was autoclaved at 121°C for 15 min. The dry residue was taken up in 100 mL of boiling distilled water. After 24 h decantation, the aqueous phase firstly degreased by petroleum ether was then extracted by chloroform ($CHCl_3$) followed by ethyl acetate (EtOAc) and finally methanol (MeOH). The organic phases were evaporated and the dry extracts were autoclaved and stored at 4°C away from light until use.

Qualitative analysis of phytochemicals

Different groups of secondary metabolites such as aldehydes, terpenoids, polyphenols including flavonoids and tannins, alkaloids, saponins and quinone substances were investigated as used by Parekh J., *et al*. (2007).

Qualitative analysis of the chemical composition by HPLC

Analysis was carried out by an HPLC (VP Shimadzu Liquid Chromatograph) at the biochemistry laboratory of the Pharmaceutical and Pharmaceutical Department of Rabat, Morocco. After filtration through a membrane (pore size of 0.45 mL), 20 µl of each extract was injected onto a C18 reverse phase column (125 × 4.6 mm). The mobile phase consisted of two solvents: solvent A, water / formic acid (95: 5; v/v) and solvent B, acetonitrile / solvent A (60:40; v/v). The elution gradient applied was isocratic type spread over 10 min with 0% B, and gradient type from 0% to 5% B (30 min), from 5% to 15% B (18 min), from 15% To 25% B (14 min), from 25% to 50% B (31 min), from 50% to 100% B (3 min), followed by rinsing and reconditioning of the column. The flow rate was 1 mL/min at 25°C. Detection was performed by a UV-Vis detector at wavelengths equal to 280 and 350 nm (Hasim K *et al*., 2008). Identification of phenolic compounds was obtained using different standards and by comparison of the retention time and the ultraviolet-visible spectra with those of the literature (Hasim *et al*., 2008; 2009).

Effects on microorganism's growth

Antibacterial activity was evaluated at Laboratory of Microbiology of hygiene and food safety department of the Institute Pasteur Tangier – Morocco.

Microbial Strains and Growth Conditions

Nine different reference strains and food-borne isolates were used for assessing the plant antimicrobial properties; including Gram-positive and Gram-negative bacteria (table 1). Fresh cultures were prepared by transferring a loop of cells from the agar slant to a test tube containing 5 mL of brain heart infusion (BHI) (BioRad) and then incubated over-night at 37°C.

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of extracts was determined by the method of Mann and Markham (1998), using resazurin as viability indicator. Different dilutions of the extracts (50; 25; 12.5; 6.25; 3.12; 1.56; 0.8; 0.4; 0.2 and 0.1 mg/mL) were prepared from a stock solution (100 mg/mL). To each well containing 50 µl of the mixture, was added 50 µl of the bacterial suspension (10^6 to 10^8 CFU/mL) prepared in Mueller-Hinton Broth medium (MHB). Plate was then incubated at 37°C for 18 to 20 h. After the first incubation step, 5 µl of resazurin (1 mg/mL) was added to each well. Reading results was carried out after further incubation for 2 h at 37°C. The MIC corresponds to the lowest extract concentration, which does not produce change of resazurin staining. Then, the optical density at 550 nm was measured (Epoch BioTek UV-Vis) for CI_{50} determination. The following formula was used to calculate the survival germs percentage (Mann and Markham, 1998):

$$S = (d_f - d_i)/(D_f - D_i) * 100$$

S: Survival percentage of germs; di: densimat value of experimental tube before incubation; Di: Densimat value control tube before incubation; Df: Densimat value after incubation control tube; di: densimat value of experimental tube before incubation; df: densimat value after incubation.

Determination of the minimal bactericidal concentration (MBC)

Plate counting agar (PCA) (BioRad) was seeded with 10 µl of samples from plate wells where there was no resazurin color change. Dishes were then incubated for 18 to 20h at 37°C. The MBC corresponds to the lowest extract concentration that gives no growth. Moreover, the ratio MBC/CMI of each sample was calculated to assess the antibacterial power.

Effects on warm-blooded animals

Acute general toxicity study

The procedures on the animals were carried out in accordance with the recommendations of the Internal Ethics Committee of the Ibn Tofail University Kenitra. This procedure was examined and approved by the Committee.

Male white rats (Albino Wistar) with an average body weight of 200±20 g were used for the acute toxicity study of the crude extract of *R. damascena* (CrE). These rats were raised under specific conditions free of pathogens at Department of Biology, Ibn Tofail University - Kenitra.

They received a rodent diet and free access to drinking water. The studies were carried out in accordance with the "Principles for the Protection of Laboratory Animals" as well as with the current version of the German Animal Protection, under ethical approval number of 2010/63/EU.

The CrE toxicity was estimated by intraperitoneal injection (ip) of a batch composed by five rats of 200 g±20 g, previously fasted for 24 hours, at a raté of 2.5 mL/200 g of weight. Another batch of five rats, receiving 2.5 mL of physiological saline, served as a control.

Determination of LD_{50} (24h)

The lethal dose that kills 50% of rats during 24 h (LD_{50}), was calculated by the graphical method. Six doses of the CrE (100, 150, 300, 500, 750 and 1000 mg/kg) were injected into six batches of five rats. These doses were between the lowest doses resulting in the death of 100% of the tested animals (LD_{100}) and the highest dose causing 0% of mortality (LD_0).

Hemolytic test

A suspension of healthy human blood was washed three times by physiological serum (v/v) and then centrifuged at 3000 rpm for 5min. The pellet was mixed with physiological serum (v/v) thus giving a suspension of 50% red blood cells. A dilution with PBS (Phosphate buffered saline) was carried out in order to obtain a 2% suspension. This one was divided into 50µl per well of a 96-well microplate. A cascade dilution of the CrE with PBS was then performed. A positive (2% suspension + distilled water) and negative (2% suspension + PBS) controls were required for the test. After gentle stirring, the microplate was incubated at 37°C for 3 h, and then left at +4°C overnight to stop the reaction. The reading of the results was done with the naked eye by comparison with the positive and negative controls.

STATISTICAL ANALYSIS

All *in vitro* experiments were conducted in triplicate and results were expressed as mean ± SD. Analysis of variance was performed by uni-varied ANOVA for the study of antibacterial and anticancer activities in the software SPSS 22Fr. CI_{50} values were determined by regression analysis. The values $p \leq 0.05$ were considered significant.

RESULTS

Analysis of the chemical composition in R. damascena petals

Phytochemical analysis of *R. damascena* showed different levels of phenolic compounds in the three fractions studied here (table 2). In fact, the highest content of phenolic and flavonoid compounds was found in the EtOAc fraction (259.82 mg GAE/g DM and 222.36 mg QE/g DM), followed by the MeOH fraction and then the $CHCl_3$ fraction.

The chemical groups present in the three fractions ($CHCl_3$, EtOAc and MeOH) were identified by chemical screening. Indeed, depending on the organic solvent used, we have shown the existence of essential oils, saponins, alkaloids, anthocyanins and aldehydes (table 3).

HPLC analysis showed variability in the chemical constituents with a richness of the CrE in flavonones and phenolic acids, and the presence of quercetin, vanillin and limonene (table 4).

Anti-oxydant activity

The antioxidant activity of *R. damascena* fractions showed significant differences between the three fractions (table 2). Moreover, the antiradical scavenging activity and the metal chelating activity were greater in the EtOAc fraction followed by the MeOH fraction, whereas the $CHCl_3$ fraction showed the lowest activities. The study of the linear correlation between the paired samples showed a strong correlation ($r \geq 0.913$), positive and highly significant ($p \leq 0.01$) between the inhibitory effect of the radical DPPH and the chelation effect of ferrous iron for the three fractions (table 5).

Toxicity study on bacterial cells

The pH of the ethanolic extract and the three other fractions was between 5.47 and 6.67 (n = 3). According to the manufacturer, the pH of the HM medium is 6.6±0.2 which is statistically ($p \leq 0.05$) similar to the obtained values. The addition of these extracts does not therefore significantly modify the pH of the HM medium. The extracts were tested against several targets, each with particular cell structures and metabolism (table 6).

The $CHCl_3$ fraction showed a bacteriostatic effect on the majority of the strains with MICs greater than or equal to 100 mg/mL and MBC >100 mg/mL. The only bactericidal effect observed was on *S. aureus*, *PN 15* and *B. cereus* strains (table 6). The EtOAc fraction showed a bactericidal effect on all the bacterial strains with the lowest values of MIC and MBC recorded for *S. aureus* strains. The effect of the MeOH fraction depends on the strain tested. The bactericidal effect was mainly observed in the case of *B. cereus*, *P 116* and *S. aureus* (S).

Table 7 showed a good effect of the EtOAc fraction against the nine strains with an CI_{50} not exceeding 40 mg/mL, followed by MeOH fraction with moderate activity, and finally the $CHCl_3$ fraction. Furthermore, it was noted that *S. aureus* and *B. cereus* were the most susceptible to the antibacterial effect of the EtOAc and MeOH fractions. However, $CHCl_3$ fraction showed more antibacterial effect on *E. coli* (S) followed by *B. cereus*.

Table 1: The bacterial strains used

Bacterial stains	Characteristics of bacteria		
	According to requirement	According to Gram	According to the sensitivity profile towards antibiotics
Staphylococcus aureus (PN15)	Non-demanding bacteria	Positive	Food isolates
Staphylococcus aureus 25923			Reference stains
Listeria monocytogenes 4032			
Bacillus cereus ATCC 14579			
Escherichia coli ATCC 25929		Negative	
Escherichia coli (TF2)			Food isolates
Pseudomonas aeruginosa			
Pseudomonas aeruginosa 195			
Salmonella enterica			

ATCC: American type culture collection.

Table 2: Total phenolic content, flavonoid content and antioxidant activity in the three organic fractions of *R. damascena* petals.

			Antioxidant Properties (CI_{50} Values ; mg/mL)	
	Polyphenols (mg AG/g DM)	Flavonoids (mg Qu/g DM)	DPPH	Metal Chelating Activity
$CHCl_3$	47.52±0.31 (c)	24.02±0.47 (c)	0.126±0.012 (c)	0.278±0.024 (c)
EtOAc	259.82±0.45 (a)	222.36±0.41 (a)	0.0262±0.001 (a)	0.0319±0.001 (a)
MeOH	176.17±0.33 (b)	94.62±0.52 (b)	0.0567±0.001 (b)	0.0755±0.004 (b)

Different letters in the same column indicate significant differences ($p < 0.01$) within conditions according to Tukey's multiple range Test.

Table 3: Qualitative determination of chemical groups in organic fractions

Fractions	Alkaloids	Leucoanthocyanins	Iridoids	Saponins	Anthraquinones	Anthocyanins	Deoxyoses	Aldehydes	Essential oils
$CHCl_3$	-	-	-	-	-	+	-	+	-
EtOAc	-	++	-	+	-	+	-	-	++
MeOH	-	+	-	+++	-	+	-	+	+
Total (N)	0/3	2/3	0/3	2/3	0/3	3/3	0/3	2/3	2/3
Total (%)	0	66.5	0	66.5	0	100	0	66.5	66.5

+++: Very abundant; ++: abundant; +: Presence of the metabolite; -: Absence of the metabolite

Table 4: Secondary metabolites composition of *R. damascena* petals

Standards	Retention time (min)
Ascorbic acid (vit. C)	3.2
Gallic acid	8
Ohbenzoic acid	52.13
Vanillin	58
Caffeic acid	58.5
P-coumaric acid	60.29
Syringic acid	63.5
Thymoquinon	74.77
Salicylic acid	80.7
Rutin	81.88
Naringinin	83.63
Tannic acid	84
Tocopherol	85.11
Carvacol	89.09
Limonen	89.64
Naringin	92
Hesperidin	94.1
Quercetin	101.1

Table 5: Pearson correlation table between the radical inhibitory effect of DPPH and the chelating effect of ferrous iron

		Inhibitory effect of DPPH			Chelating effect of ferrous iron		
		$CHCl_3$	EtOAc	MeOH	$CHCl_3$	EtOAc	MeOH
Inhibitory effect of DPPH	$CHCl_3$	1,000	0,756 *	0,723	0,913 **	0,767 *	0,589
	EtOAc		1,000	0,856 **	0,826 **	0,997 **	0,835 **
	MeOH			1,000	0,726	0,840 **	0,970 **
Chelating effect of ferrous iron	$CHCl_3$				1,000	0,827 **	0,589
	EtOAc					1,000	0,816 **
	MeOH						1,000

The correlation is significant at the 0.05 level (bilateral) ; ** : The correlation is significant at the 0.01 level (bilateral).

Table 6: Antimicrobial activity of the $CHCl_3$, EtOac and MeOH fractions

Bacterial strain	$CHCl_3$				EtOAc				MeOH			
	MIC (mg/mL)	MBC (mg/mL)	Report MBC/MIC	Decision according to (Oursou *et al.,* 2008)	MIC (mg/mL)	MBC (mg/mL)	Report MBC/MIC	Decision according to (Oursou *et al.,* 2008)	MIC (mg/mL)	MBC (mg/mL)	Report MBC/MIC	Decision according to (Oursou *et al.,* 2008)
E. coli	>100	>100	>1	Bacteriostatic	50	100	2	Bactericidal	25	>100	>4	Bacteriostatic
TF 2	>100	>100	>1	Bacteriostatic	100	100	1	Bactericidal	25	>100	>4	Bacteriostatic
S. aureus	50	100	2	Bactericidal	25	25	1	Bactericidal	50	50	1	Bactericidal
PN 15	100	100	1	Bactericidal	25	25	1	Bactericidal	50	50	1	Bactericidal
L. monocytogenes	>100	>100	>1	Bacteriostatic	50	100	2	Bactericidal	100	>100	>1	Bacteriostatic
P 116	100	>100	>1	Bacteriostatic	25	50	2	Bactericidal	50	100	2	Bactericidal
P. aeruginosa	100	>100	>1	Bacteriostatic	50	100	2	Bactericidal	100	>100	>1	Bacteriostatic
B. cereus	100	100	1	Bactericidal	25	50	2	Bactericidal	50	50	1	Bactericidal
S. enterica	>100	>100	>1	Bacteriostatic	50	100	2	Bactericidal	25	>100	>4	Bacteriostatic

Table 7: IC_{50} values of antibacterial activity of *R. damascena* fractions against nine bacterial species.

	CI_{50} Mean ± SD (mg/mL)								
	P. aeruginosa	*P 116*	*E. coli*	*TF 2*	*S. aureus*	*PN 15*	*S. enterica*	*B. cereus*	*L. monocytogenes*
$CHCl_3$	80.42±0.43 (c)	99.22±0.41 (c)	21.37±0.24 (a)	167.47±0.48 (c)	47.63±0.24 (c)	70.51±0.22 (c)	90.95±0.35 (c)	26.97±0.53 (c)	49.72±0.31 (b)
EtOAc	16.63±0.28 (a)	30.04±0.34 (a)	38.93±0.33 (c)	28.18±0.35 (a)	10.11±0.28 (a)	14.06±0.35 (a)	23.48±0.35 (a)	15.35±0.21 (a)	20.64±0.32 (a)
MeOH	47.69±0.36 (b)	39.98±0.38 (b)	24.81±0.33 (b)	30.80±0.40 (b)	21.29±0.25 (b)	27.15±0.53 (b)	27.61±0.26 (b)	21.48±0.33 (b)	49.38±0.21 (b)

Different letters in the same column indicate significant differences.

Acute toxicity study

The intraperitoneal injection of a dose of 1000 mg/kg of the ethanolic crude extract in rats, results in abdominal contortion immediately after the injection, followed by severe clonic seizure and death. These symptoms suggest the presence of toxic principles that act on the nervous system. The administration of the sub-lethal dose also caused abdominal contortion just after the injection; however, 10 to 15 minutes later, the rats became progressively normal again.

Table 8: Study of the acute toxicity of the CrE of *R. damascene*

Lethal dose (mg/mL)	CrE
LD_0	150
LD_{50}	708.97
LD_{100}	>1000

The value of the LD_{50} (24 h) was determined according to the method of Reed and Muench (1938). Six geometrically progressive doses ranging from LD_0 = 100 mg/kg to LD_{100} = 1000 mg/kg were used. The results are shown in table 8. The value of LD_{50} (24h) = 708.97 mg/kg determined graphically was qualified as a low toxicity according to the classification of Hodge and Sterner (1980).

Hemolysis test

Red blood cells were chosen as a model for the assessment of the cytotoxicity because of their simple isolation (Pagano and Faggio, 2015). The results in Table 9 indicated that the hemolytic activity of the CrE and the three fractions varies depending on their concentration. The total hemolysis of red blood cells was observed at a concentration of 10 mg/mL. However, partial hemolysis occurred at concentrations greater than or equal to 2.5

Table 9: Effects of crude extracts and their fractions at different concentrations on human erythrocytes

Concentration (mg/mL)	10	5	2.5	1.25	0.62	0.31	0.15	0.07	0.03	0.01	C-
CrE	++	+	+	-	-	-	-	-	-	-	-
CHCl3	++	+	+	+	+	+	+	+	-	-	-
EtOAc	++	+	+	+	+	+	-	-	-	-	-
MeOH	+	+	-	-	-	-	-	-	-	-	-

CrE: Crude extract; C-: Negative control; ++ : Total hemolysis; + : Partial hemolysis; - : Absence of hemolysis.

mg/mL and no hemolytic activity was observed at concentrations less than 0.31 mg/mL. The MeOH fraction showed the lowest hemolytic activity followed by the EtOAc fraction.

DISCUSSION

Phytochemical analysis of *R. damascena* petals showed high levels of phenolic and flavonoid compounds in the EtOAc fraction (259.82 mg GAE/g DM and 222.36 mg QE/g DM), followed by the MeOH fraction and then the $CHCl_3$ fraction (Table 2). In a study conducted by Error! Hyperlink reference not valid. *et al*. (2004), authors showed a total phenolic content of 276.02±2.93 mg GAE/g DM in fresh flower extract and 248.97±2.96 mg GAE/g DM in spent flower extract. However, the crude methanolic extract of the petals of the same plant from Bulgaria showed a total polyphenolic content of 93.7 mg GAE/g DM (Ginova *et al*., 2013). These differences in phenolic and flavonoid contents in rose petals may be strongly influenced by environmental conditions. They can be also attributed either to the extraction method, or to the development stage of the plant.

The analysis of the chemical families in *R. damascena* showed that the distribution of secondary metabolites depends on the organic solvent used and we were able to identify the existence of essential oils, saponins, alkaloids, anthocyanins and aldehydes (table 3). The richness of the *Rosa* genius in flavonoids, anthocyanins and essential oils has been investigated in several studies conducted on different cultivars (Error! Hyperlink reference not valid. *et al*., 2004; Vinokur *et al*., 2006; Yassa *et al*., 2015). In a study conducted on two cultivars of *R. damascena*, Memariani *et al*. (2015) showed the existence of different phenolic compounds such as, gallic acid, syringic acid and quercetin which is in accordance with our study. The molecules extracted from *R. damascena* reported to have different therapeutic effects. As an example, it was showed that the major component of rose oil has a potent antimicrobial activity against some bacteria (Yassa *et al*., 2015).

The antioxidant activity of *R. damascena* fractions showed significant differences between the three fractions (table 2). Moreover, the antiradical scavenging activity and the metal chelating activity were greater in the EtOAc fraction followed by the MeOH fraction, whereas the $CHCl_3$ fraction showed the lowest activities. The study of the linear correlation between the paired samples showed a strong correlation ($r \geq 0.913$), positive and highly significant ($p \leq 0.01$) between the inhibitory effect of the radical DPPH and the chelation effect of ferrous iron for the three fractions (table 5). These results imply that antioxidants in these fractions were able to trap free radicals and chelate metal ions at the same time.

The EtOAc fraction followed by MeOH are rich in polyphenols and flavonoids, this suggests a link between their biological activities and these components. In fact, several types of polyphenols such as tannins and flavonoids like epigallocatechin, catechin, myricetin, quercetin, (Shan *et al*., 2007), luteolin (Askun *et al*., 2009) and flavanones (Barreca *et al*., 2017), were reported to have high biological activities. The results provided here suggest that *R. damascena* petal extracts are promising sources of natural antioxidants. However, further analysis should be conducted to determine the molecules responsible for the observed activity.

The antibacterial activity of the different fractions was evaluated against nine different reference strains to assess the plant antimicrobial properties; including Gram-positive and Gram-negative bacteria (table 6 and 7). The results obtained showed that EtOAc fraction has a good effect against the nine strains with an IC_{50} not exceeding 40 mg/mL, followed by MeOH fraction with moderate activity, and finally the $CHCl_3$ fraction. Moreover, we observed that *S. aureus* and *B. cereus* were the most susceptible to the antibacterial effect of both EtOAc and MeOH fractions. For the $CHCl_3$ fraction, more antibacterial effect against *E. coli* (S) followed by *B. cereus* was observed. Shohayeb *et al*. (2014) tested the antibacterial activity in a fractionation system using ethanol, chloroform, ethyl acetate and butanol and found that the ethyl acetate fraction of *R. damascena* petals was the most active compared to the other fractions. They also showed that gram + bacteria were more susceptible to this fraction. According to the same study, *S. aureus* and *B. subtilis* were the most sensitive and the antibacterial activity of the chloroform fraction was lower compared to the other fractions (Shohayeb *et al*., 2014). In another study, Ozkan *et al*. (2004) found that *B. cereus*, *E. coli*, *E. coli O157*: *H7*, *P. aeruginosa*, *S. enteritidis*, and *S. aureus*

strains were susceptible to the methanolic extract of *R. damascena*. The presence of different flavonoids and phenolic acids in the petals of *R. damascena* may be responsible for the observed activities. In fact, different studies have reported an antibacterial effect of gallic acid, p-coumaric acid, caffeic acid and p-Hydroxybenzoic acid (Yagasaki *et al*., 2000; Kim, 2007; Lou *et al*., 2012; Heleno *et al*., 2013). On the other hand, flavonoids were reported to have the ability to alter cytoplasmic and outer membrane of bacteria and may also show activity of reducing the harm of bacteria to the host organism (Paolillo *et al*., 2011; Eumkeb and Chukrathok, 2013).

The acute toxicity of the ethanolic crude extract was conducted on rats and suggested the presence of toxic compounds that at high doses (1000 mg/kg) cause severe clonic seizure and death. However, injection of sub-lethal doses causes abdominal contortion just after the injection; however, 10 to 15 minutes later, the rats became progressively normal again. The various symptoms associated with the involvement of the central nervous system may be due to the presence of alkaloids known by their neurological effects (Wink, 2015).

The LD_{50} (24 h) which was of 708.97mg/kg was qualified as a low toxicity according to the classification of Hodge and Sterner (1980). This LD_{50} is much higher than 25 mg/kg, which according to Ramade (1979) represents the LD_{50} of a very toxic substance. Our results are in agreement with the study conducted by Achuthan *et al*. (2003) who showed that the oral administration of the partially purified acetone fraction (AF) of *R. damascena* at 50 mg/kg body weight could protect against hepatotoxicity induced by CCl4 in rats. This is consistent with our results for which the LD_0 dose was 150mg/kg (table 8).

The hemolytic test revealed low activity for the MeOH fraction followed by the EtOAc fraction (table 9). However, the $CHCl_3$ fraction had higher hemolytic activity compared to the crude extract and the two others fractions. Surprisingly, the presence of saponins, which are known for their hemolytic activity, were not been demonstrated during the phytochemical screening of $CHCl_3$ whereas their presence has been detected in EtOAc and MeOH fractions. This could be explained by the higher degree of purification of this fraction and its high concentration in chemical molecules with hemolytic potency.

CONCLUSION

In the present study, MeOH and EtOAc fractions from petals of *R. damascena* appear to be of real and potential interest by their antioxidant activities. These extracts showed a chelating power relatively similar to that of quercetin. The microbiological study of the three fractions ($CHCl_3$, EtOAc and MeOH) of the *R. damascena* plant showed a high activity of the EtOAc fraction which possesses bactericidal properties, followed by a moderate activity of the methanolic fraction. However, there was low activity for the $CHCl_3$ fraction which showed a bacteriostatic effect. The most sensitive strains were *S. aureus* and *B. cereus* while the most resistant were *P. aeruginosa* and *E. coli* (R). These results may be related to the quantitative and / or qualitative diversity of the compounds present in the fractions tested.

Based on the biological toxicity tests conducted on erythrocytes isolated from human blood and on a warm-blooded animal model, we found that the petals of this *R. damascena* were weakly toxic and can be therefore very important as diet and for clinical use without toxicity.

This study contributes to the knowledge of chemical composition and toxicity of *R. damascene*. Also, the antioxidant properties of this species seem to be the basis of her potential usefulness as medicinal plant and offer prospects for the preventive treatment of pathogenesis under conditions associated with aging and other conformational disorders.

REFERENCES

Achuthan CR, Babu BH and Padikkala J (2003). Antioxidant and hepatoprotective effects of *Rosa damascena*. *Pharm. Biol*., **41**(5): 357-361.

Askun T, Tumen G, Satil F and Ates M (2009). *In vitro* activity of methanol extracts of plants used as spices against *Mycobacterium tuberculosis* and other bacteria. *Food Chem*., **116**(1): 289-294.

Barreca D, Gattuso G, Bellocco E, Calderaro A, Trombetta D, Smeriglio A, Lagana G, Daglia M, Meneghini S and Nabavi SM (2017). Flavanones: citrus phytochemical with health-promoting properties. *Bio. Factors*, **43**(4): 495-506.

Ben Mrid R, Bouchmaa N, Bouargalne Y, Ramdan B, Karrouchi K, Kabach I and Nhiri M (2019). Phytochemical characterization, antioxidant and *In vitro* Cytotoxic Activity Evaluation of *Juniperus oxycedrus* Subsp. *oxycedrus* needles and Berries. *Molecules*, **24**(3): 502.

Dalfardi B, Heydari M, Golzari SE, Nezhad GSM and Hashempur MH (2014). Al-Baghdadi's description of venous blood circulation. *Int. J. Cardiol*., **174**(1): 209-210.

Eumkeb G and Chukrathok S (2013). Synergistic activity and mechanism of action of ceftazidime and apigenin combination against ceftazidime-resistant *Enterobacter cloacae*. *Phytomedicine*, **20**(3-4): 262-269.

Ginova A, Mihalev K and Kondakova V (2013). Antioxidant capacity of petals and leaves from different rose (*Rosa damascena* Mill.) Plantations in Bulgaria. *Int. J. Pure App. Biosci*., **1**(2): 38-43.

Hasim K, Ahmet C and Serkan S (2008). Determination of phenolic composition and antioxidant capacity of

blood orange juices obtained from cvs. Moro and Sanguinello (*Citrus sinensis* (L.) Osbeck) grown in Turkey. *Food Chem.,* **107**(4): 1710-1716.

Hasim K, Serkan S, Ahmet C and Turgut C (2009). HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. *Microchemical. J*., **91**(2009): 187-192.

Heleno SA, Ferreira ICFR, Esteves AP, Ciric A, Glamočlija J, Martins A, Sokovic M and Queiroz MJRP (2013). Antimicrobial and demelanizing activity of *Ganoderma lucidum* extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. *Food Chem. Toxicol*., **58**(2013): 95-100.

Hodge AC and sterner JH (1980). Etudes de toxicite: quelques donnees fondamentales (A.K. DONE). *Tempo Medical Afrique.*, **7**(1980): 18.

Kim YJ (2007). Antimelanogenic and antioxidant properties of gallic acid. *Biol. Pharm. Bull*., **30**(6): 1052-1055.

Lesjak MM, Beara IN, Orcic DZ, Anackov GT, Balog KJ, Francicsovic MM and Mimica-Dukic NM (2011). *Juniperus sibirica* Burgsdorf. as a novel source of antioxidant and anti-inflammatory agents. *Food Chem*., **124**(3): 850-856.

Lou Z, Wang H, Rao S, Sun J, Ma C and Li J (2012). p-Coumaric acid kills bacteria through dual damage mechanisms. *Food control*, **25**(2): 550-554.

Mahboubi M (2016). *Rosa damascena* as holy ancient herb with novel applications. *J. Tradit. Complement. Med*., **6**(1): 10-16.

Mann CM and Markham JL (1998). A new method for determining the minimum inhibitory concentration of essential oils. *J. Appl. Microbiol.,* **84**(4): 538-544.

Memariani Z, Amin G, Moghaddam G and Hajimahmoodi M (2015). Comparative analysis of phenolic compounds in two samples of *Rosa damascena* by HPLC. *Int. J. Biosci.,* **7**(1): 112-118.

Nayebi N, Khalili N, Kamalinejad M and Emtiazy M (2017). A systematic review of the efficacy and safety of *Rosa damascena* Mill. with an overview on its phytopharmacological properties. Complement *Ther. Med*., **34**(2017): 129-140.

Ozkan G, Sagdiç O, Baydar NG and Baydar H (2004). Antioxidant and antibacterial activities of *Rosa Damascena* flower extracts. *Food. Sci. Technol. Int.,* **10**(4): 277-281.

Pagano M and Faggio C (2015). The use of erythrocyte fragility to assess xenobiotic cytotoxicity. *Cell. Biochem. Funct.,* **33**(6): 351-355.

Paolillo R, Carratelli CR and Rizzo A (2011). Effect of resveratrol and quercetin in experimental infection by *Salmonella enterica* serovar Typhimurium. *Int. Immunopharmacol.,* **11**(2): 149-156.

Parekh J, Darsharna J and Chanda S (2007). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antimicrobial activity. *Turk. J. Bio.* **29**(2007): 203-210.

Ramade F (1979). Ecotoxicologie, Ed., Masson, Paris, Switzerland, p.5.

Ramdan B, Ramdan R, El Karbane M, El Maadoudi M, Ben Mrid R and Nhiri M (2019). Anti-glycation study of hydro-alcohol and aqueous extracts of Moroccan plant species. Nhiri M *et al*., *Int. J. Res. Pharm. Sci.,* **10**(2): 1-12.

Reed LJ and Muench H (1938). A simple method of estimating fifty percent endpoints. A*m. J. Hyg*., **27**(3): 493-497.

Shan B, Cai YZ, Brooks JD and Corke H (2007). The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. *Int. J. Food Microbiol*., **117**(1): 112-119.

Shohayeb M, El-Sayed SAH, Bazaid SA and Maghrabi I (2014). Antibacterial and antifungal activity of *Rosa damascena* MILL. essential oil, different extracts of rose petals. *Global J. Pharmacol*., **8**(1): 1-7.

Tavirani MR, Fayazfar S, Keshel SHMB, Rezaee MZR, Tavirani M and Khodarahmi R (2013). Effect of essential oil of *Rosa damascena* on human colon cancer cell line SW742. *Gastroenterol Hepatol Bed Bench*, **6**(1): 25-31.

Vinokur Y, Rodov V, Reznick N, Goldman G, Horev B, Umiei N and Friedman H (2006). Rose petal tea as an antioxidant rich beverage: cultivar effects. *Food Sci*., **71**(1): 842-847.

Wink M (2015). Modes of action of herbal medicines and plant secondary metabolites. *Medicines*, **2**(3): 251-286.

Yagasaki K, Miura Y, Okauchi R and Furuse T (2000). Inhibitory effects of chlorogenic acid and its related compounds on the invasion of hepatoma cells in culture. *Cytotechnology*, **33**(1-3): 229-235.

Yassa N, Masoomi F, Rankouhi SR and Hadjiakhoondi A (2015). Chemical composition and antioxidant activity of the extract and essential oil of *Rosa damascena* from Iran, population of Guilan. *DARU*, **17**(3): 175-180.