https://doi.org/10.48047/AFJBS.6.15.2024.12175-12196

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Chemical composition and biological activities Assessment of crude extract of roots of *Paeonia algeriensis* Chabert.

Wafa Nouioua [1] Sofiane Gaamoune [2] and Ouarda Djaout [3]

[1]: Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Natural Life and Sciences, University Ferhat Abbas Setif 1, Algeria

[2] : National Institute of Agricultural Research – Setif – Algeria.

[3]: Center of Scientific and Technical Research in Physicochemical Analyzes (CRAPC), 42004 Tipaza, Algeria

nouioua.wafa@yahoo.fr

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 25 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.12175-12196

Abstract

Paeonia algeriensis Chabert is an Algerian endemic species habitually used by local people in traditional medicine. This study tried to express phytochemicals composition and biological activities of the species. CG/Ms was used to determine molecular composition, antioxidant activity was assessed by three tests (DPPH, reducing power and lipid peroxidation), antimicrobial activity was tested with four bacterial strains and one yeast, the anti-inflammatory activity was carried out by the human red blood cell (HRBC) membrane stabilization method and the *In-vivo* healing wounds activity by excision model. The result show a perfect antioxidant activity, a powerful antibacterial effect an important anti- inflammatory power at a low concentration and total healing wounds effect in just 13 days of experiment.

Keys word: *Paeonia*; lipid peroxidation; DPPH; reducing power; HBRC; antimicrobial; healing.

Introduction

The name *Paeonia* (*Paionia*) of the plant commemorating Paeon, was given to the peony by the ancient Greeks. The plant Paeonia is also known as ‘the queen of herbs’ a title deserved on account of the beauty of its flowers and its medicinal repute [1]. The genus *Paeonia* (peony) belong to the Paeoniaceae family and includes 179 scientific plant names of species rank, among which 36 are accepted species names according to The Plant List database [2]. According to Polunin, 1969, the genus *Paeonia* has been found in Asia, Europe, and North America. *P. mascula* is a circum Mediterranean species that grow in semi-shaded habitats on rich organic soils [3]. China is the central region of the origin, evolution, differentiation development, and diversity of this genus. The genus *Paeonia* is currently divided into three

sections: Sect. Moutan (shrubs that are native to China and widely distributed in East Asia, including 11 species, 2 subspecies, and 1 variety), Sect. Oanepia (herbaceous perennials that are mainly distributed in America and Mexico, including 2 species), and Sect. Paeonia (herbaceous perennials that are distributed from Spain and North Africa eastwards to China and Japan, including 22 species and 13 subspecies) [2]

In addition to ornamental utilization of peony species because of their attractive flowers, most of them have been also utilized as medicinal plants. For instance; some *Paeonia* species were recorded to be used against internal diseases, pains, and epilepsy [4]. The roots of the plant have been used traditionally for their healing and antiseptic activities, for cleansing wounds and as an antispasmodic drug [5].

Paeonia species are known to be a rich source of monoterpenes possessing a 'cagelike' pinane skeleton [6]. Furthermore, other monoterpenoids, flavonoids, polyphenols (including phenol and gallic acid derivatives), steroids, and triterpenoids were previously described in this genus [7].

The purpose of this study was to develop the chemicals and biological powers of the peony Algerian species (*Paeonia algeriensis* Chabert).

Material and method:

Plant material:

Roots of *Paeonia algeriensis* Chabert = *Paeonia mascula* subsp. *atlantica* (Coss.) Greuter & Burdet, were taken from Kefrida forest at 36° 34′ 14″ N, 5° 17′ 24″ E, determined and dried under shad for ulterior use.

Preparation of extract

Leaves of the three species were powdered and macerated three times in 80% methanol (the best extraction solvent) for 24 hours each, at the laboratory temperature (1:10 w/v, 10 g of dried herb). The resulting extracts were collected, filtered and evaporated under vacuum. The dry extract was stored at a temperature of -18 °C for later use.

Determination of Total Phenolic Content

For total polyphenol dosage, Foline Ciocalteu method was used [8]. Briefly, 0.2 mL of extract was mixed with 1 mL of the diluated Folin-Ciocalteu (1/10 v/v). The solutions were allowed to stand for 4 minutes at 25 °C before 0.2 mL of a saturated sodium carbonate solution (75 mg/mL) was added. The mixed solutions were allowed to stand for another 120 minutes before the absorbance was measured at 765 nm. For the calibration, Gallic acid was used as a standard. Polyphenols content was indicated as mg equivalent of Gallic acid per grams of dry extract (mg EAG/GE).

Determination of total flavonoids content

The flavonoids content in crude extract were estimated by the Aluminium chloride solution according to the method described by Bahorun *et al*., (1996) [9]. Briefly, 1 mL of the methanol solutions of the extracts were added to 1 mL of 2 % $AlCl_3$ in methanol. After 10 minutes, the absorbance was determined at 430 nm. Quercetin was used as a standard. Results were expressed as mg equivalent Quercetin per gram of extract (mg EQ/GE).

GC-MS

Analyses were carried out on a Hewlett Packard Agilent 6890 plus gas chromatograph, coupled with a Hewlett Packard Agilent 5973 mass spectrometer, equipped with a 30m HP-5MS (Agilent Technology, Inc. Wilmington, DE 9808-1610) capillary column (5% phenyl and 95% dimethyl-polysiloxane) with 0.25mm i.d and 0.25 mm film thicknesses. Injector temperature was set to 250 ºC; the organic layer solution (1 mL) was introduced into the column in the split mode (10:1) and the helium carrier gas flow rate was set at 0.5 ml/min. The oven program temperature was first fixed to 150 ºC for 2 min then increased to 250 ºC at 50 ºC/min and held for 2 min with a total run time of 6 min. The mass spectrometer detector (MSD) was used in selected ion monitoring (SIM) mode and a signal at m/z 144 was recorded. The volatile molecules were identified according to the NIST 02L database and confirmed by the external standard.

DPPH Assay

The donation capacity of extract was measured by bleaching of the purple-coloured solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato et al., (1998) [10]. One millilitre of the extract at different concentrations was added to 0.5 mL of DPPH-methanol solution. The mixtures were shaken vigorously and left standing at the laboratory temperature for 30 minutes in the dark. The absorbance of the resulting solutions was measured at 517 nm. The antiradical activity was expressed as IC_{50} (micrograms per millilitre), calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/ A_0] \times 100$$

Where:

A_0: the absorbance of the control.

A_1: absorbance of the sample. Butylated hydroxytoluene (BHT) was used as standard [11].

Reducing power

The method of Oyaizu (1986) [18] was used to carry out the reducing power activity. Briefly, 2,5 mL of the extract was mixed with 2.5 mL of sodium phosphate buffer (pH 6.6 ; 200

mmol/L) and 2.5 mL of potassium ferricyanide (10 mg/mL). The mixtures were incubated at 50 °C for 20 minutes. After cooling, 2.5 mL of trichloroacetic acid (100 mg/mL) were added; the mixtures were centrifuged at 200g for 10 minutes. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 1 mg/mL ferric chloride, and the absorbance was measured at 700 nm against a blank. EC50 value (mg extract/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. BHA was used as a reference standard [12].

Inhibition of lipid peroxidation

Inhibition of lipid peroxidation in the egg was determined by the method of (Ohkawa et al., 1979) [13]. Egg homogenate (0.5 ml, 10% in distilled water, v/v) and 0.1 ml of each fraction were mixed separately in a test tube and the volume was made up to 1 ml, by adding distilled water.

Finally, 0.05 ml FeSO4 (0.07 M) was added to the above mixture and incubated for 30 min, to induce lipid peroxidation.

Then, 1.5 ml of 20% acetic acid, 1.5 ml of 0.8% TBA (w/v) in 1.1% sodium dodecyl sulfate (SDS) and 0.05 ml 20% TCA were added, vortexed and heated in a boiling water bath for 60 min. the final mixtures were cooled , mixed with 5.0 mL of butanol and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm [14].

Antimicrobial screening:

Bacteria Strains were obtained from the American Type Culture Collection: Gram-positive bacteria (*Bacillus subtilis* ATCC6633 and *Methicillin-resistant Staphylococcus aureus* (MRSA) *ATCC 43300*), Gram-negative bacteria (*Klebsiella pneumonia* ATCC700603 and *Escherichia coli ATCC25922)* and one yeast: *Candida albicans* ATCC1024. Muller Hinton agar and Sabouraud were used for bacteria culture and yeast respectively.

Antibacterial assay

Agar disc diffusion method was employed for the determination of antibacterial activities of the extracts [15] [16]. Briefly, a suspensions of the tested microorganism (10^8 CFU / mL) were spread on the solid media plates. Then, filter paper discs were disposed on the surface impregnated with 10 µL (100 mg/mL) of the extract and incubated for 24 hours at 37 C°. Gentamicin (10 µg/disc) was used as a standard and dimethyl sulfoxide (DMSO) as a control.

The parameters explained by Alves *et al*. (2000) [17] were taken in consideration (< 9 mm inactive, 9 – 12 mm less active, 13 – 18 mm active and > 18 mm very active).

Antifungal assay

Disc diffusion method was chosen for the antifungal activity experiments with slight modifications [15]. *Candida albicans* suspension was obtained in physiological saline 0.9 % from a culture in Sabouraud (incubated before 24 hours at 37 °C), adjusted to 10^5 CFU / mL.

Strain yeast suspension (100 µL) were spread out over petri dishes. Then, impregnated paper discs (contain 10 µL of extract) were disposed on the surface. Amphotericin and dimethylsulfoxide DMSO were used as standard and control. Inhibition zones were determined after incubation at 27 °C for 48 hours.

Anti-inflammatory activity (Human Red Blood Cell membrane stabilization method)

Human Red Blood Cell membrane stabilization method (HRBC) suspension was prepared by washing blood cells thrice. The volume of blood was reconstituted as 10 % v/v suspension with normal saline.

The principle involved here was stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The mixture contain 1 mL phosphate buffer (pH 7.4, 0.15 M), 2 mL hypo saline (0.36 %), 0.5 mL HRBC suspension (10 % v/v) and 0.5 mL of plant extract or DICLOFENAC SODIUM. The control was distilled water to produce 100 % haemolysis.

The mixtures were incubated at 37 °C for 30 minutes and centrifuged at 2500 rpm for 5 minutes. The absorbance of haemoglobin content in the suspensions were estimated at 560 nm. The percentage of haemolysis of HRBC membrane can be calculated as follows:

Haemolysis (%) = (Optical density of Test sample / Optical density of Control) ×100

However, the percentage of HRBC membrane stabilization can be calculated as follows:

Protection (%) = 100 – [(Optical density of Test sample / Optical density of Control) × 100] [18].

Healing wounds activity:

- **Ointments and Animals Groups**

Wound healing activity of the freshly made ointment was prepared with crude methanolic extract of the roots of *Paeonia algeriensis* mixed with soft paraffin according to the protocol

of Okore *et al*. (2004) [19]. The negative control was left untreated while the standard was Madecassol 1% hydrocotyl cream (Bayer).

The animals (New Zealand breed rabbits (*Oryctolagus Cuniculus*)) weighing 1.4 – 1.6 kg were acquired from Specialized Agricultural Medium Technology Institute. The animals were placed in individual cages, kept under standard condition (12h/12h dark and light, controlled temperature environment (18 to 20 C°) and were fed *ad libitum*).

Nine rabbits were grouped into three groups of three animals each as follows:

Group A: Served as the reference standard treated with Madecassol;

Group B: Considered as control;

Group C: animals were treated with the 1% w/w methanol crude extract of the roots of *Paeonia algeriensis.*

- **Surgical procedure (excision wound model)**

The dorsal shaved skin area of the tested animals were cut a full thickness wounds circle of 27mm × 27mm under sterile conditions and local anaesthesia according to the method described by Biswas et al., 2004 [20] with modifications.

The percentage of reduction in wounds area were expressed by the following equation:

Wound healing (%) = (Healed area/ total area) ×100.

Percentage of area contraction = 100 - Wound healing (%).

Wound healing activity was assessment by wound contraction percentage. The wound area was measured each three day during fifteen days, by placing a transparent paper over the wound and tracing it out [21], area of this impression was calculated using the Digimizer version 4.0.0.0

Statistical analysis

Results were expressed as the mean ± standard deviation. Data was analysed using t test of Student and Fisher test with the criterion of $P < 0.05$ to determine any significant differences

between the crude extract and standards. The used software was Graphpad prism 8 Demo Software.

Results:

The used extraction method give 54.8 % of yield of which 16,27±0,81 mg EAG/gE of polyphenols and 6,44±0,02 mg EQ/gEof flavonoids. However, CG/Ms analysis detect 14 molecules (table 1), three of which are majority molecules: Benzoic acid (42.7038 %), 5-Hydroxymethylfurfural (28.75 %) and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (16.9304 %). Others are in minors quantities: 3-Isopropyl-4-methyl-1-pentyn-3-ol (3.1335%), 4H-1-Benzothiopyran-4-one, 2,3-dihydro-8-methyl (2.1476) and 6,7-Dioxabicyclo[3.2.2]nonane (1.1522 %). The rest of the molecules are in trace amounts(figure 1).

Table 1: molecular composition of methanolic crude extract of the roots of *Paeonia algeriensis.*

Retention Time	Compound Name	Area	CAS	Area Percent
14.103	Furaneol	401121.6	3658-77-3	1.0748
14.32	6,7-Dioxabicyclo[3.2.2]nonane	590585.2	283-35-2	1.5824
14.527	Octadecane, 6-methyl-	65566.4	10544-96-4	0.1757
14.737	Propanoic acid, 2-oxo-, methyl ester	430028	600-22-6	1.1522
15.073	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6318638	28564-83-2	16.9304
15.173	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	285634	28564-83-2	0.7653
15.847	Benzoic acid	15937580	65-85-0	42.7038
16.01	5-Hydroxymethylfurfural	10729850	67-47-0	28.75
16.167	3-Isopropyl-4-methyl-1-pentyn-3-ol	1169451	5333-87-9	3.1335
17.033	1,6:3,4-Dianhydro-2-O-acetyl-á-d-galactopyranose	353376.8	0	0.9469
17.637	Ethyl 2-hydroxybenzyl sulfone	85107.2	53380-27-1	0.228
22.53	4H-1-Benzothiopyran-4-one, 2,3-dihydro-8-methyl-	801516	29373-02-2	2.1476
26.233	n-Hexadecanoic acid	73404	57-10-3	0.1967
28.913	Phenylglyoxylic acid, 2-methylbutyl ester	79370	0	0.2127

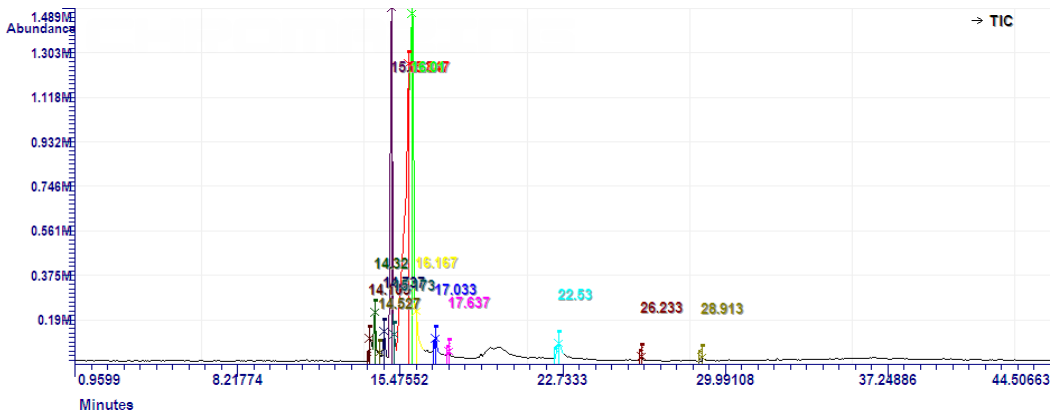

Figure 1. GC-MS chromatogram of methanolic crude extract of the roots of *Paeonia algeriensis*

Antioxidants act as a radical scavenger, electron donor, hydrogen donor, peroxide decomposer, enzyme inhibitor, synergist, and metal chelating agent [22]. The antioxidant activity of methanolic crude extract of the roots of *Paeonia algeriensis* determined by DPPH free radical was recognized to be due to the hydrogen or electron donating abilities of phytochemicals (figure2).

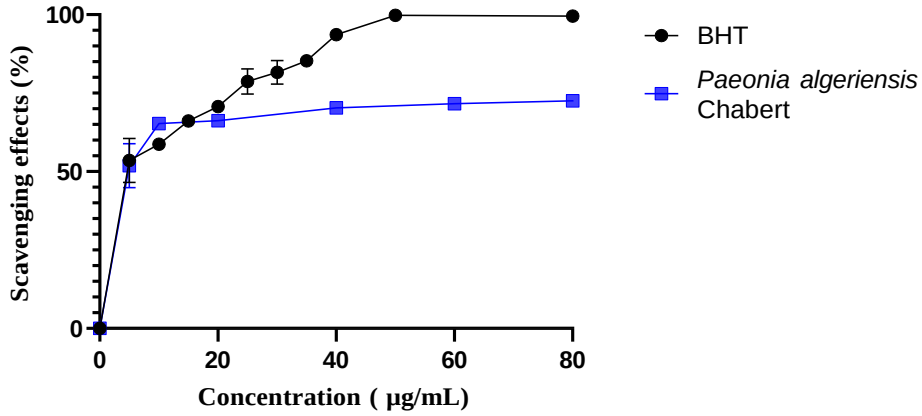

Figure 2: scavenging effect of DPPH and methanolic crude extract of the roots of *Paeonia algeriensis*

The scavenging ability of the extract was found to be a concentration dependent manner reach a maximum of 75, 91±2, 00 %**** at 200 µg/mL compared to 99,81±0,05 % at 50 µg/mL. However the IC_{50} of the extract was much better than BHT: 1,92±0,49 µg/mL** compared to 5,48±1,01 µg/mL in the case of BHT.

Assessment of potassium ferricyanide colorimetric is another method for antioxidant activity evaluation of methanolic crude extract of the roots of *Paeonia algeriensis*. The obtained results are recapitulated in figure 3:

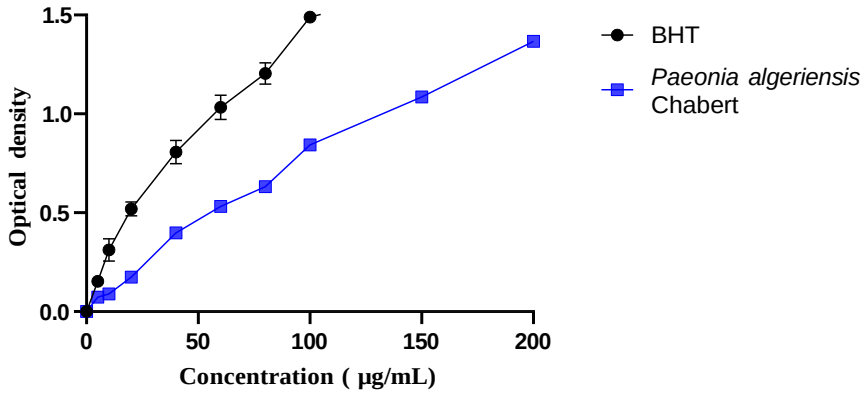

Figure 3: reducing power of standard and methanolic crude extract of the roots of *Paeonia algeriensis*

The EC_{50} of the extract was relatively low compared to the standard (62,40±0,76 µg/mL****) against 15,00±0,36 µg/mL in the case of BHA.

Lipid peroxidation is one of the most important outcomes of free radical-induced tissue damage. Oxidative processes initiate the peroxidation of lipids with an increase in MDA concentrations [23]. MDA is a sensitive biomarker of the level of membrane lipid oxidation induced by the overwhelming release of free radical [24]. The result of crude extract of the roots of Paeonia algeriensis against MDA formation are shown in figure 4:

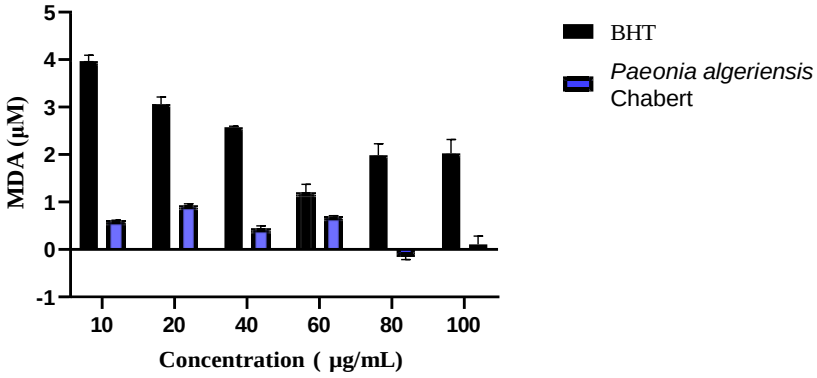

Figure 4: lipid peroxidation test malondialdehyde (MDA) of standard and methanolic crude extract of the roots of *Paeonia algeriensis.*

The antimicrobial activity of methanolic crude extract of the roots of *Paeonia algeriensis* reveals an important results: 17,50±1,08 mm, 11,67±0,24 mm and 15,33±1,25 mm of inhibition area against MRSA, *Bacillus subtilis* and *Klebsiella pneumonia* respectively (figure 5), compared to 30,00±0,82 mm in MRSA (using Vancomycin), 15,92±0,28 mm in case of *Klebsiella pneumonia* and 29,95±0,30 mm in the case of *Bacillus subtilis* (using Gentamycin).

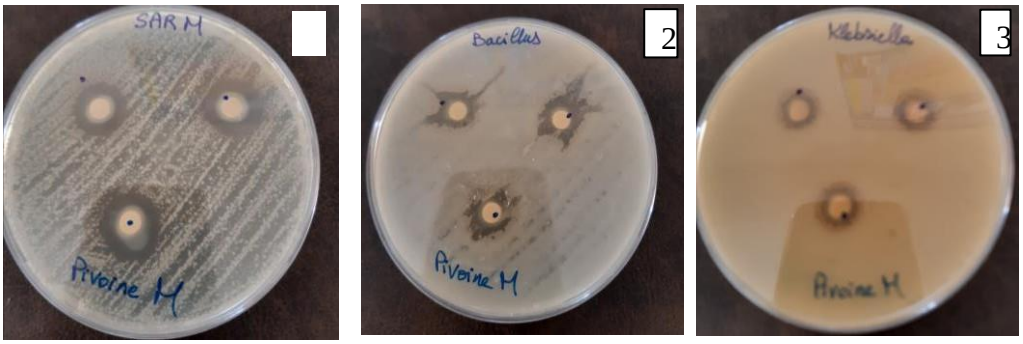

Figure 5: antibacterial activity of methanolic crude extract of the roots of *Paeonia algeriensis* against: (1) *MRSA*, (2) *Bacillus subtilis* and (3) *Klebsiella pneumonia*

Inflammation is the tissue or organ injury resulting in symptomatic pain, swelling, temperature, and redness [25]. Therefore, associated with membrane lipid peroxidation and several sequential pathological conditions. Therefore, regulation of lysosomal membrane damage is the rate-limiting step in minimalizing the inflammation. Several plant-derived compounds and their secondary metabolites mediate to synthesized nanoparticles which enhances the cell interactions to stabilize the cell membrane [25]. In the current study, the crude extract of the roots of *Paeonia algeriensis* show significant membrane stabilization activity in the presence of hypotonic solution. Under hypotonic stress, haemolysis was inhibited by increasing concentrations of this extract until 100 µg/mL, beyond this concentration haemolysis increased until 83,93±4,34 %**** at 500 µg/mL compared to the standard 3,76±0,10 % at the same concentration .

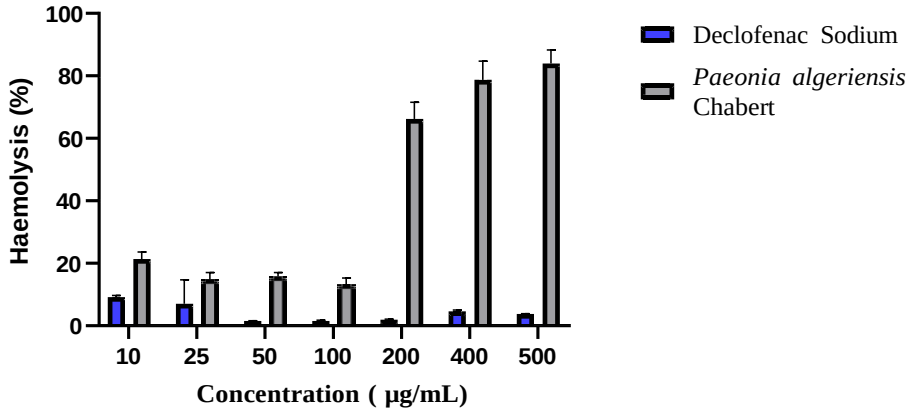

Figure 6: The percentage inhibition of hypotonicity induced haemolysis of HRBCs (%) of standard and crude extract of the roots of *Paeonia algeriensis*

The wound healing activity was evaluated by excision wound model and the experimental animals were observed for 15 days (table 2, figure 6).

Table 2: Wounds contraction area in mm^2 of three treated rabbits groups.

Time in days	Group of Madecassol 1% (wounds surface in mm^2)	Group of *Paeonia algeriensis* ointment (wounds surface in mm^2)	Group of Control (wounds surface in mm^2)
1	301,17±9,14	394,97 ±63,03	687,82±24,48
3	225,33±13,34	266,52 ±106,17	347,94 ±17,15
6	211,87±17,06	141,24 ±92,90	292,59±44,05
9	52,60±1,20	11,88±5,90	115,97±4,72
12	10,47±2,32	2,40±1,74	71,75± 6,97
15	1,95±0,98	0,00±0,00	37,06±1,00

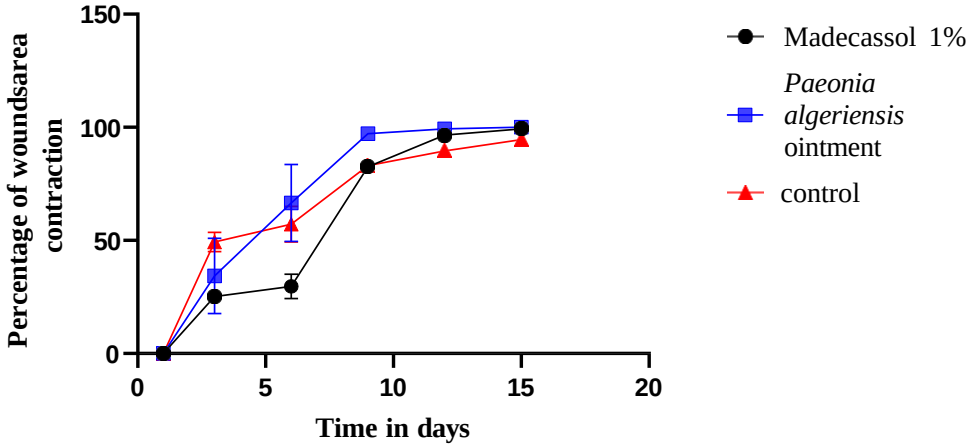

Figure 7: percentage of wounds contraction in the three experimental groups (standard, control and *Paeonia algeriensis* ointment)

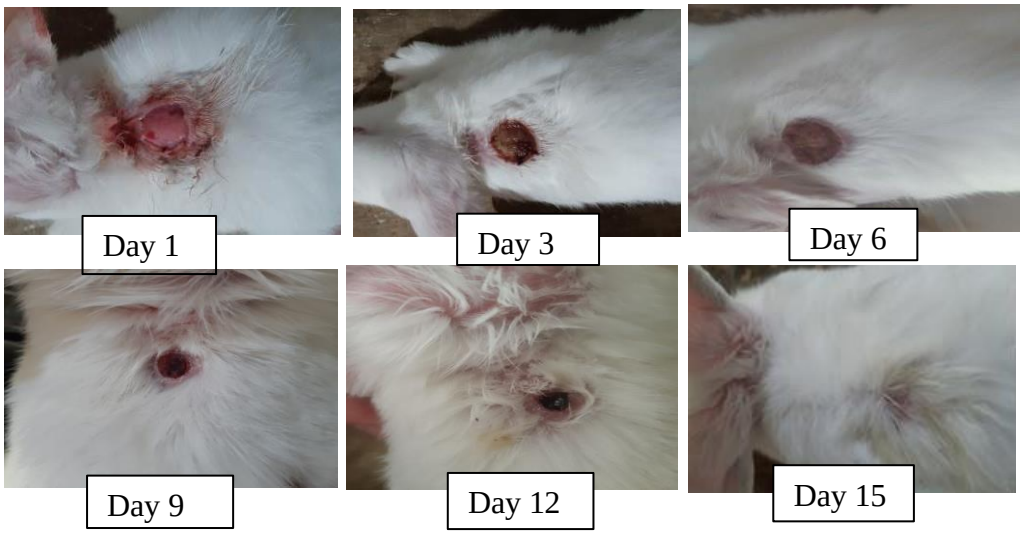

Figure 8: healing wound during 15 days of experiment of the rabbits group treated with *Paeonia algeriensis* ointment.

Crude extract of the roots of *Paeonia algeriensis* ointment ointments reduced significantly ($p \leq 0.05$) the level of the wound area and enhanced significantly ($p \leq 0.05$) the percent of wound contracture until the 100 % of healing (figure 8), as compared with the basal ointment and control groups.

Discussion

The tests of total phenolic content and flavonoids of crude extract of the roots of *Paeonia algeriensis* prove that there are medicinally active constituents. Furthermore, the CG/Ms analysis was used in order to investigate phytoconstituants contain in crude extract reveal the

presence of *three* majors molecule constitute 88, 38% of the total detected molecules. Benzoic acids (42.70%) is a C6–C1 aromatic carboxylic acids that serve as precursors for a wide variety of essential compounds and natural products playing crucial roles in plant fitness [26]. 5-Hydroxymethylfurfural (HMF) is the second majors molecule (28.75 %), a multifunctional molecule because it is at the same time an aromatic aldehyde, an aromatic alcohol and a furan ring system. HMF is a versatile intermediate that can be further transformed into a high value-added [27]. The compound 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) consists flavonoid fractions has received much attention for its antimicrobial activity [28]. It has been reported as a major contributor to the anticancer effects [29], mutagen antimicrobial, anti-inflammatory, and antioxidant capacity [30]. Others molecules at a low concentration were detected like Furaneol (1.0748 %) a flavour compound, Propanoic acid, 2-oxo-, methyl ester or Pyruvic acid (1.1522 %), an important organic acid, has been widely used in the food, agrochemical, and medicine fields [31] [32].

Antioxidants from natural products detoxify the toxins, removes excessive ROS, actively involves in anti-lipid peroxidation and scavenging of free radicals [33].The antioxidant activities of Crude extract of the roots of *Paeonia algeriensis* have been examined against DPPH radicals, the reducing power and lipid peroxidation tests. It might be connected to the chemical composition which includes bioactive phytochemicals.

The DPPH method was used for these studies because the DPPH free radical is stable and unaffected by certain side reactions of polyphenols, its direct measurement of inhibition, and simplicity and quick analysis [34] [35]. In this study the obtained results indicate a powerful substance better than standard explained by the presence of flavonoids and polyphenols considered as a good source of antioxidant. Moreover HMF showed an important DPPH radical scavenging activities [36].

The antioxidant activity was further proved by measuring the reduction process of ferric (Fe^{+3}) to ferrous (Fe^{+2}) under the action of extracts that transformed the yellow colour of test solution to green [37]. Reducing power assay indicate an important results but still lower than standard due to the identified phytoconstituants like DDMP as a strong antioxidant [38] and HMF [36]. However, in case of Benzoic acids, Carboxyl group is an electron-withdrawing group, which does not benefit radical scavenging [39], may explain the low capacity to reduce (Fe^{+3}) to ferrous (Fe^{+2}).

Lipid peroxidation has been reported to be a marker of oxidative stress [40] [41]. It alters the organization of the membrane by inducing changes in fluidity and permeability [42]. Lipid peroxidation of biological membranes can cause alterations in fluidity, reductions in membrane potential, increased permeability to H^+ and other ions, and eventual membrane rupture leading to release of cell and organelle contents. Cytotoxic aldehydes resulting from lipid peroxidation can block macrophage action, inhibit protein synthesis, inactivate enzymes, cross-link proteins, and can lead to the generation of thrombin [43]. In our study MDA concentration was dependent manner in standards where maximum of concentration reach 3,97±0,11mM at 10 µg/mL then decrease to 1,20±0,16 mM at 60 µg/mL. However, the extract show a value inferior to 1 mM whatever the concentration (maximum of 0,93±0,03 mM^{***} at 20 µg/mL) indicate a powerful capacity to eliminate reactive molecules such as MDA and 4-hydroxynonenal (HNE). Oxidative processes related to lipid peroxidation might have occurred in parallel with a severe depletion on lipophilic antioxidants, mainly due to carotenoids [44], and others molecules related to phytochemicals detected by CG/Ms analysis.

The result of antimicrobial activity has shown strong activity against MRSA and *Klebsiella pneumonia*. Using agar disc-diffusion method, the extract was selective against both gram-positive bacteria and gram-negative bacteria. According to the 2019 CDC report, MRSA is one of the most common antibiotic-resistant bacterial pathogens and is estimated to cause more than 323,000 cases and 10,600 deaths annually in the United States alone [45]. MRSA often results from the horizontal acquisition of *mecA* gene by the methicillin-sensitive *Staphylococcus aureus* (MSSA). The gene is encoded for penicillin-binding protein (PBP2a) that confers resistance to all b-lactam antibiotics [46]. However, *Klebsiella pneumonia* is a gram-negative bacterium responsible for pneumonia, high morbidity and mortality due to excessive neutrophil and macrophage infiltration and severe lung injury [47]. The resistance of this bacterium to antibiotic is threatening to human health [48]. Due to the complex pathogenicity of the diseases, present vaccines and treatments available for *Klebsiella* infections are currently unsuccessful [49]. Hence, *Paeonia algeriensis* potentially acts against these two publicly dangerous strains by their phytoconstituents specially DDMP [28] [30].

The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzymes which are responsible for the conversion of arachidonic acid to prostaglandins. Since human red blood cell (HRBC) membranes are similar to these lysosomal membrane components, the prevention of Hypotonicity induced HRBC membrane lysis was taken as a measure in estimating the anti-inflammatory property of various extracts [50]. Thence, the studied extract

prove an anti-inflammatory activity but no so powerful as the standard. The extract was concentration dependent manner until the concentration of 100 µg/mL, where only 13,09±2,15 %* of haemolysis was registered beyond which the extract become pro-inflammatory substance. This result is related to molecular composition of the extract, like 5-HMF's which can modulate the innate immune responses through anti-inflammatory or anti-allergic effects [51] [52]. Further, DDMP reveal anti-inflammatory power [30]. Thus, may explain the antihaemolytic effect of the extract.

The *In-vivo* study of the healing wounds power of *Paeonia algeriensis* roots crude extract was performed to find the percentage of wound closure. The wound healing is a complex process that involves the synchronization and activation of coagulatory and inflammatory events, epithelialization, fibrous tissue accretion, deposition of collagen, wound contraction, tissue granulation and remodelling [53]. During healing, contraction plays a crucial role as it decreases the dimension of the wound and hence shortens the healing time. Moreover, contraction reduces the extracellular matrix amount needed to repair the defect and helps re-epithelisation by reducing the distance travelled by migrating keratinocytes [54]. Our results indicate a remarkable wounds contraction in control group during the first three days explained probably by animals' saliva by liking. However, this process slow down during the period between 4 and 15 days of experiment. Healing process occurs by immunological activities of victim itself, but various risk factors such as infection and week immunity may cause delay in healing has brought attention to promote this process [55] [56]. Thus may explain the result of the control group.

Topical applications of the standard and *Paeonia algeriensis* ointments present an important results. In which, the extract show 100 % of contraction after 13 days compared to the standard which still unhealed until the end. Applying the extract directly on the affected wound cannot bring the desired effect as it does not stay longer on the wounded skin of the experimental animals. Ointment is necessary to achieve a sustained drug release at the application sites [57]. In the present study, the phytochemical play an important role in healing process due to their antioxidant (powerful scavenging effect) and antimicrobial activities (bacterial mutagenic and anti-inflammatory effects of DDMP [30]). Moreover, the lipid peroxidation is an important process in several types of injuries like burns, infected wound, skin ulcer etc. Hence, any drug that inhibit lipid peroxidation is believed to increase the viability of collagen fibrils, which in turn results in an increase in the strength of collagen fibre by increasing the circulation, preventing the cell damage and promoting the DNA

synthesis [58]. Thus, may explain the capacity *Paeonia algeriensis* roots crude extract to heal wounds.

Conclusion

Paeonia mascula subsp. *atlantica* (Coss.) Greuter & Burdet or *Paeonia algeriensis* Chabert is an endemic herbaceous species of peony that naturally occurs in Eastern North of Algeria. The assessment study of its roots by extracting the major phytoconstituents using diluted methanol was necessary to understand their medicinal capacities often used by the residents. Crude extract of the studied species show a perfect antioxidant capacities (DPPH test, reducing power and lipid peroxidation), an important antibacterial effect especially against MRSA and an anti-inflammatory power at a low concentrations. These activity acts synergistically to exercise a perfect healing wounds activity. More investigation are needed to reveal their secrets.

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Acknowledgements

For the authors

Funding

No funding

Ethics declarations

Ethics approval and consent to participate

This study was following European Union Guidelines (2010/63/ EU) approved by the Committee of the Algerian Association of Experimental Animal Sciences (88-08/1988).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.