Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 11, 2015

Original Article

IN VITRO EVALUATION OF BIOLOGICAL ACTIVITIES OF *PISTACIA LENTISCUS* AQUEOUS EXTRACT

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Received: 11 Jul 2015 Revised and Accepted: 22 Sep 2015

ABSTRACT

Objective: The aim of this study was to study the antioxidant potential and the antibacterial activity of leaves aqueous extract of *Pistacia Lentiscus* as well as the protective effect of this extract against the haemolysis in hypotonic condition, in oxidative stress and in the existence of saponin injury.

Methods: We studied the antioxidant capacity through the DPPH assay, H_2O_2 scavenging activity, Ferric-reducing power (FRAP) assay, total antioxidant assay and the antibacterial activity using the disc diffusion method. We also investigated the haemolytic activity with the spectrophotometric method.

Results: The result showed that the aqueous extract had a good antioxidant capacity, which was calculated as IC_{50}. IC_{50} of the aqueous extract was found to be 9.89±0.7µg/ml for DPPH scavenging, 200±18.02µg/ml for H_2O_2 scavenging assay, 54.06±12.66µg/ml for Ferric-reducing power (FRAP) and 500±22.3 µg/ml for total antioxidant capacity. The aqueous extract also inhibited the growth of the tested bacterial strains with a maximum inhibition zone of 30.33±5.5 mm observed on *Pseudomonas aeruginosa* for wood-seed and a moderate activity against all other strain. The haemolytic analysis showed that the aqueous extract is not toxic for the human erythrocytes and protects them against the oxidative and osmotic stress and also against saponin injury.

Conclusion: The results of our study suggest that the aqueous extract of leaves of *Pistacia lentiscus possess* potent anti-haemolytic activity, are a good source of natural antioxidant.

Keywords: *Pistacia lentiscus*, Antioxidant, Haemolysis.

INTRODUCTION

Natural antioxidants have attracted increasing interest mainly because of the preventive effect they exhibit against various disorder caused by oxidative stress [1]. Polyphenols are substances that are capable of reducing the oxidation. The mechanisms by which they can inhibit the process of oxidation include scavenging free radicals, chelating of free metals, and inhibition of enzymes responsible for the production of the free radicals and the prevention of antioxidant defense systems. Fatini *et al.* [2], report the anti-cancer effects of polyphenols and their ability to modulate multiple signalling pathways involved in cancer [2]. Polyphenols extracted from green tree may have great potential as clinical treatment in chronic kidney diseases [3].

There is increasing evidence that diets rich in plants could be beneficial to Man and preventive to some diseases. Thus, many individuals rely on plant products for medicinal purposes.

Pistacia lentiscus is an evergreen shrub, it is a small tree growing up to 1-8m tall, it belongs to the Anacardiacea family [4]. It is evergreen sclerophyll, largely distributed in dry/warm areas of the Mediterranean region, well adapted to hard conditions due to its exceptional high resistance, it plays a key role in phyto-stabilization of contaminated mines sites with heavy metal (Cd, Pb and Zn) [5].

A number of studies have shown beneficial effects of different parts of *Pistacia lentiscus. Pistacia lentiscus* fatty oil possesses anti-lipid properties at least in reducing total cholesterol and triglycerides [6]. Chio mastic derived from *Pistacia lentiscus* could be considered as a conglomeration of effective anti-cancer drug, attributed to their capacity to inhibit cell proliferation through extrinsic and intrinsic apoptosis signaling pathways [7]. In his study, Azalzeh [8] found that *Pistacia lentiscus* is highly effective at inhibiting larval escheatment [8]. The anti-bacterial property of *Pistacia lentiscus* is also proved [9].

Erythrocytes are highly susceptible to attack by reactive oxygen species. This oxidative stress is one of the major actions in some pathology of hemoglobin. The phytochemicals compounds can either protect erythrocytes or increase their resistance to oxidative reaction. Phenolic compounds may protect against oxidative damage by (ROS) and free radicals, as they act as exogenous antioxidant system [10].

The originality of our study lies in the fact that most researches on *Pistacia lentiscus* targeted either the essential oil which valorization and the exploitation are very expensive, or extracts by solvents, which often cannot be used because of their toxicity. The aqueous extracts offer a solution that is both easy and especially safe for Human health. Thus the aim of this this study, is to investigating *Pistacia lentiscus* aqueous extract biological activities, the *in vitro* antioxidant potential; the capacity to protect red blood cells in hypotonic condition, from oxidative injury induced condition, the activity against saponin injury and antibacterial activity against referenced bacterial strains. The total phenolic content was also determined.

MATERIALS AND METHODS

Extract procedure

The leaves, of *Pistacia lentiscus* were collected in Tizi-Ouzou, a region in the north of Algeria, in (October-December) 2014. These leaves grew up in a shadowy place, and then ground into powder (dry weight of plant). 20g of plant sample were steeped with 200 ml of distilled water in 250 ml flasks. The flasks were shacked at 8 rpm in room temperature during 24 h followed by rapid filtration through four layers of gauze and a second filtration through Watman N °01 filter paper. The resulting solution was lyophilized. The percent (w/w) extraction yield is calculated. The powder was stored at (-18 °C) in a desiccant until required. The powder was dissolved

the in the adequate solvent for the different tests, this constitutes an aqueous extract (AE).

Determination of total phenolic compounds

The total phenolic content was estimated by the Folin-Ciocalteu method [11]. In this assay, 200ul of diluted sample at 40µg/ml were added to 1 ml of Folin-Ciocalteu reagent diluted in distilled water at 1/10. After 4mn, we added 800ul of saturated sodium carbonate solution (75g/l). After 2 h of incubation at room temperature, the absorbance was measured at 765 nm. Gallic acid was used as a standard. The results were expressed as Gallic Acid Equivalent per grams of dry weight (GAE/g extract).

Determination of antioxidant capacity

Determination of DPPH free radical scavenging activity

The free radical scavenging activity, of aqueous extract of *Pistacia lentiscus* and standard reference compound (i.e. ascorbic acid), was analysed by the DPPH assay as described by Sadegh *et al.* [12]. 3.75 ml assay of varying concentrations of aqueous extract (5.75-6.5-7.25-8-8.75-9.5-10.25 µg/ml) were dissolve in ethanol, then, mixed with 0.25 ml of DPPH prepared in ethanol at 0.8 mM. The mixture was vortexes and incubated for 30mn. The optical density of the solution was measured at 517 nm and compared to a control without extract.

The DPPH radical scavenging activity was calculated from the absorption value using the following equation:

$$\text{Radical scavenging activity \%} = \frac{\text{OD CONTROL} - \text{OD SAMPLE}}{\text{OD CONTROL}} * 100$$

The IC_{50} value was determined from the graph of scavenging activity against the concentrations. This value defined as the concentration extracts necessary to inhibit 50 % of the initial DPPH radical, expressed in µg/ml.

Hydrogen peroxide scavenging activity

Hydrogen scavenging ability of the extract was estimated according to the method of Jayaprakasha *et al.* [13]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer pH 7.3. 3.4 ml of different concentration of plant extract in distillate water (200-300-400-500-600 µg/ml) were added to 0.6 ml of H_2O_2 solution. After 10mn incubation, the absorbance value was measured at 230 nm, against a blank solution containing phosphate buffer without H_2O_2. Ascorbic acid was used as a positive control. IC_{50} value was determined from the graph of scavenging activity against the concentrations. These values defined as the concentration extract which has 50 % scavenging.

Ferric-reducing power (FRAP) assay

The Ferric reducing power of sample was determined using a FRAP essay of Fejes *et al.* [14]. This method valuates the ability of a substance to reduce $Fe^{3+}$to Fe^{2+}, which is measured by the formation of the coloured complex with potassium ferricyanide that can be read by spectrophotometer at 700 nm, proportional to the amount of Fe^{2+}. For this assay, different concentration of extract (15.62-31.25-62.5-125-250-500 µg/ml) which are prepared in distilled water are mixed with 2,5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(Cn)_6$). The mixture is incubated at 50 °C for 20mn, and then 2.5 ml of trichloroacetic acid (10%) are added to the mixture, and centrifuged at 3000rpm for 10mn. We mixed the upper of the solution (5 ml) with 5 ml of distilled water. Finally, the absorbance is measured at 700 nm after addition of 1 ml of $FeCl_3$ (0.1%), the standard compound was ascorbic acid and phosphate buffer (pH 6.6) as the blank solution. Increasing absorbance of the reaction mixture indicated an increasing in the reducing power. The EC_{50} value is the effective concentration, which has the absorbance at 0.5 for reducing power [15].

Total antioxidant assay by phosphomolybdate method

The total antioxidant capacity of the extract was determined by phosphomolybdate method using ascorbic acid as the standard [16]. An aliquot of 0.1 ml of the extract (15.62-31.25-62.5-125-250-500 µg/ml) was combined with 1 ml of reagent (1.6 mM sulphuric acid, 28Mm sodium phosphate and 4Mm ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 °C for 90mn. After the samples were cooled to room temperature, and the absorbance was measured at 695 nm. An increasing absorbance of the reaction mixture indicated an increasing in the antioxidant capacity.

Haemolysis assay

Preparation of red blood cell suspension

The blood was obtained from healthy consenting donors by vein puncture and collected into tubes containing heparin as anticoagulant. Samples centrifuged immediately at 2000rpm for 10mn at 4 °C and then the plasma is discarded carefully. Erythrocytes was washed three times with phosphate buffered saline (PBS) and re-suspended in PBS to obtain desired haematocrit [17].

Aqueous extract treatment of red blood cells

In order to determine the effect of the aqueous extract of *Pistacia lentiscus* on red cell blood (RCB), 1 ml of erythrocytes suspension (2% haematocrit) was incubated with an equal volume of extract dissolved in PBS at different concentrations (1000, 500, 250, 125, and 62.5 µg/ml). Distilled water was used as positive control (100% lyses) and PBS as negative control. The mixtures was shacked gently while incubation for 30mn at 37 °C and the aliquots of reaction mixture was centrifuged at 2000rpm for 10mn at 4 °C to separate the erythrocytes. The percentage of haemolysis was determined by measuring the absorbance (A) of the supernatant at 540 nm, corresponding to haemoglobin liberation, and compared with that complete haemolysis (B) obtained with erythrocytes incubated in distillate water (100% haemolysis) in the same conditions as the extract. The haemolysis percentage was calculated using the formula: A/B*100 [18].

Protective effect of aqueous extract to haemolysis caused by saponin

To determine the effect of the aqueous extract on the haemolysis induced by saponin, 1 ml of erythrocyte suspension at 2% haematocrit was mixed with 0.5 ml of PBS solution containing aqueous extract at 1000 and 500µg/ml. Then, 0.5 ml of PBS containing 100 µg/ml of saponin was added. The essay for erythrocytes in presence of saponin without extract is realised. The reaction mixtures were shaken gently while being incubation at 37 °C for 30mn. In all of the experiments, a negative control is prepared with erythrocytes in PBS without saponin. The aliquots of reaction mixture are centrifuged at 2000 rpm for 10mn at 4 °C to separate the erythrocytes. The percentage of haemolysis was determined by measuring the absorbance of the supernatant at 540 nm and calculated as previously [18].

Determination of osmotic stability of human erythrocytes

In this test, the effect of the extract in the erythrocyte membrane stability in the osmotic pressure is determined as described by Mariana *et al.* [19] with few modifications. 40 µl of washed erythrocytes suspension were added to 1 ml of (0-0.1-0.3-0.5-0.7-0.9 and 1%), NaCl solution in distillate water (control) or 1 ml of the same concentration of NaCl solution in different concentrations of the extract (250-500-1000 and 3000µg/ml). The reaction mixture was gently shacked during the incubation at 37 °C for 30mn. Following this step, the absorbance was measured at 540 nm.

Protective effect of aqueous extract against oxidative effect caused by HOCl

In this study, the *in vitro* oxidative haemolysis of human erythrocytes was used as a model to study the free radical inducing damage of biological membrane and the protective effect of the aqueous extract of *Pistacia lentiscus*. For this, 1 ml of Red blood cells (5%) were incubated for 15mn at 37 °C with 1 ml of extract at different concentration (1000, 500, 250, 125 µg/ml).

A HOCl of a diluted solution in PBS was added, whose concentrations (0.15, 0.075 and 0.1375). This reagent contains approximatively 1:1

ratio of HOCl and is subsequently referred to as HOCl. After 30mn of incubation, an aliquot of the mixture was centrifuged at 2000rpm for 10mn. After this, we added 5 ml of PBS, and we evaluated the haemolysis by spectrophotometer at 540 nm [20].

Antibacterial susceptibility test

Bacterial strains

The antibacterial test was carried out using referenced strain: *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853) and *Enterobacter faecalis* (ATCC 49452). *Bacillus cereus* (ATCC10876), *Citrobacter freundii* (ATCC8090), *Proteus mirabilis* and *Klebsiella pneumonia* (700603) were obtained from the Laboratory of Natural Substances at the University of Tlemcen (Algeria).

Antibacterial activity

The antibacterial activity *in vitro* was screened using the disc diffusion method [21]. For this, a sterile Muller Hinton agar plates were inoculated by the method of streak with inoculums of the tested bacteria suspension. The bacteria suspension from young colonies of 18 to 24 h was made in sterile distilled physiological water for each strain and its turbidity was adjusted to 0.5 McFarland (10^8 CFU/ml). Then, sterile paper discs of 6 mm diameters were impregnated with 15 µl of plant extract, dried and delicately deposed on the medium. We tested each extract in triplicate with the presence of the sterile disc dampened with DMSO as a negative control and we used an antibiotic, trimethoprime/sulfamides, as a positive control. Inoculated plates with bacteria were incubated at 37 °C for 24h. The antibacterial activity was evaluated by measuring the area of inhibition against the tested microorganism and the average of the three measurements was calculated. If the diameter of inhibition area is higher than 14 mm, we considered that we have a high antibacterial activity.

Statistical analysis

The results are expressed as mean±SD. A Student's test was used to analyse level of statistical significance between groups $P \leq 0.05$ statistically significant.

RESULTS AND DISCUSSION

Polyphenols

Phenolic content could be used as an indicator of antioxidant properties. The amount of total phenolic in extract was determined by spectrophotometry according to the Folin-Ciocalteu procedure, has been expressed as mg equivalent to Gallic acid per g dry weight (standard curve equation: y= 0.019x-0.035 R^2=0.991). We found that the aqueous extract have a good amount of phenolic compounds, with 119.77±0.32 mg GAE/g dry weight of plant material.

In comparison to other plants using the same solvent, the aqueous extract of *Pistacia lentiscus* extract had higher concentration of phenols than that with *Satureja calamintha* (12.6±0.775 mg/g) [22] but less than that with *Rubutus kulesziae* [23] (136.04±0.67 mg GAE/g) and similar to that with *Uncaria tomentosa* (109.84±9.63 mg/g) [24].

Antioxidant activity

In this study, the antioxidant potential of the aqueous extract of *Pistacia lentiscus* was measured by different chemical assays: DPPH radical scavenging activity, H_2O_2 scavenging activity, ferric-reducing antioxidant power (FRAP) assay, and total antioxidant assay by phosphomolibdate method. We compared the antioxidant capacity of the extract with ascorbic acid as reference.

DPPH scavenging activity

The DPPH is a stable nitrogen-centred free radical with a maximum absorption at 517 nm that can readily undergoes reduction by an antioxidant. The scavenging DPPH scavenging ability of the aqueous extract and ascorbic acid is shown in fig. 1. At concentrations from 5.75 to 10.25µg/ml, the scavenging activities of the extract were 7.29% to 52.31%, while the scavenging activities of ascorbic acid were 19% to 92.09%. The quality of antioxidant in the extract was determined by using IC_{50} values, denoting the concentration of the sample required to scavenge 50% of the DPPH free radical, it was calculated from the graph of percentage scavenging DPPH versus concentration. The IC_{50} values for ascorbic acid and aqueous extract were 6.71±0.19 and 9.86±0.37µg/ml respectively. The scavenging DPPH activity observed with our extract is better than observed with aqueous extract of *Pistacia inteferrima* with IC^{50} 11.5µg/ml [25]. This activity is mainly due to their redox properties, which plays an important role in absorbing and neutralizing free radical, quenching singlet and triple oxygen, or decomposing peroxides. We found that aqueous extract of *Pistacia lentiscus* have a good radical scavenging activity and it is depending on concentrations as shown in fig. 1. The scavenging capacity of the extract of *Pistacia lentiscus* on DPPH was possibly due to the hydrogen donating ability of polyphenolic compounds present in the extract. Polyphenols are very valuable plant constituents in the scavenging of free radicals due to their several phenolic hydroxyl groups [26].

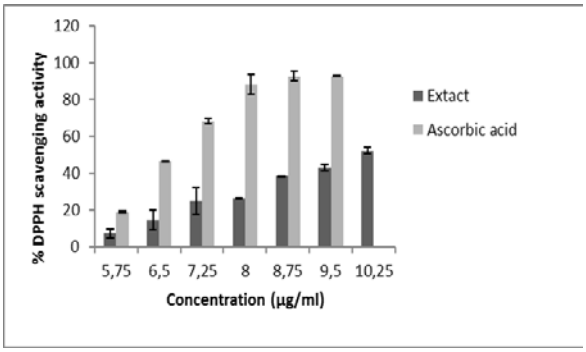

Fig. 1: DPPH radical scavenging activity of ascorbic acid and aqueous extract of *Pistacia lentiscus.* Each value is represented as means±SD (n=3)

Hydrogen peroxide scavenging activity

Fig. 2 depicts the scavenging capacity of the aqueous extract of *Pistacia lentiscus* against H_2O_2. The concentration range (200 to 800µg/ml) of aqueous extract was capable of scavenging H_2O_2 in a concentration-dependent manner. Later, when compared with ascorbic acid, the extract was less effective for scavenging H_2O_2. The ascorbic acid was capable of scavenging the H_2O_2 in the concentration range (40 to 200µg/ml), the H_2O_2 in the concentration-dependent manner. IC_{50} values, denoting the concentration of the sample required scavenging 50% of the H_2O_2, it was calculated from the graph of percentage scavenging versus concentration. IC^{50} of the standard compound is less than the IC_{50} of the extract that exhibit respectively 183.66±12.89 and 200±18.02 µg/ml. The values obtained were lower than the values obtained with the *Bierberstinia multifida* plant which presented IC_{50} 1364±54µg/ml [27].

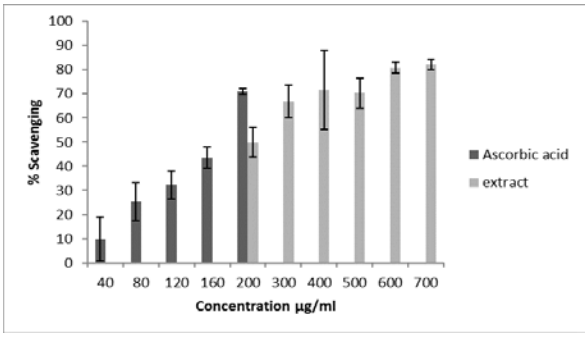

Fig. 2: Hydrogen peroxide scavenging activity of ascorbic acid and aqueous extract of *Pistacia lentiscus*. Each value is represented as means±SD (n=3)

Ferric-reducing power (FRAP) assay

It has been recognized that transition metal ions as iron is an important catalyst for the generation of the first few free radical to initiate the radical chain reaction or the mediates lipid peroxidation. Chelating agent may reduce free radical damage.

FRAP assay is a simple and direct method to assess antioxidant power. Increasing absorbance at 700 nm indicates an increase in reducing ability. In fig. 3, we found a dose-response relationship in reducing ability of the extract as the positive control (ascorbic acid). The result showed that ascorbic acid is more efficient (P≤0.05) in the reducing ability than the aqueous extract. The EC50 value is the effective concentration, which has the absorbance at 0.5 for reducing power it was calculated from the graph of absorbance at 700 nm versus concentration. Reducing an ability of the extract (EC_{50} = 54.06±12.66µg/ml) was higher than ascorbic acid (EC_{50}=38.92±3.16µg/ml). The values obtained were similar to those obtained in the same experiment with *Atalantica ceylanica* which presented IC50 87.7µg/ml [28].

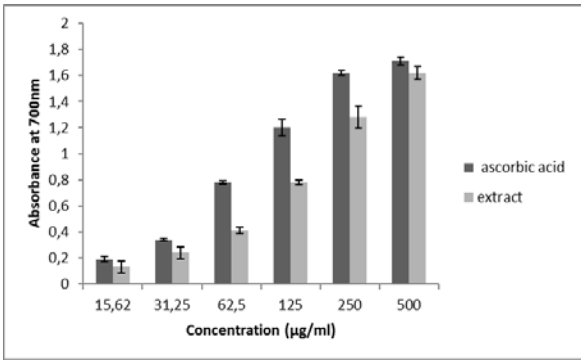

Fig. 3: Ferric-reducing power (FRAP) of ascorbic acid and aqueous extract of *Pistacia lentiscus*. Each value is represented as means±SD (n=3)

Total antioxidant assay by phosphomolybdate

The total antioxidant capacity test evaluated the ability of a sample to donate electron, neutralizing thus the free radical such as ROS. As can be observed in fig. 4, the extract and the ascorbic acid presented total antioxidant ability in a concentration-dependent manner. The extract presented a good antioxidant capacity in the range concentration (15.62 and 500 µg/ml), however, lower than the standard. The extract and ascorbic acid exhibit respectively 500±22.3 and 240±12.04 µg/ml

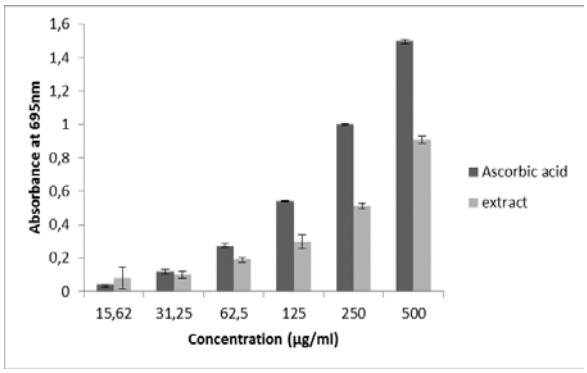

Fig. 4: Total antioxidant assay of ascorbic acid and aqueous extract of *Pistacia lentiscus*. Each value is represented as means±SD (n=3)

Haemolysis assay

Haemolytic activity could be used as the primary tool for studying the toxicity of the drug. Human cell blood treated with gradual concentration of AE (1000-62.5µg/ml) at 37 °C up to 30mn, in table 1, we noted a very low haemolytic effect. The haemolysis passes from 5.05±1.56 to 8.32±3.66% when the concentration of the extract evaluate at 62.5 to 1000µg/ml. The low haemolytic effect of the extract suggests the low/no toxicity of the extract in this range concentration.

Table 1: **Haemolysis effect of plant extract on human erythrocytes**

Concentrations of extract (µg/ml)	% of haemolysis
1000	8.320±3.660
500	5.670±0.490
250	6.200±1.900
125	5.970±1.330
62.25	5.050±1.560
Control	1.790±0.780

Each value is represented as means±SD (n=3)

Protect effect of aqueous extract to erythrocytes treated with saponin

Saponin can interfere with biological surface such as cell membranes. This latter may result in haemolysis or cytotoxicity because of perturbation or loss of the cell membrane integrity [27]. The haemolytic properties of saponin are generally due to the interaction between the saponin and the sterols of erythrocytes membrane [29].

It is visibly clear from fig. 5 that incorporation of the AE in erythrocyte membrane enhances the stability of intact erythrocytes against haemolysis, in presence of haemolytic agent (saponin). This observation suggests that erythrocyte membrane becomes more ordered in the presence of AE, which can rationalize their anti-haemolytic activities. Here, we observe that efficiency of anti-haemolytic activity is maxima for the erythrocytes incubation with 100µg/ml of saponin in the presence of 1000µg/ml of AE. In the anti-haemolytic effect, it demonstrates changes in shape and size of human erythrocytes incubated with extract of *Uncaria tomentosa*, and it reveals remarkable morphological changes, which were characteristic to echinocyte formation [25].

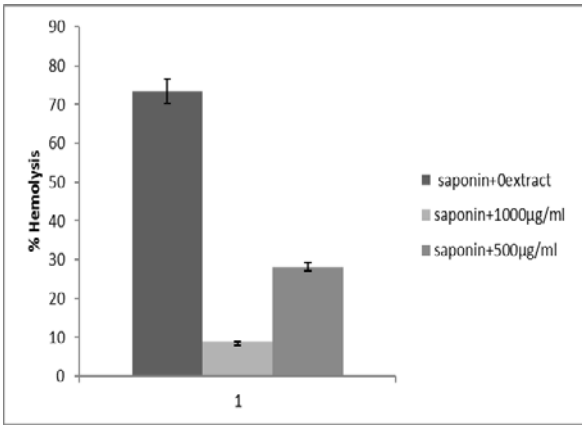

Fig. 5: Suspension of erythrocyte treated with 100µg/ml with the presence of aqueous extract at 1000 or 500 µg/ml of extract compared with control without presence of extract. Each value is represented as means±SD (n=3)

Determination of osmotic stability of human erythrocytes

We measured the extent of protection of erythrocytes after 30mn incubation of erythrocytes at 37 °C with 3 mg/ml of extract and without (control). In this study as shown in fig. 6, the percentage of

the control and test samples showed a sigmoidal relationship with increasing concentrations of saline solution.

Suwalsky *et al.* [29] Reports that the anti-haemolytic activity of flavonoids interacts with membranes by insertion in the lipid bilayer and protect erythrocytes from hypotonic lysis [19]. Polyphenol was able to localize the plasma membrane and increase red blood cells integrity. The plant extract could potentially affect osmotic stability through perturbation of any of three parameters: H, H_{50} and dS where:

H = A_1-A_2 where A_1 and A_2 are the mean maximal and minimal absorbance values of the sigmoid.

H_{50} is the NaCl concentration that causes 50% of haemolysis.

dS the amplitude of the haemolysis transition between A_1 and A_2

A stabilizing effect could be associated with an increase in dS and decreases in $H_{50;}$ whereas a destabilizing effect would be indicated by a decrease in dS and an increase in H_{50} [30].

Presence of aqueous extract of *Pistacia lentiscus* affected the parameter of osmoses (table2). A statistically significant decrease in H_{50} and an increase in dS was observed in the presence of the extract at 3000µg/ml, indicating the property of stabilizing erythrocyte membranes. Wloch and *al*., suggested that this increased osmotic resistance against hypotonic stress may be a consequence of the multi-morphological states of red blood cell [31].

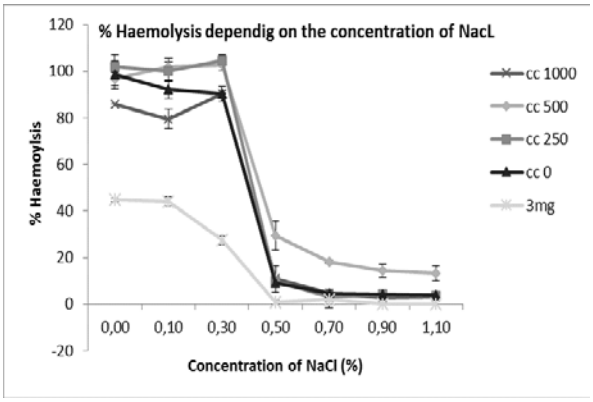

Fig. 6: Comparison of osmotic stability curves of human erythrocytes in the absence (control) and in presence of aqueous extract at 250-500-1000 and 3 mg/ml. Each value is represented as means±SD (n=3)

Table 2: **Effect of the plant aqueous extract on the human erythrocytes osmotic stability**

Concentration of extract (µg/ml)	H	H_{50}	dS
Control	1.73±0.020	0.32±0.052	0.11±0.072
250	1.75±0.045	0.32±0.098	0.12±0.033
500	1.55±0.680	0.25±0.078	0.08±0.01
1000	1.45±0.220	0.32±0.055	0.10±0.012
3000	0.75±0.012	0.30±0.022	0.30±0.054

Each value is represented as means±SD (n=3)

Protective effect of aqueous extract to oxidative effect caused by HOCl

We evaluated the protective effect of aqueous extract of *Pistacia lentiscus* on human erythrocytes exposed *in vitro* to the oxidative stress induced by HOCl. HOCl is an extremely toxic biological oxidant generated by neutrophils and monocytes, it is considered as one of the most important factors causing tissue injuries in inflammation [32]. Fig. 7 shows the effect of aqueous extract in varying concentrations (1000, 500, 250 and 125µg/ml) on human erythrocytes exposed to radical initiator HOCl (0.15, 0.075 and 0.0375%).

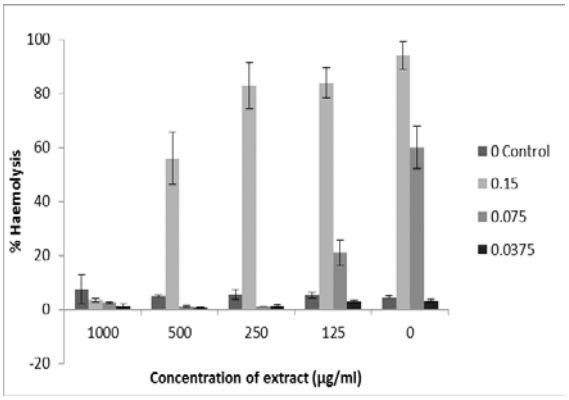

Fig. 7: Suspension of erythrocyte treated with different concentration of HOCl with the presence of aqueous extract at 1000-500-250-and 125 µg/ml of extract and without presence of extract. Each value is represented as means±SD (n=3)

The presence of gradual concentration of HOCl, in an absence of extract, causes gradual haemolysis. We observe 94.32±5.06, 60±7.97 and 3.35±0.43% in the presence of respectively 0.15-0.075 and 0.0375% of HOC. In the presence of aqueous extract of *Pistacia lentiscus*, we note significantly decreasing of the haemolysis induced by the concentrations of HOCl. We observe 7.47% of haemolysis caused by 0.15% of HOCl in the presence of 1000µg/ml of the extract and 94.32-+5.06% in the absence of the extract.

We observed significant reducing haemolysis in the presence of the different concentration of the extract. The authors of many works consider the effective protection of biological membranes against oxidation depends on the capacity of the polyphenolic substance binding to membranes [32]. Plant extract of *Primula heterochroma* demonstrates notable anti-haemolytic activity. It binding to the lipids and proteins of red blood cell membranes than it inhibit lipid peroxidation and also increases reed cell integrity against haemolysis [33].

Antibacterial activity

Antibacterial activity is shown in (table3) which indicated that all fractions inhibit the microorganisms tested. A maximum inhibition zone of 30.33±5.5 mm was observed on *Pseudomonas aeruginosae* for wood-seed. While moderates activity was obtained against all strain. Several researchers have mentioned that extracts have antibacterial activity. *Pistacia lentiscus* L. has found to be effective against *Sarcina lutea*, *Staphylococcus aureus* and *Escherichia coli* [34]. In another search, Hamad *et al.* [35] mentioned that the ethanolic extract obtained from *Pistacia lentiscus* showed a moderately high activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*. In the same way *Pistacia lentiscus* extract obtained by decoction showed the best antimicrobial activity against *Sarcina lutea, Staphylococcus aureus* and *Escherichia coli* [36].

Table 3: Antibacterial activity of aqueous extract of different part of *Pistacia lentiscus*, expressed by diameter (mm) of inhibition zone

	Le	Ws	Is	Atb
Staphylococcus aureus	10.33±0.5	12±1.00	9±1.00	23.33±4.72
Bacillus cereus	10.00±0.0	7.33±1.15	6.33±0.57	38.33±5.73
Pseudomonas aeruginosa	12.00±0.0	30.33±5.50	9.33±4.04	7±0.00
Proteus mirabilis	11.00±1.7	11.33±2.08	7±0.00	8.66±2.08
Listeria monocytogenes	09.33±0.5	7.33±0.57	10±1.00	7±0.00
Klebsiella pneumoniae	09.00±1.0	11.33±1.15	7.33±2.30	0.00
Enterobacter faecalis	09.33±0.5	11.33±1.52	9.33±2.08	30.5±0.7
Escherichia coli	09.33±2.3	0.00	0.00	0.00
Salmonella typhimurium	09.00±0.0	0.00	0.00	0.00
Citrobacter freundi	11.00±1.0	0.00	0.00	0.00

Le: Leaves (1.5 mg/disc), Ws: Wool-seed: (0.55 mg/disc), Is: immature-seed (2.25 mg/disc), Atb: antibiotic: trimethoprime/sulfamides. Each value is represented as means±SD (n=3)

CONCLUSION

The aqueous extract of *Pistacia lentiscus* contained a high level of phenolic compound and has an effective antioxidant in different assay including DPPH radical, hydrogen peroxide scavenging, reducing power and total antioxidant capacity. In addition the aqueous extract protected human erythrocytes against hypotonic shock, against oxidative stress and in saponin injury. While moderate antibacterial activity was observed. It is also suggested that the aqueous extract is viewed as a potential source of natural antioxidant, which can provide protection to human erythrocytes.

CONFLICT OF INTERESTS

The authors have no conflict of interest directly relevant to the content of this article.

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