ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Chemical Composition, Toxicity and Antioxydant Activity of *Pistacia atlantica* desf. subsp Oil.

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ABSTRACT

Despite the development of research, few who are implementing the biological activity of oil has never been studied. The biochemical composition of the seeds of *Pistacia atlantica* presents richness in lipids, which has shown a high tenure in mono-insatured fatty acids: oleic 39.41% and linoleic 15.06% and satured: palmitique 43.64%. The antioxidant activity of seed oil from our study was tested *in vitro*, using two free radicals DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). Results show a strong antioxidant activity, in other side, tests kinetic of growth and respiratory metabolites don't show any anomaly.

Keywords: *Pistacia atlantica Desf*, DPPH, ABTS, antioxidant activity, toxicity.

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ISSN: 0975-8585

INTRODUCTION

From a vegetable or animal original, fatty acids are fluid materials, creamy insoluble in water and alcohol. Plants that produce are very varied. The food, but also animal feed and industrial uses are their main uses [1]. In plants suppliers of oils, fat stores are found mostly in seeds is the case of Pistachio. Recent decades have shown that many plants used in traditional medicines from various countries contain specific antioxidants that explain at least part of their healing properties.

MATERIALS AND METHODS

PLANT MATERIAL

The genus *Pistacia* belonging to the Anacardiaceae family, includes many species very answered in the Mediterranean and the middle [2]. The fruits of *Pistacia atlantica* Desf bétoum (El Khodiri) are edible drupes of a pea-sized, slightly oval and flattened, used for culinary and medicinal. They are rich in dense and energetic oil. In our context, we thought to enhance the species *Pistacia atlantica Desf* which grows wild in Algeria, and is used by more people. Oil of bétoum represents a specific gravity of 0.918 to 15 ° C, medium, yellow with smell and taste pleasant, solidifies between 5 and 10 ° C [3]. Seeds of PA Djelfa region south of Algiers (319 km) are harvested in September 2010.

EXTRACT PREPARATION

To study the lipid fraction, was performed Folsh method [4]. 25 g of crushed seeds with a coffee grinder are placed in 100 ml of chloroform/methanol (v/v 2/1). After 1 h of stirring, we add to the filtrate KCl solution at 9%. The organic phase was evaporated using a Buchi Rotavapor an R-200 at 40 ° C.

GC-MS ANALYSIS

Fatty acid analysis is performed after a diversion of fatty acids in milk fat (triacylglycerol, phospholipid, cholesterol esters). A mass close to 20 mg of the sample is placed in a tube with screw cap then supplemented with 0.5 ml of heptane. After stirring, a volume corresponding to 0.2 ml of NaOH 2 mol / l in methanol is added to the tube; it is then heated in a water bath, stirred and then added 0.2 ml of HCl 2 mol / l. After further stirring, the mixture is poured into a glass tube, abandoned in a place to settle. 100 ml of the supper phase is removed, placed in a glass tube and evaporated in a ventilated. Finally the contents of the tube are shown in 50µl of heptane. After extraction with heptanes, methyl esters were then analyzed by GC-MS.

ANTIOXIDANTS TESTS

The measurement of overall antioxidant potential is addressed by assessing the ability to trap free radicals by the original composition.

*DPPH TEST

The method is based on the reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl) alcoolé wich has a Violet dense color with an absorbance of 517 nm. By measuring the change in absorbance in the presence of lipid extract [5-9]. Different concentrations of oil are prepared.50 ml of each solution was added to 1 ml of ethanol containing DPPH with a concentration of 100µl. After 30 minutes in a room temperature, it binds the absorbance (Shimadzu UV / Vis-type mark-Perkin-Elmer, UV-Vis spectrophotometer lambda1). The power of inhibition is calculated from the following relationship:

$$PI\% = (1 - A_{ext} / A_{witness}) \times 100$$

PI %: percent inhibition
A_{ext} : The absorbance of the solution of DPPH in the presence of the extract
$A_{witness}$: The absorbance of the solution of DPPH in the absence of the extract

ISSN: 0975-8585

***ABTS TEST**

The antioxidant activity was evaluated by fading **[10]** ABTS (2,2 '-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid). The ABTS has a blue color with an absorbance of 734 nm. The procedure is the same steps as the DPPH.

CYTOTOXICITY ASSAYS IN PARAMECIA

KINETICS OF CELLS GROWTH

The physiological parameter studied is *Paramecium*-growth kinetics, which is carried out by measuring optical density OD at wavelength λ = 600nm versus time [11, 12].Different concentrations of *Pistacia atlantica* oil (10uM, 20µM, 30µM), were added to the culture media when the initial cells density is 2.10^3 cell/mL [13] and growth kinetics is followed over time for both witnesses for processing. We also used two control tests, chloroform-control to investigate the impact of chloroform on cells (the oil is soluble in chloroform) and a control test with only paramecia.

CALCULATION OF PERCENTAGE OF RESPONSE

This is a calculation that evaluates the response of the protists exposed to xenobiotic, based on the following equation:

$$\% \text{ of response} = [(N_C - N_E) / N_C] \times 100$$

N_C: Number of control cells.
N_E: Final number of cells treated

% of response is the response percentage of *Paramecium* (%); C_N is the number of control cells (cell ml^{-1}) and E_N is the number of treated cells (cell ml^{-1}). Positive values of response percentage indicate an inhibition of growth, while negative values indicate a simulation of growth [14].

TOTAL PROTEIN

The protein assay for paramecia treated and the controls is carried out according to the method of [15]. This setting is performed by measuring optical density OD at the wavelength λ = 595 nm.

RESULTS AND DISCUSSION

Yield: Oil represents a yield of 47.64% dry matter (Table 1).
GCMS**:** The analysis of the oil into fatty acids and sterols showed a high content of palmitic and oleic acids.

Table 1: Chemical composition of oil.

Compounds	T R (mn)	%
Palmitic acid	22.012	43.64
9,12-octadecanoic linoleic acid (Z, Z)	30.140	15.06
9-octadecanoic oleic acid (Z)	30.930	39.41
Stearic octadecanoic acid	33.316	0.97
Sulfuric cyclohexylmethy lpentadécyle acid	38.570	0.92
Total		100

ISSN: 0975-8585

ANTIOXIDANT ACTIVITY

DPPH TEST

A linear relationship (Figure 1) was found between oil concentration in the solution of DPPH and ABTS, the percent inhibition with a correlation coefficient R^2 equal to 0.991, shown in Figures 1 and 2.We have used this linear relationship to calculate the inhibitory concentration of oil. The curve shows a strong inhibitory capacity of the oil with a concentration of 110mg/ml (4.4g/100g dry matter).

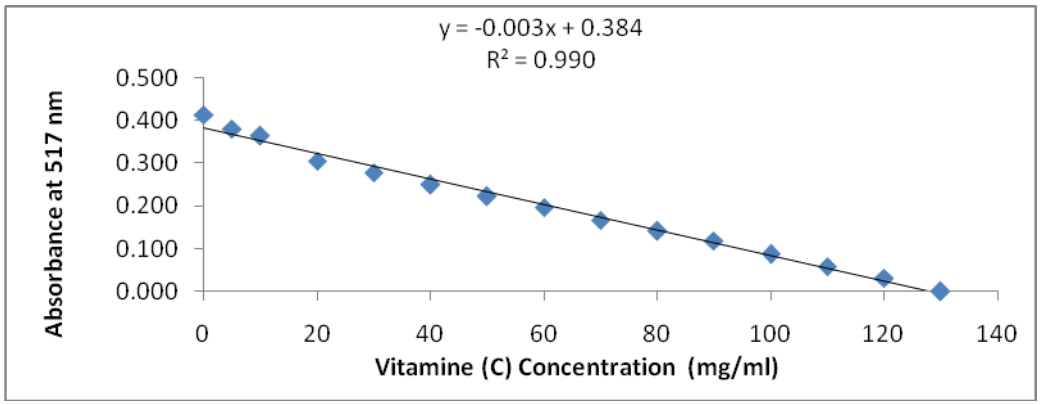

Figure 1: Antioxidant effect of oil (DPPH test)

ABTS TEST

The curve (Figure 2) shows a strong inhibitory capacity of oil with a concentration of 194 mg/ml (7.76g/100g dry matter). This strong inhibition of oil is related to its fatty acid composition and sterols that have the ability to scavenge free radicals for their functional groups (aldehyde, ketones ...).

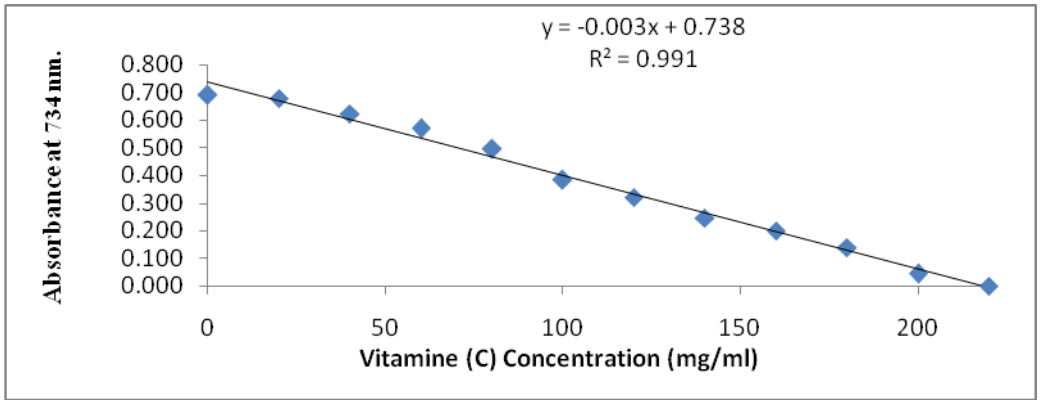

Figure 2: Antioxidant effect of oil (ABTS test)

TOXICITY TESTS

CELLS GROWTH

Figure (3) illustrate the effect of treatment of paramecia with vegetable oil of *Pistasia atlantica*. Our results showed normal growth of control cells with an exponential growth phase between time T0 and 1h. In cells treated with different concentrations of vegetable oil (P*istasia atlantica*), we showed a slight disturbance of growth, dose dependent and highly significant for the highest concentration (30µM). This can be explained either by the fact that the oil of *Pistacia atlantica* did not able to penetrate inside the cells, and is adsorbed on the membrane, in fact, the cell membrane, is the first barrier during the absorption of xenobiotics and plays an

ISSN: 0975-8585

important role in the process of tolerance [16]. This result goes in the same direction as Bouhdid. (2009) [17] demonstrated that in a loss of potential and membrane permeability as a result of essential oils of *O. compactum* and *C. verum* on *Ps. aeruginosa* and *Staph. Aureus*, or by the fact that the oil of *Pistacia atlantica* penetrated into cells and has been metabolized and eliminated especially for lower concentrations.

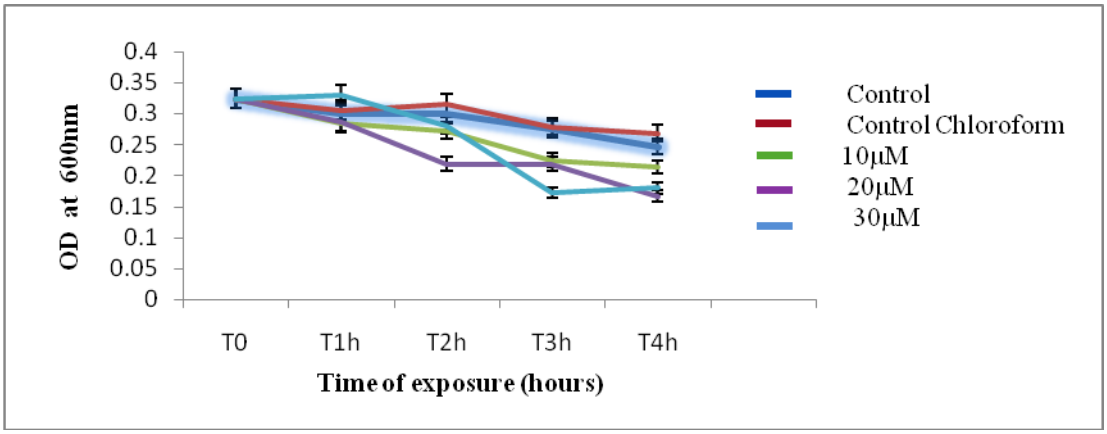

Figure 3: Cells growth measurement of paramecia treated with vegetable oil of *Pistasia atlantica*.

VARIATION OF TOTAL PROTEIN

Table (2) shows that the rates of total protein in paramecia treated with the highest concentrations of oil: 20µM and 30µM are substantially lower (respectively 0.23% µg / ml, 0, 15µg / ml) compared to controls and controls chloroform (approximately 0.23% µg / ml and 0,22µg / ml). This dose dependent decrease is parallels to evolution of the number of paramecia. Indeed, the number of cells is proportional to the total protein. This result confirms the work of Piccini *et al.* (1994) **[18]** ; Masaya *et al*. (2002) **[19]** ; Redouan-Salah. (2004) **[20]** , who demonstrated that protists are able to synthesize a variety of specific proteins and enzymes to the detoxifications of xenobiotics and that they have a major role in the stress against microorganisms. Thus it turns out that our oils do not appear to be toxic.

Table 2: Effects of vegetable oil (*Pistacia atlantica*) on total protein rate of *Paramecium*.

	Times / Traitements	T0h	T1h	T2h	T3h	T4h
Total protein Rate µg/µl ± SE	**Control**	0.023 ±0.001	0.025 ±0.012	0.023 ±0.007	0.023 ±0.001	0.022 ±0.026
	Chloroform-control	0.022 ±0.001	0.023 ±0.002	0.023 ±0.002	0.024 ±0.005	0.017 ±0.002
	T_1	0.020 ±0.003	0.023 ±0.000	0.024 ±0.001	0.029 ±0.001	0.019 ±0.004
	T_2	0.023 ±0.001	0.023 ±0.024	0.023 ±0.001	0.022 ±0.001	0.023 ±0.000
	T_3	0.015 ±0.008	0.022 ±0.003	0.021 ±0.001	0.021 ±0.002	0.016 ±0.011

T1: Treated with 10µM of oil
T2: Treated with 20µM of oil
T3: Treated with 30µM of oil

ISSN: 0975-8585

PERCENTAGE OF RESPONSE

Figure (4) shows that the percentage of response in Paramecium after 6 hours of exposure to the vegetable oil (*Pistasia Atlantica*) confirm the evolution of growth curve. The percentages of response obtained are dose dependent, -45% for the treatment with 10uM, 39% to 20µM, and -28% for 30µM. Thus this parameter confirms the evolution of paramecia growth treated with different concentrations of *Pistasia atlantica* oil, when the negative response percentage confirms the cells growth stimulating.

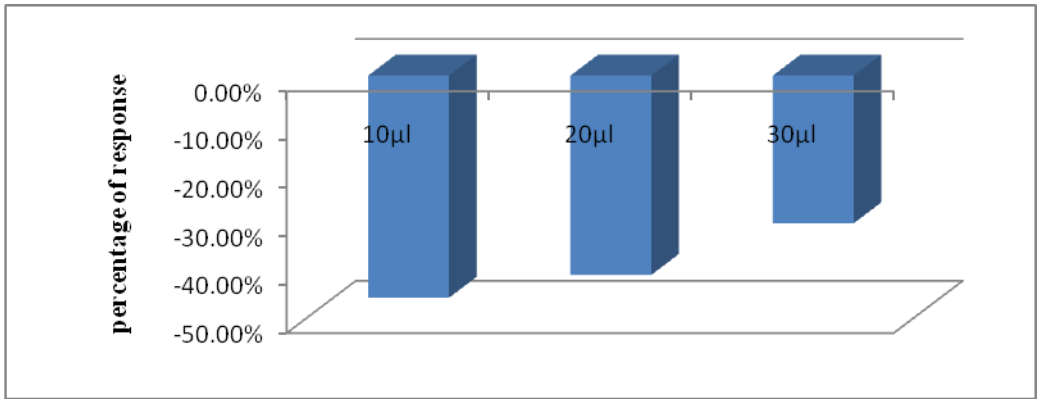

Figure 4: Response percentage of paramecia treated with vegetable oil of *Pistasia atlantica*

CONCLUSION

Biochemical analysis of the seeds of pistachio Atlas brought out their oil content especially unsaturated fatty acids (oleo-linoleic) with dietary and nutritional interests are well established [21]. The fraction of unsaturated fatty acids is present in major proportion, and gives oil a high nutritional value or of physiological importance of this fatty acid and that of linoleic acid in preventing some metabolic disorders, cardiovascular diseases and cancer[22, 23]. Presence of linoleic acid protects oil the oxidation phenomenon during heat treatment and therefore predisposed to potential heaters [23]. This specificity makes these fatty acids ability to be used in various fields (Pharmaceutical, Food Processing) by integrating these oils to medicines and food and other cosmetics. Moreover pistachio Atlas is widely used by the Algerian steppe nomads in their daily diet and their livestock [24]; In this work, we have demonstrated the biological activity of the essential oil *Pistasia at*lantica, this activity was reflected in a slight inhibition of growth and a low mortality rate and dose dependent, this gives the oil a low toxicity, suggesting its use for therapeutic and dietary end.

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