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Antibacterial activity and physicochemical characteristics of *Pistacia atlantica* extracts

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ABSTRACT

This work focused on the physicochemical study, phytochemical screening and evaluation of the biological activity of the fruits and leaves of Pistacia atlantica in occidental Algeria (Bechar region).The phytochemical screening shows that this plant is rich in secondary metabolism: flavonoids, tannins, alkaloids, steroids in the leaves we record against lack alkaloids, saponins, steroids and starch and presence of polyphenols in fruit. The performance of the fixed oil of Pistacia atlantica fruit extracted by Soxhlet is around 77%, which is higher compared to the traditional method (3.33%). Leaves result a small amount of 2.2% by the first method. We have used the Disk method to determine the antibacterial activity which was revealed compared to six strains gram positive and gram negative. All tested strains were susceptible against the aqueous extract with an 8mm zone of inhibition, except Pseudomonas aeruginosa given 14mm diameter, fixed oils against the fruit has an antibacterial potency against only grams negative strain.

Keywords: antibacterial activity, physicochemical analysis, phytochemical screening, aqueous extract, fixed oil, *Pistacia atlantica.*

INTRODUCTION

The pistachio is native to Central Asia. In Turkey since(7000 years BC.), It was introduced in Italy in the first century(BC), and later, its cultivation spread to other Mediterranean countries and the USA in 1854 **[1].**The common name of this species is Elbetoum or Btouma in local Arab and Iggth in Berber, refers to the Atlas mountains where the species grows. This tree is commonly known as wild pistachio, pistachio false, globular fruit, small red-brown, containing a green seed, fruits are called "Godim" by local people, label due to the predominance of ripe dark green **[2].**

Pistacia atlantica is a medicinal plant par excellence; this is proven by the results obtained on its therapeutic use in traditional medicine and pharmaceuticals**[3].**The different parts of the plant are used in the treatment of oral diseases, eczema, paralysis, diarrhea, throat infections, kidney stones and asthma. It is considered an astringent, anti-inflammatory, antipyretic, antibacterial and antiviral**[4],[5].** The plant is also used in the preparation of certain cosmetic products such as soap famous**[6].**This study provided a better understanding of the phytochemistry *Pistacia atlantica*, and also demonstrated the value of this plant as a source of new natural products and to promising biological and pharmacological activities in phenolic compounds have compose which are secondary metabolisms **[7].**Also, the objective of this work is a contribution to the enhancement of *Pistacia atlantica*, which is widespread in the Bechar region (fig1) via a physicochemical study, a phytochemical screening and analysis of antibacterial activity.

Figure1Atlantics pistachio (*Pistacia atlantica*) of Algeria occidental

MATERIALS AND METHODS

Ethnobotanical study: For the purpose of Pistachio Atlas or "Btom" in traditional medicine, we made visits to herbalists, traditional healers and druggists in the city of Bechar, the table 1 present the Traditional use of different parts of Pistachio Atlas in the town.

Table 1 Traditional use of different parts of Pistachio Atlas in the town of Bechar

The parts of the plant	Utilisation ethnobotanique	
	Usages	Methodof Preparation
fruits	▪ Fruits having anti-oxidant capacity for internal use orally, recommended for treating: Stomach Cardiovascular problems Kidney diseases	Infusion and decoction
Leaves	▪ The leaves usually for internal use, orally to treat certain diseases as appropriate: ▪ Bacterial infections ▪ Dental pain ▪ Conjonctivitis	Can take either as tea, decoction, or even chewing Infusion and pure or mixed with honey powder.
Bark of the trunk	Recommended in cases of stomach aches and stomach pains.	By decoction
Galls	Galls of pistachio are used as anti-diarrheal.	As powder
Oils	▪ For internal use, oral: tooth care For external use, apply on chest against the cough and bronchitis, the healing of skin wounds and hair care.	The extracts or liquid fruit oils

II.1 Sampling:

The plant was harvested in the region which is called Djedida located about 80 km north of Bechar. The leaves of *Pistacia atlantica* are harvested during the month of March 2015while fruits were harvested in September, 2014.The leaves and fruits are *Pistacia atlantica* follows dried in a dry and airy place in the shade for 10 to 15 days, after drying, they are ground and stored in small vials sterilized and sealed glass.

II. 2 Extraction

II.2.1 Aqueous extract

The aqueous extract of the leaves of *Pistacia atlantica*, is carried out by maceration method, it consists in placing 20g of plant material with 300ml distilled water under stirring for 24 h at room temperature. The mixture is then filtered and passed through a rota-vapor to recover the aqueous extract.

II.2.2 fixed oil

The fixed oil of *Pistacia atlantica*is obtained according to two methods, the first is traditional and the second is provided by Soxhlet;

-The mature fruit of 300 g quantities are crushed and added dates 200g (Hmira) blocks then mix the two components until a homogeneous paste, pressing the dough by hand to extract the fixed oil of *Pistacia atlantica*.

-The extraction is carried out by soxhlet which allows for continuous extraction of a solid by a solvent.

II.3Physicochemical characteristics

II. 3. 1 Phytochemical screening

Such as alkaloids (salt, bases), saponins, flavonoids, tannins and sterols and steroids. These phytochemicals tests are based on the solubility tests, constituents of the plant, against organic solvents of different polarity: water, ethanol, diethyl ether and reactions colorings and precipitation **[8].**

II. 3. 2 Mineral elements

In a jar; Dissolve 0.1 g of ash leaves *Pistacia atlantica* obtained with 20 ml of distilled water. For better dissolution of the ash, it is preferable to add a few drops of nitric acid if necessary; putting the mixture under stirring for a few minutes until complete dissolution of the ash, from this solution, we performed the assay of the following minerals: calcium, potassium, sodium, lithium and barium.

II. 4Antibacterial activity of the fixed oil and aqueous extract of *Pistacia atlantica*

For the implementation of microbiological tests, we used six bacterial strains. These are Gram positive: *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 10876) and *Listeria monocytogenes* (ATCC 15313), and gram negative *Escherichia coli* (ATCC 25922), *Pseudomonas aerugenosa* (ATCC 27853) and *Acenitobacterboumanii* (ATCC 19606).

Antibacterial tests should be performed from young bacterial cultures (18-24 hours). The tested strains were subcultured in petri dishes containing nutrient agar and incubated at 37 °C for 18 hours were suspended in sterile physiological saline (0.9%). The turbidity of the inoculum was adjusted to 0.5 McFarland, which corresponds to an inoculum of 10^8 CFU / ml**[9].**

We have prepared for the aqueous extract and the fixed oil *Pistacia atlantica*, the following dilutions: 50%, 25%, 12.5%, 6.25%, 3.13% and 1%.

We used disk method; put 6 disks that are sterilized and impregnated by the fixed oils or the aqueous extract on a 90mm diameter box. The disks should be spaced 24 mm center to center. After incubation at 37 °C for 24 h the inhibition zones formed around the discs were measured using a caliper**[10].**

RESULTS

The yieldsof the *Pistacia atlantica* extracts are shown in figure 2:

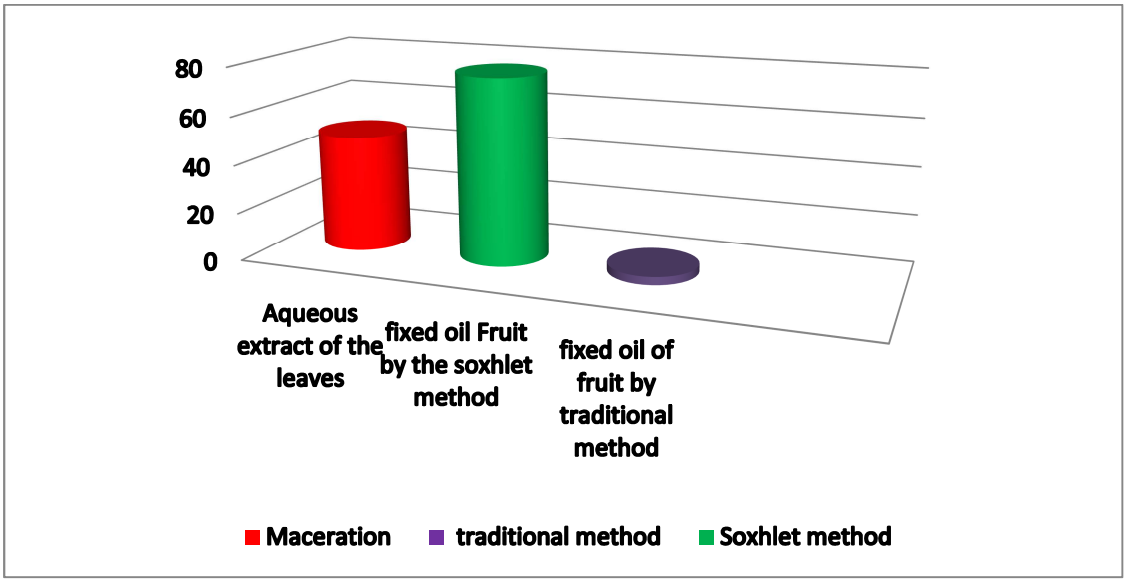

Figure 2Yieldof the *Pistacia atlantica* extracts

From Table 2, we observe that the fruits of *Pistacia atlantica* are rich in flavonoids and tannins. Starches, alkaloids, sterols, steroids and saponins are absent. The leaves have a high concentration of flavonoids and tannins, alkaloids, sterols with an absence of starch, and a low concentration of saponins.

Table 2 The results of phytochemical screening

Extracts	Species considered	*P. atlantica*	
	Chemical families	Fruits	Leaves
	Polyphenols	+	+
Ethanol extract	Alkaloïds sels	-	-
	Alkaloids bases	-	++
	Flavonoïds	+++	++
	Tanins	+++	++
Extrait l'éther diéthylique	Sterols et steroïds	-	++
	Saponins	-	++
Extrait aqueux	Amidon	-	-

Our results coincide with the work of Kawashty& al 2000, put in evidence that the existence of flavonoids, tannins, alkaloids and saponins in the aerial part (fruit and leaves) of two species of *Pistacia***[11]**. Highlighting minimal amounts of tannins in *P. lentiscus* and *P. weinmannifolia*, which is obtained by Romani & al, (2002)? Other classes such as sterols and steroids were found on average quantity in the leaves of *P. atlantica***[12].**

The result obtained shows that this plant is a promising source of polyphenols mostly in *Pistacia atlantica*leaves, which is less important in fruits, which coincide with the results of **[13].**

The results obtained for each element are shown in Table 3;

Table 3 concentration of minerals leaves *Pistacia atlantica*

Mineral elements	Ca	K	Na	Li	Ba
Concentration (mg/l)	0,0014	0,078	0,008	0,0001	Traces

From the table 3, we observe a fairly high concentration of potassium (Na), sodium (Na) and calcium (Ca), with a significant presence of lithium (Li), traces of barium (Ba) were detected in the *Pistacia atlantica*, any concerns about suspected human or animal health.

The fixed oil yield extracted by Soxhlet method from the *Pistacia atlantica* fruit is 77%, it's higher than the fixed oil of *Pistacia atlantica* fruits extracted by traditional method which is equal to 3.33%.We find that the performance of the fixed oil fruits (Soxhlet) is more important than the fixed oil fruit *Pistacialentiscus* (38.8%) and the results of the work of Acheheb& al(2012) tests to optimize oil yield fruit *Pistacia atlantica* is 44.17%.**[14]**&**[15].**

The aqueous extraction yield by macerating of *Pistacia atlantica* leaves is equal to 48.34%, according to the results of Bougattra& al (2010), the aqueous extraction yield is equal to 28% *Rosmarinus officinalis* L and it is equal to 32.24% for *Rhetinolepislondiodes*coss**[16].** These yields are lower compared to aqueous extract of *P. atlantica*; this value can be caused by different conditions: Climate, geography, genetics of the plant, the extraction method used.

The total sugar content and fat content of the *Pistacia atlantica* leaves and fruit, is shown in figure 3:

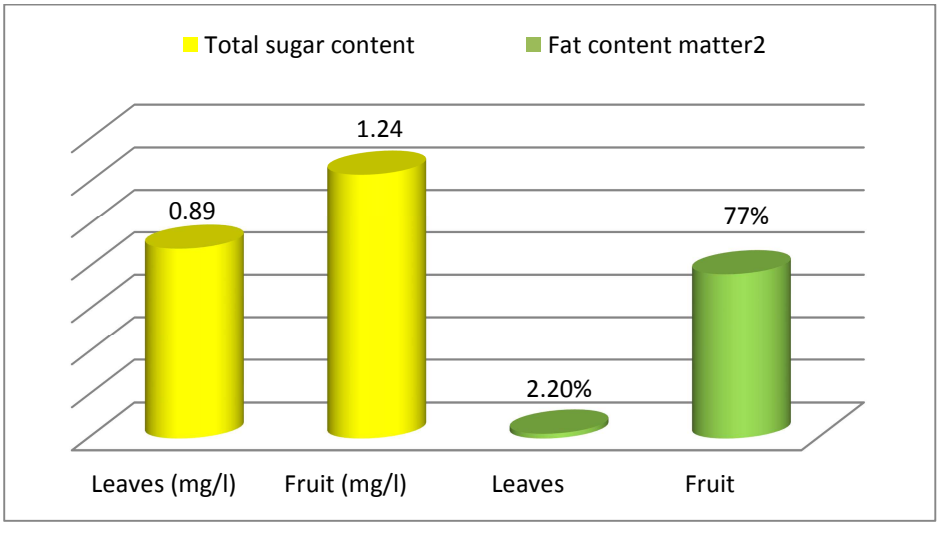

Figure 3 Total sugar and fat content of the leaves and fruit of *Pistacia atlantica*

The total sugar content of *Pistacia atlantica* fruitis equal to 1.24 mg; it is higher than the total sugar content of *Pistacia atlantica* leaves (0.89 mg), the fat content of *Pistacia atlantica* fruit (77%) is more important than the fat content of *Pistacia atlantica* leaves (2.20%)

The figure 4 presents the rate of the mineral and moisture of *Pistacia atlantica* leaves*:*

Figure 4Rateof the mineral and moisture of *Pistacia atlantica* leaves

The inorganic matter content of the fruits of *Pistacia atlantica* is equal to 5.2%, on the other hand it equals to 3.14%in the leaves. What almost identical to the mineral content of fruits of *Pistacialantiscus* which it equals to 5% and 5.14% obtained for the fruits of *Juniperusphoenicea.*

The humidity results show that the aerial parts (leaves) of *Pistacia atlantica* has an average moisture content of 58.69% compared to the moisture content of*Matricariapubescens* which it is about 21% and for *Cotulacinerea* it is equal to 28.4% **[17].**

All tested strains were susceptible against the aqueous extract with a diameter of8mm of the inhibition zone for the concentration 50% (*Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa* and *Escherichia coli*)) and the same concentration symbolizes the powerful effect on *Bacillus cereus* and *Acenitobacterboumanii* strains with the following two diameters:11mm and 14mm of the inhibition zone, this effect is due to the richness in phenolic compound.

About the fixed oil extracted of*Pistacia atlantica* fruit by soxhlet method has an antibacterial potency against grams negative bacteria (*Escherichia coli, Acenitobacterboumanii* and *Pseudomonas aerugenosa*) and by the traditional method, we recorded an effect only on two bacteria (*Escherichia coli* and*Acenitobacterboumanii*); The diameters operated by the application of extracts (aqueous extract and fixed oils) clearly show that the inhibition zones decrease with decreasing concentration of extract applied: 50٪,25٪,12.5٪,6.25٪,3.13 ٪,1٪. The tables(4, 5 and 6)show the results ofthe antibacterial effect of the aqueous extract of the leaves and the fruits fixed oils.

Table 4 Results of the antibacterial effect of the aqueous extract of *Pistacia atlantica* leaves

Strains tested	Concentration of the aqueous extract					
	50٪	**25٪**	**12.5**	**6.25٪**	**3.13٪**	**1٪**
E. coli	+	+	+	-	-	-
S. aureus	+	+	+	+	+	-
L.monocytogenes	+	+	+	+	+	-
A.boumanii	+	+	+	+	+	+
B.cereus	+	+	+	+	-	-
P.aeruginosa	+	+	+	+	-	-

Table 5 Results of the antibacterial effect of the fixed oil of *Pistacia atlantica* fruits (Traditional method)

Strains tested	Concentration of fixedoil					
	50%	25%	12.5%	6.25%	3.13%	1%
E. coli	+	+	+	-	-	-
S. aureus	-	-	-	-	-	-
L. monocytogenese	-	-	-	-	-	-
A.boumanii	+	-	-	-	-	-
B.cereus	-	-	-	-	-	-
P.aeruginosa	+	+	+	-	-	-

Table 6 Results of the antibacterial effect of the fixed oil of *Pistacia atlantica* fruits (Soxhlet method)

Strains tested	Concentration of fixedoil					
	50%	25%	12.5%	6.25%	3.13%	1%
E. coli	+	+	-	+	-	-
S.aureus	-	-	-	-	-	-
L. monocytogenes	-	-	-	-	-	-
A.boumanii	+	+	+	+	-	-
B.cereus	-	-	-	-	-	-
P.aeruginosa	+	+	+	-	-	-

CONCLUSION

The results allowed us to highlight the richness of fruit by tannins, flavonoids. The presence of flavonoids, alkaloids, saponins, tannins and steroids in the leaves against starch, saponins and alkaloids are classes of chemical families totally lacking in fruit. The most important performance is that of fixed oils of *Pistacia atlantica* fruit have the following percentage: 77%, relative to the sheets which are 2.2% by the method of Soxhlet.

A physico-chemical analysis gives a water content value equal to 58.69%, and a fruit ash content equal to 5.2% which is larger than the sheets containing 3.14%. The aqueous extract is active on all strains tested and confirmed the effectiveness of this plant to treat diseases of bacterial origin. Fixed oils of the fruits exhibit antibacterial properties against gram-negative bacteria: *Pseudomonas aerugenosa, Acenitobacterboumanii and Escherichia coli* and no antibacterial effect against Gram positive bacteria: *Staphylococcus aureus, Listeria monocytogenes* and *Bacillus cereus.*

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