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Topic

Optimization of bioactive molecules (phenolic compounds) extraction from *Pistacia atlantica fruits*

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Dedication

I dedicate this modest work:

To my dear father and mother, who have always supported me with their valuable advice, patience, and encouragement throughout my academic journey, May God protect them and reward them for all the good they have done for me.

To my dear sisters: Asma, Soumia, and Asia, as well as her husband, for their constant support and sincere encouragement.

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Ahmed

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Abbreviations list:

GAE: Gallic Ecid Equivalent

CCD: Central composit disign

DPPH: 2,2-diphényl-1-picrylhydrazyle

TPC: Total phenolic content

DW: Dry weigh

AA: Antioxidant activity

IR: infrarouge

Ac: Absorbance of the control

As: Absorbance of the sample

OES: oil extracted by Soxhlet

OER: oil extracted at room tempreture

UAE: ultrasound assisted extraction

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Introduction

Pistacia atlantica is a species that grows in arid and semi-arid regions, where it is well adapted to withstand strong winds and prolonged dry periods (**Belhadj, 2001**). However, the population and density of ***Pistacia atlantica*** are in decline due to multiple threats. In this context, a global decrease of 25% is projected over the next 100 years, mainly due to overexploitation, poor health of mature trees, insect infestations, and significant habitat degradation (**Rankou et al., 2018**).

Since early civilization, people have valued various parts of *P.atlantica*, such as its resin and fruits. Its abundant essential oils and fixed oils have been widely used in traditional medicine and beauty products. Since then, Atlas pistachio fixed oil is incorporated into several cosmetic product to benefit from its medicinal properties (**El Zerey-Belaskri et al., 2022**). The fruits are locally known as ***El Khodiri***. They are traditionally mixed with mashed dates to prepare date energy balls, which are often consumed with buttermilk (**Belhadj, 2001**). This resilient tree and its various parts were highly were also valued and used for multiple purposes. It was especially known for its medicinal properties, as people relied on it to relieve digestive issues particularly stomach ailments and to support kidney function by treating renal disorders. In addition, it was applied to wounds to promote healing, and used to soothe persistent coughs (**Mahjoub et al., 2018**). Besides, thanks to its high resilience against extreme arid climate, *P. atlantica* is an appropriate tree for the reinforcement of the Algerian green dam, which is the sole barrier to the desert advance (**Benlabiod et al., 2023**).

Phenolic compounds, primarily flavonoids and phenolic acids, are secondary metabolites abundantly present in fruits. Their rising importance is largely due to their strong antioxidant properties and the observed link between their intake and the prevention of various diseases. The health-promoting effects of these phytochemicals depend on consistent consumption and their bioavailability. Research has highlighted the significance of regularly including fruits in the diet, particularly in reducing the risk of diseases related to oxidative stress (**Haminiuk et al., 2012**).

Despite the growing interest in natural bioactive compounds for food processing, cosmetics, and medicine, the efficient extraction and preservation of these molecules remain challenging. Conventional methods are simple and widely used, but they often involve long extraction time, large solvent volumes, and high temperatures that can degrade heat-sensitive compounds (**Mungwari et al., 2024**). In contrast, non-conventional methods like UAE offer

greater efficiency, and better preservation of thermolabile molecules. Still, optimizing these techniques is a key research concern. Parameters such as solvent type, extraction time, temperature, particle size, and solvent-to-material ratio greatly influence both yield and quality (**Bhadange et al., 2024**). This raises a central question: how can we optimize these factors to maximize yield and preserve the biological activity of bioactive molecules? Especially for saharian and subsaharian national bio resource like *P. atlantica*.

Response surface methodology (RSM) is a widely used approach in setting experiments for optimization purposes. It offers significant advantages over traditional one-factor-at-a-time methods by allowing the simultaneous evaluation of multiple variables and their interactions. It reduces the number of experiments needed, saving time and resources, while providing deeper insight into the effects of each factor. RSM also helps identify optimal conditions more accurately through predictive models and visual tools like contour and surface plots, making it highly effective for process optimization (**Bezerra et al., 2008**).

In this context, the present study was focused on the investigation of the optimal method of the extraction of phenolic content and the antioxidant capacity of *P. atlantica* extract. Therefore, this document reports all the investigation done in order to fulfill the objectives of the study:

I Literature review on P. atlantica, its ecobiology, phytochemistry and socioeconomic importance, with a focus on the extraction method of bioactive molecules;

II The used methodology for oil extraction and UAE RSM optimization of phenolic content extraction and antioxidant activity;

III The results and discussion of the main findings.

PART I: BIBLIOGRAPHY SYNTHESIS

I. Overview of *Pistacia atlantica* plant and its applications

I.1. The history and localization

Pistacia atlantica has a long history of use, dating back to ancient times in the Middle East and Mediterranean region. *Pistacia atlantica* has a large geographic range (**Figure1**), where it spread over the North Africa (morocco, Algeria, Tunisia), Canary Island, Turkey, and a section of Eurasia from the Iranian Plateau (**Rankou et al., 2018**).

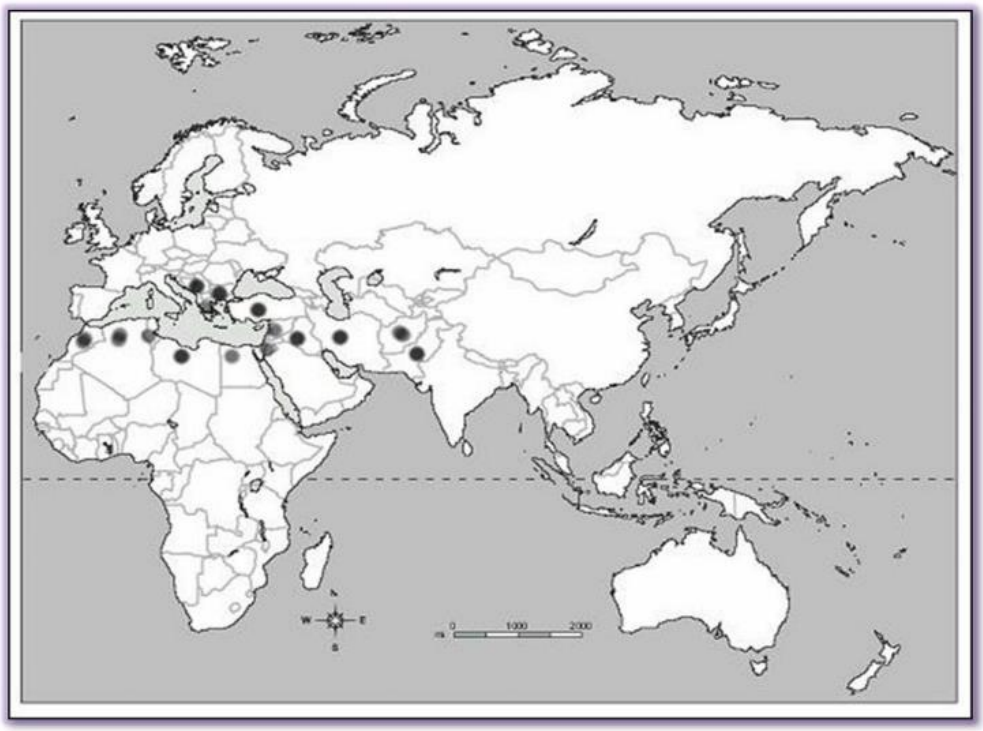


Figure 1 : Distribution map of the Atlas pistachio tree in the world (Zohary, 1952)

Pistacia atlantica is identify in 18 Algerian states, distributed across three region (Figure 2) East region, Central region, and West region (**Taib. 2021**). However, its optimum founds at arid and semi-arid regions, where it thrives in oueds and dayas (**Harfouche et al,. 2005**).

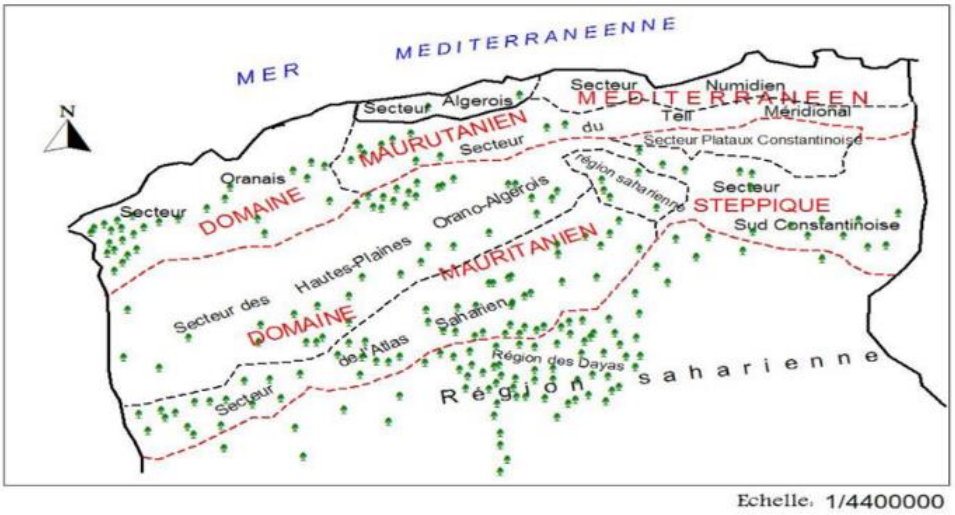


Figure 2 : Distribution of the Atlas pistachio in Algeria (Monjauze, 1980)

I.2. Botanical characteristics

The scientific name of Atlas pistachio is *pistacia atlantica Desf*, from the anacardiacées family known as '' Betoum or Botma''. Their dimension is around 15 to 25m of height in the average of 200 years old (**Harfouche et al., 2005**). In Algeria, *P.atlantica* is often found in association with *Ziziphus lotus*, which helps protect young plants from grazing animals and harsh winds (**Belhadj, 2001**). The Atlas pistachio (***Pistacia atlantica***) is a dioeciously plant. Its flowers are a patulous and reddish, arranged in terminal clusters for male trees and axillary clusters for female trees. Its forms are compact, giving it exceptional plasticity against drought. The drupes measure approximately 6–8 mm in length and 5–6 mm in width. The wood of ***Pistacia atlantica***, is dense, and highly durable. It is excellent for fuel wood and charcoal production (**Monjauze., 1980**).

I.3. Plant Characteristics and Ecology

Pistacia atlantica is a thermophilous xerophyte that thrives in dry, stony, or rocky hillsides, edges of fields, roadsides, near the base of dry stone walls, and other similar habitats (**Tzakou et al., 2007**). It can grow at altitudes ranging from below 1,500 meters up to 2,000 meters (**Monjauze., 1980**), where the growth of *P.atlantica* is too slow in the nature, but it is so fast in irrigated plantation (30 cm/year, sometimes more) (**HARFOUCHE., et al 2005**). Plant reproductive process, such as pollen germination, pollen tube growth, and fruit set, is affected

by temperature, which is one of the most important environmental factors (**Kakani et al., 2005**). In a study conducted by **Kakani et al., 2010**, the effect of temperature on *in vitro* pollen germination and pollen tube growth was investigated in various pistachio species, including ***Pistacia atlantica***. The researchers found that the optimal temperature for ***P. atlantica*** pollen germination was 22.3°C, with germination rates significantly decreasing at temperatures below 6.7°C or above 39.8°C. After germination, fruit development proceeds through distinct maturation stages, during which the fruits exhibit characteristic color changes: initially red during the immature phase, transitioning to dark red during intermediate maturation, and finally turning black or dark green upon reaching full maturity. These ripening stages are largely documented, especially the evolution of fruit pigmentation due to the biosynthesis of several pigments such as chlorophyll, carotenoids, anthocyanin, etc. (**Bellomo et Fallico., 2007**).

The fruits of the Atlas pistachio tree (Figure 3) are ovoid drupes with a wrinkled, dark green pericarp and a hard stone that accounts for 55.6% of the total weight (**Acheheb., 2013**).

Figure 3: *Pistacia atlntica* fruits

Furthermore, it is important to note that fruit maturation on the same tree does not occur uniformly; some fruits complete their maturation prematurely, while others do so later. Consequently, it is not surprising to observe mature fruits as early as the beginning of August, and immature fruits still present by the end of September (**Yousfi et al., 2017**).

II. Uses and Applications of *P. atlantica*

Pistacia atlantica, with its various constituents and properties, has been used for many different purposes. One of these constituents is its resin produced by the bark, it is a mastic resin used by local populations as chewing gum, and in pharmacy for making ointments

(**Monjauze, 1980**). Recent studies have shown that the essential oil of this resin has an antibacterial activity, particularly against ***E. coli***, ***S. aureus***, and ***S. pyogenes*** (**Ghalem et al., 2009**)**.** In addition to the resin, the oil extracted from the fruit is also valued for its phytocosmetic properties. Rich in unsaturated fatty acids, phenols, palmitoleic acid, and vitamin E, it possesses exceptional softening and healing properties for the skin (**Acheheb, 2013**). Moreover, the dried fruit methanolic extract has also a notable activity against *candida albicans, saccharomyces cerevisiae,* and *candida glabrata* (**Falahati et al., 2015**). Another study realized by **Achili et al., (2020)**, on leaves and steam extracts from *P.atlantica*, showed antioxidant, anticholinesterase and antiproliferative activities, which could open up new applications for pharmaceutical and food industries.

III. Fruit composition and Bioactive molecules of *P.atlantica*

III.1. Fruit composition

The composition of *P.atlantica* is presented in table I according to **Benhassaini et Bendahmane., (2007**).

Table I: The entire composition of *P.atlantica* fruit (Dry matter) (**Hachemi and Bendahmane., 2007**)

Parameters %	Algerian north ecotype
Moisture	21.26 ± 1.24
DM (dry matter)	78.74 ± 0.48
Starch	5.43 ± 0.35
Crude fibers	12.60 ± 0.71
Crude oil	39.80 ± 1.37
Crude proteins (N×6.25)	10.39 ± 0.66
Ash	5.54 ± 0.11

The composition presented in table I showed that *P. atlantica* fruits are oleaginous where it present a considerable oil yield with 39.8%.

A histological examination of Atlas pistachio fruits, under light microscopy conducted by **Acheheb, (2013**), revealed the presence of oil secretion channels (Figure 4). The oil channels in the seed coat were found to be more abundant compared to those in the kernel.

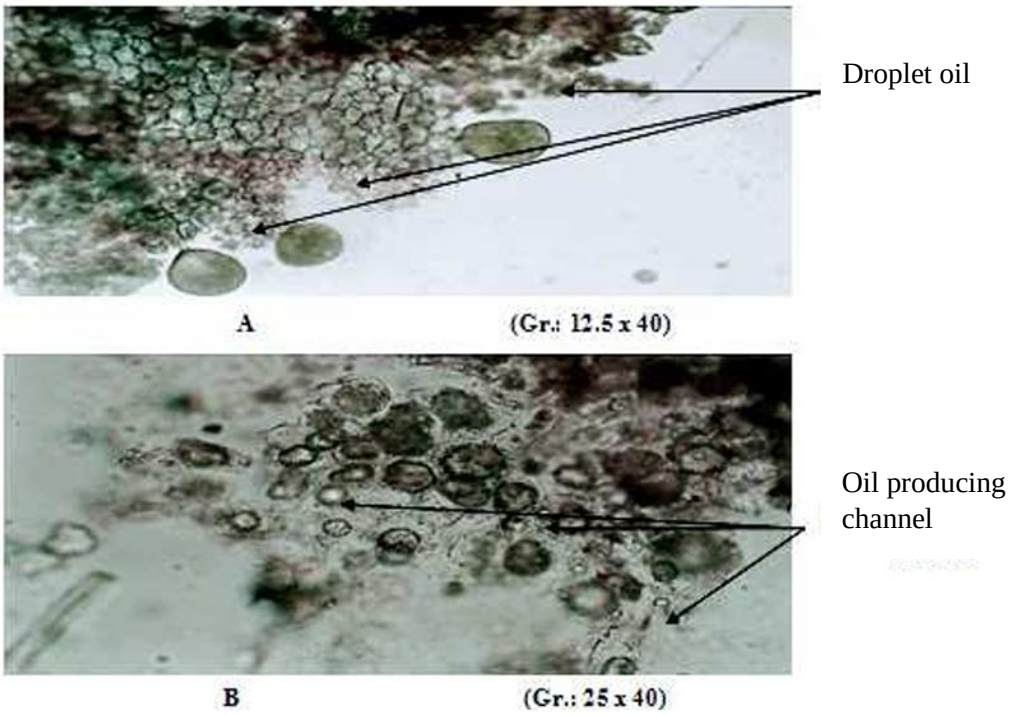


Figure4: Cross section of the seed of the atlas pistachio tree (A, B) (Acheheb, 2013)

The oil content of *Pistacia atlantica* fruits at ripening stage can reach up to 46 % (**Yousfi et al., 2017)**, depending on the ecotype and environmental conditions. This oil is predominantly composed of unsaturated fatty acids, with 39 to 49% of oleic acid, and 23.6 to 31% of linoleic acid being the most abundant (**Labdelli et al., 2019)**

III.2. Phytochemistry

The *P. atlantica* fruit composition is rich of bioactive molecules that give it many important biological activities, such as the anti-inflammatory, antioxidant, and antimicrobial, etc. several studies reported a large phytochemical range of biomolecules in the fruit leaves and bark of *P. atlantica* (essential oil, polyphenols, vitamins, etc.) (**Achili et al., (2020**); **Hatamnia et al., (2014); Falahati, et al., (2015))**. About 30 compounds in the essential oil, were identified, where the main compounds were monoterpenes especially α-Pinene with a considered yield (71.79 %) (**Fathollahi et al., 2019**).

A study conducted by **Ghalem et al., (2007**) focused on biochemical profiling where Phytosterols were found in significant amounts in *P. atlantica*. They reported that β-sterol is the predominant compound within the sterols classes, representing approximately 91% of the total sterol composition. In fact, its level is comparable to those in leading vegetable oils such as soybean, corn, sunflower, and canola, which are benchmark sources for these bioactive compounds.

Moreover, the phenols are also well known as an important compounds and contribute strongly to the total antioxidant activity of *P. atlantica* **(Benmohamed et al., 2023).** These authors recorded that all studied unripe fruits of *P. atlantica* from four different regions contained a large amount of phenolic content ranged from 213.7059 (mg GAE/g) to 227.0392 (mg GAE/g). Molecular identification of the phenolic constituents in the fruit extracts revealed the presence of Luteolin, luteolin 7-glycoside, chlorogenic acid, kampferol, naringin and naringin 7-glycoside **(Mahjoub et al,. 2018**).

IV. **Extraction of phenolic compounds**

Multiple methods are available and widely used to extract bioactive components from plant material for various fields of utilization, including pharmaceutical, food, and chemical industries.

IV.1. Extraction conditions and parameters

The efficiency of both conventional and non-conventional extraction methods largely depends on critical input parameters. Therefore, a thorough understanding of the plant matrix, the chemistry of bioactive compounds, and scientific expertise is crucial (**Azmir et al., 2013**). In this context, **Bouterfas et al,. (2014**) showed that different experimental conditions has a notably influence on the values of total phenolic and total flavonoide compounds which the organic solvent type, solvent concentration, extraction time and extraction temperature are the main factors. The polarity index and the solubility of phenolic compounds in the extraction solvents is one of those parameters that effect the extractability and recovery yield of a particular component like phenolic compounds, which are often extracted in higher amounts in more polar solvents (**B Iloki-Assanga et al., 2015**).

IV.2. Advances in extraction techniques

The development of novel extraction techniques has been made possible by new technological innovations. Modern extraction techniques are not only improving the speed, precision, and environmental impact of extraction processes, but they are also creating new avenues for the investigation of novel bioactive compounds derived from bioavailable materials (**Sigauke et al., 2024**). The main important methods are listed below:

- **Microwave-assisted extraction (MAE)**

The mechanism of MAE is based on exposing the analytes to the solvent, where microwave treatment induces a rapid temperature rise and an increase in internal pressure, leading to the disruption of the cell structure (**Dhobi et al., 2009**). The principle difference between conventional heating and microwave heating is the direction of heat and mass transfers that occur during the process, with varying temperatures through the bulk of the liquid as shown in (Figure 5) (**Karmakar et al., 2019**).

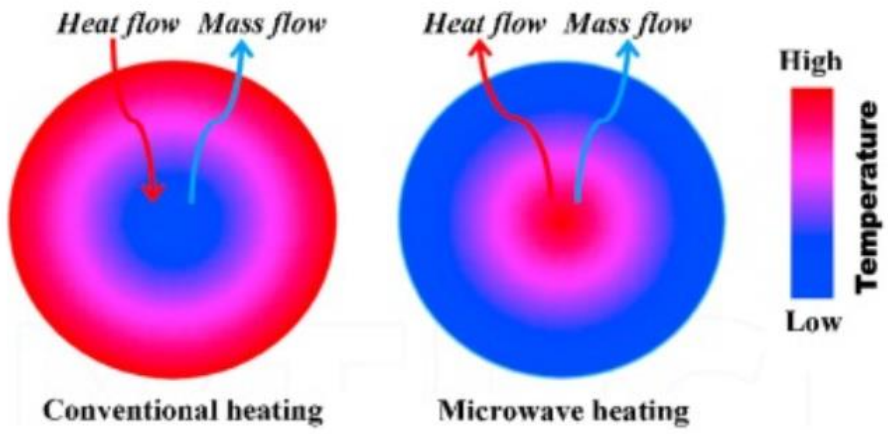


Figure 5: Differences between conventional and microwave heating in terms of heat transfer and mass transfer gradients (**Karmakar et Halder., 2019**)

- **Supercritical fluid extraction**

Supercritical fluid extraction (SFE) is a method that use the unique properties of supercritical fluids (Figure 6). The carbon dioxide is used as the mainly fluid for the SFE method. It is an ideal solvent for thermally labile compounds because of its low critical temperature. (**Kim et al., 2012**). In addition, it has low viscosity that allows a better diffusivity of the solute, and the density values of SF enables substantial solvation power (**Niraj Vyas,**

2009). The principle of supercritical fluid (SFE), as an extraction method, is based on the properties of both gas and liquid when it is above its critical point (**Sigauke et al., 2024**)

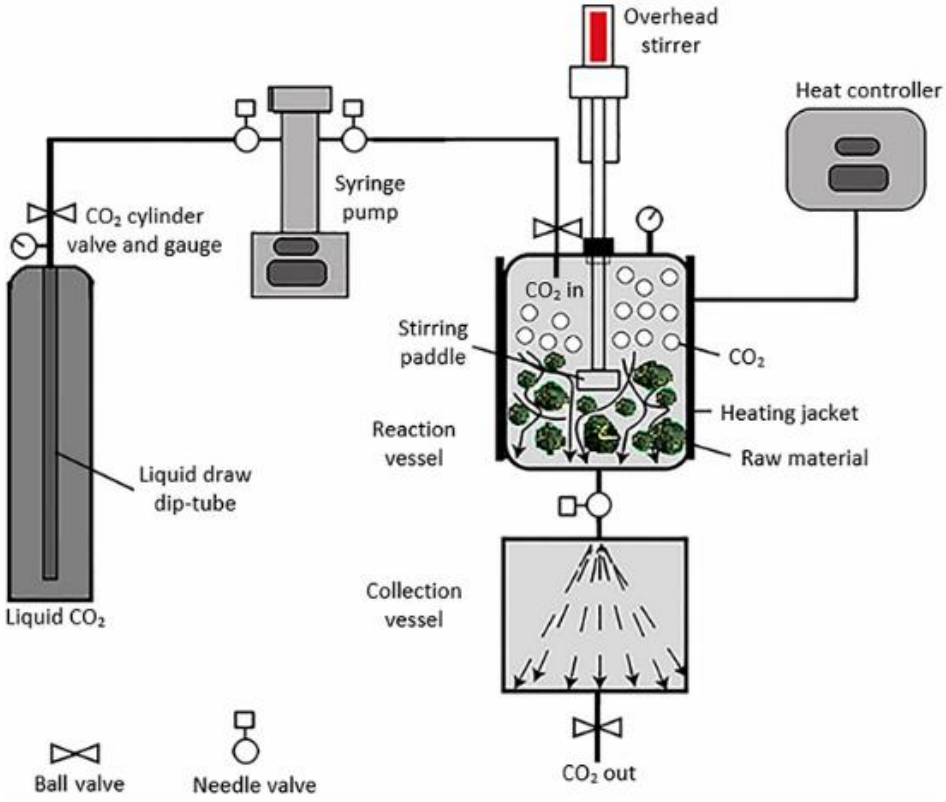


Figure 6: the supercritical fluid extraction system (Khaw et al., 2017)

- **Ultrasound assisted extraction**

Ultrasound-assisted extraction (UAE) is a widely studied and recognized method for the efficient extraction of bioactive compounds (**Zhu et al., 2024**). UAE is typically based on the mechanism of acoustic cavitation, which involves the collapse of microbubbles in the solvent (Figure 7). This phenomenon generates compression and expansion forces within the matrix, leading to cell wall disruption and enhanced permeability, thereby improving the extraction yield of the desired compounds (**Iqbal et al., 2021**).

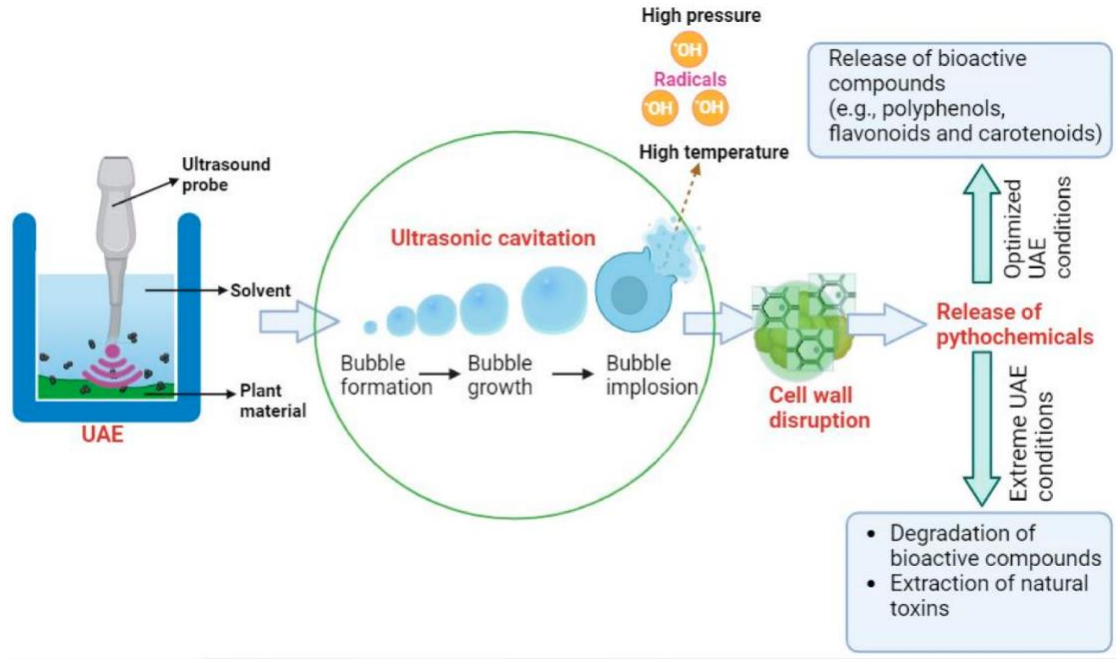


Figure 7: Mechanism of the UAE of bioactive compounds from plant materials (Demesa et al., 2024).

Some advantages of the UAE method over conventional extraction (CE) should be considered, such as time extraction and mass transfer. UAE could also be a nondestructive method of bioactive molecules. In this context, high amount of TPC is obtained in a short extraction time, and cavitation produced during the UAE improves the mass transfer of solutes in the solvent due to cell wall disruption and microstreaming effect (**Herrera-Pool et al., 2021**).

Material and methods

Part II: Material and method

I. Plant material

Pistacia atlantica fruits used in our study were collected randomly from Ghardaia region (Oued-Enssa) on September 2024. Collected fruits were mainly at the ripe stage and stored at 10°C.

II. Oil extraction

II.1. Conventional liquid-solid extraction

The fresh fruits was grind. Then 50 g of the powder were mixed with hexane (150 ml). The mixture was stirred for 48 hours at room temperature. Then the oil was separated by using rotary evaporator at 40°C and stored in the dark at low temperature (10°C), according to the method described by Yousfi et al., (2017) with some modification. The results are expressed with percentage of lipid content in the dried samples.

II.2. Soxhlet extraction

The oil was extracted from 25 g of sample per cartridge (with a total of 225 g) using a Soxhlet apparatus and 150 ml of 99% hexane, heated in the apparatus at 65 °C for three hours. The hexane phase was separated from the oil by using rotary evaporator at 40 °C, and then the sample was stored in the dark at 10 °C. The results are expressed with percentage of lipid content in the dried samples.

III. Oil and powder characterization

Conventional and Soxhlet extracted oils samples were characterized by determining their acidity value (mg KOH/g) and peroxide index (meq O2/Kg) by using standard procedure of AFNOR (2001). The moisture and the ashes of the powder were determined respectively by using IR desiccator until the weight stabilization and the mineralization of 9.03 g in an oven at 500°C for 3h, according to the classical methods (A.F.N.O.R. 2001).

IV. Optimization of phenolic compounds extraction

IV.1. Experimental design

The optimization of phenolic compound extraction and antioxidant activity is modeled through response surface methodology (RSM). A central composite design (CCD) was used to evaluate the effect of three independent variables: ultrasound amplitude, extraction time, and solvent concentration. These independent factors were set at low (− 1), central (0), and high (+ 1) levels respectively as following : 40, 70, and 100 % for ultrasound amplitude (X1), 0.5, 6.25, and 12 min for time (X2), and 20, 55, and 90% for ethanol concentration (X3). The different combinations of the three levels of the three factors according to CCD are shown in (**Table II**). Seventeen experiments were realized, and the central point is repeated three times. Experimental data were fitted to a second-order polynomial model as expressed in the Eq. (1):

$$y = \alpha^0 + \sum_{i=1}^{\alpha} \alpha_i x_i + \sum_{i=1}^{\alpha} \alpha_{ii} x_i^2 + \sum_{i=1}^{\alpha} \sum_{j=i+1}^{\alpha} \alpha_{ij} x_i x_j \ (i \neq j) \qquad (1)$$

Where y is the response variable, xi and xj represent the independent factors affecting the response, α0, αi, αii, and αij are the regression coefficients, and α is the number of factors.

Table II: The different combinations of the three levels of the three factors according to CCD

N°	Configuration	Variables		
		X_1	X_2	X_3
1	−−+	40	0.5	90
2	−++	40	12	90
3	00a	70	6.25	20
4	00A	70	6.25	90
5	++−	100	12	20
6	0	70	6.25	55
7	A00	100	6.25	55
8	−+−	40	12	20
9	0a0	70	0.5	55
10	0	70	6.25	55
11	−−−	40	0.5	20
12	+−+	100	0.5	90
13	+++	100	12	90
14	0A0	70	12	55
15	+−−	100	0.5	20
16	a00	40	6.25	55
17	0	70	6.25	55

X_1, Ultrasound amplitude (%); X_2, Time (min); X_3, Ethanol concentration (%); TPC, total phenolic compounds (mg GAE/g DW); DPPH, DPPH inhibition percentage (%).

IV.2. Ultrasound assisted extraction

After extracting the oil from the ground fruits, the delipidated powder was extracted using 96% methanol at a 1:10 ratio. The extractions were carried out according to the planned experimental design (the CCD 17 experiments). For each extraction, the suspension was centrifuged after the ultrasound assisted extraction (UAE), then the supernatants were recovered for determination of antioxidant activities and total phenolic compounds contents (TPC).

IV.3. Determination of total phenolic content

According to Singleton and Rossi., (1965) the TPC was determined by using Folin ciocalteu reagent. 1ml of diluted folin ciocaltu (1/10) were added to 200 µl of the extract, and

800 µl of sodium carbonate Na_2 Co_3 (7.5%). After 30 minutes of incubation in dark, the absorbance was read at 760 nm by UV-Visible spectrophotometer. The results of TPC were expressed as milligrams gallic acid equivalent per gram of dry weight (mg GAE/g DW). The calibration curve is presented in annex1.

IV.4. Determination of DPPH radical scavenging

The free radical scavenging activity of the extract was determined using the DPPH method as Dudonne et al., (2009) describe it. One hundred microliters of the sample dilution were added to 3 mL of DPPH solution prepared in ethanol (60µM). Ethanol was used as a control. After 30 minutes of incubation in the dark, the absorbance was measured at 515 nm. The percentage of inhibition was calculated using the following formula:

$$DPPH\ (\%) = [(A_C - A_S) / A_C] \times 100$$

Where A_C is the absorbance of the control (ethanol with DPPH), and A_S is the absorbance of the sample extract with DPPH.

IV.5. Statistical analysis

JMP® software (version 10.0, SAS Institute Inc.) was used for the construction of the experimental design, the analysis of the mathematic model, and the construction of 3D plots. All experiments were performed in triplicate and expressed as the mean ± standard deviation. The level of significance was considered to be $p < 0.05$.

Results and discussion

PART III: RESULTS AND DISCUSSION

I. Physical characteristic of *Pistacia atlantica*

The collected *Pistacia atlantica* fruits are characterized by an ovoid shape and thin pericarp with a black or green color at full ripening stage (A), dark red at intermediate stage (B) and red at unripe stage (C) as illustrated in **figure 8**.

Figure 8: Different stages of ripening of *Pistacia atlantica* fruits (A,B and C)

The dimensions and average weight were determined from 10 harvested fruits, as shown in **Table III**.

Table III: dimension of the harvested fruits.

Length	7.12 ± 0.63 mm
Width	4.39 ± 0.47 mm
Weight (average)	126 ± 8.39 mg

The morphological characteristics of the collected fruits are quite similar to those reported by **Monjauze., (1980)** who studied Algerian *P. atlantica* as a spontaneous growing trees in arid regions.

II. Ashes and moisture of *P. atlantica* fruits

The ashes obtained after incineration were 0.296 ± 0.023 g which represent 3.2 % of the fresh matter. This finding is lower when compared to the ashes content reported by **Hachemi and Bendahmane (2007**), who analyzed Northwest Algerian *P. atlantica* fruits (5.54±0.11 %).

The moisture content of the sample was approximately 6.30%. According to the same author, our results are too lower than their percentage (21.26%). This could be explained by the climatic conditions (arid environment) and the possible genetic variability inside the same specie. In addition, the fruits were harvested on spontaneously grown trees without any irrigation (**HARFOUCHE., et al 2005**). Otherwise, the moisture depends also on the storage condition and the ripening stage of the fruits (**Paull, 1999**).

III. Chemical properties of *P. atlantica* oil

Oil obtained from the fruits is slightly aromatic with a clear yellow color. Chemical properties and content of the oils obtained from two different extraction methods are shown in **Table IV**. The extraction yield using Soxhlet was 32.37 %, and higher than extraction by maceration at room temperature: 26 %. This difference can be attributed to the higher efficiency of the Soxhlet method, which operates at elevated temperatures and continuously recycles fresh solvent, thereby enhancing the mass transfer rate and improving oil recovery (**Mandal et al., 2021**).

Table IV: Chemical properties of oil extracted by two different method.

Parameters	**OES**	**OER**
Acidity index (mg KOH/g)	13.52 ± 0.05	14.86 ± 0.73
Peroxide index (meq O2/Kg)	8.67 ± 1.31	5.17 ± 1.25
Oil (%)	32.37 %	26 %

OES: Oil extracted by Soxhlet; **OER**: Oil extracted in a room temperature

The extraction yield obtained by OER is near to the interval values reported by Yousfi et al., (2017), who studied the extraction yield of *P. atlantica* fruits at different stages of ripening from different sites in the region of Laghouat, with a yield varied from 27.7 % to 46.06%.

Whereas the extraction yield obtained by OES belong to the same interval values. This difference may be due to the effect of rainfall and temperature conditions (**Hilou et al., 2022**).

In addition, whatever the extraction method (OES, OER), the fruits used in our study are mainly at the ripening stage (80 %), which could explain the lower value of oil content in the studied fruits.

The acidity index of OES and OER were found to be 13.52 ± 0.05 mg KOH/g and 14.86 ± 0.73 mg KOH/g respectively. These values are comparable to those reported by Youssfi et al. (2017) for *P.atlantica* fruits at the ripening stage from various sites, which ranged from 4.2 to 15.15 mg KOH/g. The peroxide index of OES and OER obtained is 8.67 ± 1.31 meq O2/Kg, and 5.17 ± 1.25 meq O2/Kg respectively. Comparing to those reported by Achheb (2013), which was (8.350 ± 0.120 meq O2/Kg) our results are slightly lower.

Acidity index is directly related and proportional to the release of fatty acids from the oil triglycerides. This phenomenon could be triggered by multiple factors such as enzymatic hydrolysis (lipases activities), microbial proliferation or a mineral (metal Ion) catalysis during the storage and/or fruit processing (Mattson et al., 1966, Corma et al., 2005). Regarding to the peroxide index, this parameter indicates the oxidation and/or the peroxydation status of the oil. Like the acidity index, this parameter should be as lower as possible. It is mainly increased by high temperature, Oxygen concentration and light (Ahmed et al., 2019).

IV. **Optimization of TPC extraction and antioxidant activity**

The choice of ethanol and water mixture and ultrasound-assisted extraction (UAE) was based on their proven effectiveness in extracting phenolic compounds. Ethanol, particularly in aqueous mixtures between 50% and 80% (v/v), it offers an ideal balance its moderate polarity improves the solubility of phenolic, while the water content facilitates the breakdown of plant cell walls, enhancing extraction yield (**Lohvina et al., 2021**). The decision to use UAE stems from its advantages as a green and highly efficient technique. Through acoustic cavitation generated by ultrasonic waves, UAE disrupts cell structures and enhances solvent penetration, allowing for greater mass transfer and higher phenolic recovery at lower temperatures and shorter times than conventional methods (**Medina Torres et al., 2017**). These parameters were therefore chosen to optimize both the efficiency and sustainability of the extraction process.

IV.1. Model fitting and statistical validation

The response variables: total phenolic content (TPC) and antioxidant activity (DPPH), obtained using a central composite design (CCD), and the studied factors (X_1, X_2, and X_3) are presented in **Table V**. The results showed that concentration of TPC and DPPH inhibition rate are varied from 7.56 to 21.42 mg GAE/g DW, and from 29.93 to 67.17 % respectively.

Table V: Matrix of runs and experimental and calculated values for TPC and DPPH of *P. atlantica* fruits.

N°	Configuration	Variables			Experimental		Predicted	
		X_1	X_2	X_3	TPC	DPPH	TPC	DPPH
1	−−+	40	0.5	90	7.56 ± 0.34	29.93 ± 0.96	6.94	28.79
2	−++	40	12	90	10.05 ± 0.10	34.46 ± 1.59	10.22	35.08
3	00a	70	6.25	20	13.50 ± 0.11	46.19 ± 1.51	13.29	46.11
4	00A	70	6.25	90	12.16 ± 0.10	40.42 ± 1.76	12.13	40.39
5	++−	100	12	20	16.82 ± 0.35	47.2 ± 3.58	17.50	48.37
6	0	70	6.25	55	17.17 ± 0.49	48.97 ± 4.39	17.42	55.38
7	A00	100	6.25	55	21.42 ± 1.18	49 ± 1.13	20.37	47.85
8	−+−	40	12	20	10.74 ± 0.05	42.73 ± 1.88	10.58	43.85
9	0a0	70	0.5	55	14.5 ± 1.53	50.53 ± 0.50	15.18	54.97
10	0	70	6.25	55	17.53 ± 1.21	57.29 ± 1.73	17.42	55.38
11	−−−	40	0.5	20	9.99 ± 0.08	25.49 ± 1.00	9.80	23.87
12	+−+	100	0.5	90	11.43 ± 0.43	37.8 ± 1.22	11.66	36.71
13	+++	100	12	90	17.78 ± 0.34	30.36 ± 1.62	18.04	32.01
14	0A0	70	12	55	19.68 ± 0.84	67.17 ± 1.57	18.76	62.61
15	+−−	100	0.5	20	13.72 ± 0.06	39.97 ± 3.05	13.62	39.38
16	a00	40	6.25	55	13.73 ± 0.35	40.59 ± 4.71	14.55	41.62
17	0	70	6.25	55	17.05 ± 0.39	59.66 ± 1.34	17.42	55.38

X_1, Ultrasound amplitude (%); X_2, Time (min); X_3, Ethanol concentration (%); TPC, total phenolic compounds (mg GAE/g DW); DPPH, DPPH inhibition percentage (%).

The reliability of the models was evaluated using the coefficient of determination (R^2), which ranges from 0 to 1. As shown in **Table VI**, the model achieved high predictive accuracy, with R^2 values of 0.98 for TPC and 0.94 for DPPH inhibition, indicating that the second-order polynomial model adequately describes the behavior of the responses. Furthermore, the

adjusted R^2 values were 0.95 for TPC and 0.86 for DPPH, confirming that the models fit strongly to the experimental data while accounting for the number of variables.

Table VI: Analysis of variance for determination of optimization model fit (Adjustment resume).

	DPPH	**TPC**
R^2	0.940	0.981
R^2 Adjusted	0.864	0.957

The *p*-values of both models were less than 0.05, indicating that the models are statistically significant and that the selected variables have a meaningful effect on the responses. In addition, the lack-of-fit p-values (**Table VII, VIII**) were 0.8549 for DPPH and 0.0689 for TPC, which are both higher than 0.05. This suggests that there is no significant lack of fit, and thus the models provide an adequate representation of the experimental data.

Table VII: ANOVA table of TPC models for *P. atlantica* fruits.

Total phenolic contents (mg GAE/g DW)					
Source	**DF**	**Sum of squares**	**Square of means**	**F Ratio**	**Prob. > F**
Model	9	228.728	25.414	40.146	<0.0001*
Error	7	4.431	0.633		
Total model	16	233.159			
Lack of fit	5	4.307	0.861	13.803	0.0689
Pure error	2	0.125	0.062		
Total error	7	4.431			

DF, Degrees of freedom; * statistically significant values ($p < 0.05$).

Table VIII: ANOVA table of DPPH models (**DPPH inhibition rate (%))**

Source	DF	Sum of squares	Square of means	F Ratio	Prob. > F
Model	9	1847.301	205.256	12.263	0.0016*
Error	7	117.166	16.738		
Total model	16	1964.468			
Lack of fit	5	54.128	10.826	0.344	0.8549
Pure error	2	63.038	31.519		
Total error	7	117.166			

DF, Degrees of freedom; * statistically significant values ($p < 0.05$).

IV.2. Interactive Effects of Extraction Parameters on TPC and AA

Table IX presents the parameter estimations of the fitted models for TPC and AA (DPPH inhibition), highlighting the linear, interaction, and quadratic effects of the extraction variables.

- Antioxidant Activity

Ultrasound amplitude (X_1) and extraction time (X_2) had significant positive linear effects on DPPH, with p-values of 0.047 and 0.021, respectively. This indicates that increasing either parameter enhances antioxidant activity, likely due to improved mass transfer and disruption of plant cell walls (**Iqbal et al., 2021**). However, the quadratic terms for amplitude (X_1^2, $p = 0.004$) and ethanol concentration (X_3^2, $p = 0.002$) showed significant negative effects. This suggests that excessively high levels of these parameters may reduce DPPH activity, potentially due to the degradation of sensitive antioxidant compounds caused by prolonged exposure of the bioactive molecules to ultrasound waves (**Dzah et al., 2020**).

In contrast, Ethanol concentration (X_3) showed a non-significant linear negative effect ($p = 0.063$), suggesting a slight tendency to reduce DPPH activity. Additionally, the interaction between all parameters (X_1–X_2), (X_1–X_3), and (X_2–X_3), were also not significant and negative ($p<0.05$), implying no synergistic effects between those factor pairs ($p = 0.099$, $P = 0.231$, and $p = 0.050$) respectively. Furthermore, the quadratic effect of time showed a non-significant and positive effect on DPPH (X_2^2, $p = 0.215$).

- Total phenolic component

All linear terms for ultrasound amplitude (X_1) and extraction time (X_2) were highly significant and positive ($p < 0.0001$ and $p = 0.0002$, respectively), confirming their roles in enhancing the TPC extraction. This indicates that increasing both amplitude and time improves phenolic compound recovery. Additionally, the interaction between amplitude and time (X_1–X_2) was significant and positive ($p = 0.0283$), indicating a synergistic effect, where their combination, enhances extraction more than each factor individually. Furthermore, the quadratic effect of ethanol concentration (X_3^2) was highly significant and negative ($p < 0.0001$), implying that both excessively low and high ethanol concentrations reduce TPC yield.

The ethanol concentration (X_3) exhibited a borderline of non-significant negative effect ($p = 0.0546$), suggesting that higher ethanol levels may slightly reduce TPC. Additionally quadratic terms for X_1^2 were non-significant and positive ($P = 0.9392$), and X_2^2 were non-significant and negative (0.3853). Regarding, the interactions effect of X_1–X_3 ($P = 0.4501$) and X_2–X_3 ($P = 0.0617$) were both not significant and positive.

In summary, ultrasound amplitude and extraction time are the most influential factors in enhancing both antioxidant activity and phenolic compound yield. However, these benefits reach a plateau or may decline at excessive values, as indicated by the negative quadratic effects. Ethanol concentration requires careful optimization, as its impact is nonlinear and sensitive to interaction effects. These results underscore the importance of fine-tuning extraction conditions to maximize the recovery of functional compounds while preserving their integrity.

Table IX: Parameters estimation of TPC and DPPH models (**DPPH inhibition rate (%)**).

TPC (mg GAE/g DW)				
Term	**Estimate**	**SE**	***t*-Ratio**	**Prob>|t|**
Intercept	17.421	0.340	51.17	<0.0001*
X_1	2.910	0.252	11.57	<0.0001*
X_2	1.788	0.252	7.11	0.0002*
X_3	-0.580	0.252	-2.31	0.0546
X_1- X_2	0.775	0.281	2.76	0.0283*
X_1- X_3	0.225	0.281	0.8	0.4501
X_2- X_3	0.625	0.281	2.22	0.0617
X_{1*} X_1	0.038	0.486	0.08	0.9392
X_{2*} X_2	-0.450	0.486	-0.93	0.3853
X_{3*} X_3	-4.712	0.486	-9.69	<0.0001*

DPPH (mg GAE/g DW)				
Term	**Estimate**	**SE**	***t*-Ratio**	**Prob>|t|**
Intercept	55.384	1.751	31.640	<0.0001*
X_1	3.113	1.294	2.410	0.047*
X_2	3.820	1.294	2.950	0.021*
X_3	-2.861	1.294	-2.210	0.063
X_1- X_2	-2.748	1.446	-1.900	0.099
X_1- X_3	-1.898	1.446	-1.310	0.231
X_2- X_3	-3.423	1.446	-2.370	0.050
X_{1*} X_1	-10.647	2.499	-4.260	0.004*
X_{2*} X_2	3.408	2.499	1.360	0.215
X_{3*} X_3	-12.137	2.499	-4.860	0.002*

X_1, Ultrasound amplitude (%); X_2, Time (min); X_3, Ethanol concentration (%); SE, Standard Error; * Statistically significant values ($p< 0.05$).

The mathematical quadratic model, at $p < 0.05$, is written in the Eq. 2, 3 and represented in response surface plots (Figure 9)the based on the statistical analysis of the CCD data and the relationship between the dependent factors and the (TPC and DPPH) response

$$Y_{TPC} = 17.421 + 2.910\ X_1 + 1.788\ X_2 + 0.775\ X_1X_2 - 4.712\ X_3^2 \quad \text{(Eq. 2)}$$

$$Y_{DPPH} = 55.384 + 3.113\ X_1 + 3.820\ X_2 - 10.647 X_1^2 - 12.137\ X_3^2 \quad \text{(Eq. 3)}$$

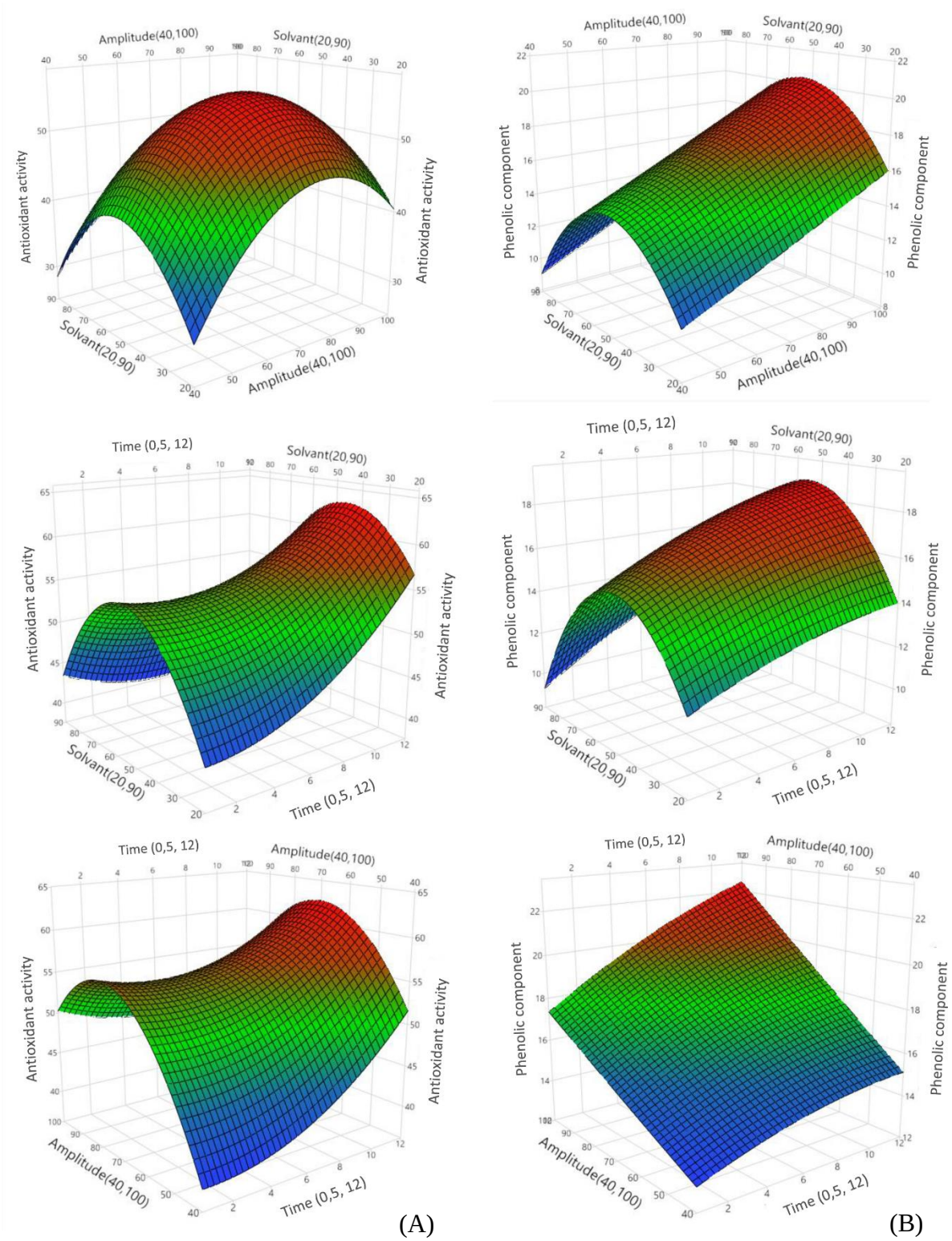


Figure 9: Response surface plots showing the combined effects of the extraction parameters on TPC (B) and antioxidant activity (A).

IV.3. Optimization results

The optimal response was calculated with the JMP profiler (Table X).

Table X: Optimal extraction conditions, predicted values and experimental results for TPC and DPPH Inhibition of *P. atlantica* fruits.

Parameters	Optimal values
Optimized parameters	
Ethanol concentration (%, v/v)	53%
Ultrasound amplitude (%)	86%
Time (min)	12
Optimized variables	
Estimated TPC (mg GAE/g DW)	20.67
Experimental TPC (mg GAE/g DW)	22.71 ± 0.413
Estimated DPPH inhibition percentage (%).	60.3 %
Experimental DPPH inhibition percentage (%).	63.44 % ± 2.133

The calculated TPC and DPPH inhibition optimal values were 20.67 mg GAE/g DW and 60.3 % respectively, which was achieved under optimal UAE using 53%, 86%, and,12 min for ethanol concentration, Ultrasound amplitude, and time, respectively. The TPC extracted and DPPH inhibition were assessed under the optimal conditions and the results were compared to the estimated optimal values, to validate the developed models. The experimental result of TPC under these optimal conditions was 22.71 ± 0.413 mg GAE/g DW, and the DPPH inhibition was 63.44 ± 2.133%, which were slightly higher to the estimated value (20.67 mg GAE/g DW, 60.3%), and the Student's t-test reveals a non-significant difference between the experimental and estimated TPC and DPPH inhibition values.

Conclusion

Conclusion

The aim of this study was to investigate the potential value of *P. atlantica* fruits through their physicochemical characterization and optimization of phenolic compounds extraction and their antioxidant capacity.

The fruits were ovoid, with an average length of 7.12 ± 0.63 mm and a width of 4.39 ± 0.47 mm, with an average weight of 126 ± 8.39 mg. The moisture and ash contents were 6.30% and 3.2%, respectively.

Oil was extracted using both Soxhlet and conventional method (maceration). The Soxhlet method yielded 32.37% of oil, whereas the conventional extraction at room temperature (with stirring for 48 hours) yielded 26%. The physicochemical properties of both oils were evaluated: the Soxhlet-extracted oil showed an acidity of 13.52 ± 0.05 mg KOH/g and a peroxide value of 8.67 ± 1.31 meq O_2/kg, while the conventionally extracted oil exhibited an acidity of 14.86 ± 0.73 mg KOH/g and a peroxide value of 5.17 ± 1.25 meq O_2/kg. These results for either extraction yield or physicochemical properties are consistent with values reported in the literature.

UAE Optimization of Phenolic compound extraction by RSM, specifically total phenolic content (TPC) and antioxidant activity (DPPH inhibition), demonstrated significant improvements with optimal values of 22.71± 0.413 (mg GAE/g DW) and 63.44 ± 2.133 % Respectively. The experimental values closely matched the predicted values, with correlation coefficients R^2 of 0.98 for TPC and 0.94 for DPPH inhibition. The optimal extraction conditions were determined to be 86% ultrasound amplitude, 53% ethanol concentration, and 12 minutes of extraction time. Among these variables, ultrasound amplitude and extraction time were identified as the most influential factors affecting the extraction yield of bioactive compounds from *P. atlantica* fruits.

In order to have a wider view of the bioactive potential of *P. atlantica* fruits, more investigations should be performed such as the detailed mineral profile of the ashes; the phytochemical composition of the extract and the chemical profiling of the extracted oil (fatty acid composition, tocopherol content and other lipophilic active compounds). This would provide a deeper insight into its nutritional and functional properties. Moreover, the antioxidant-rich extracts obtained through optimized ultrasound-assisted extraction should be assessed for their biological activities, including anti-inflammatory, antimicrobial, and cytoprotective

effects, to support their potential applications in the food, pharmaceutical, and cosmetic industries.

References

V. References

A.F.N.O.R (Association Française de Normalisation), Norme francaise, FT-60-2000 (2001).

A.F.N.O.R (Association Française de Normalisation), Norme francaise, V 04 – 401 (2001).

A.F.N.O.R (Association Française de Normalisation), Norme francaise, V 04 – 404 (2001).

Acar, I., & Kakani, V. G. (2010). The effects of temperature on in vitro pollen germination and pollen tube growth of Pistacia spp. Scientia Horticulturae, 125(4), 569-572.

Acheheb, H. (2013). Valorisation de l'huile des graines de Pistachier de l'atlas (Pistacia atlantica Desf.) (Défend d'une thèse de doctorat à l'Ecole Nationale Supérieur Agronomique de EL Harrach).

Achili, I., Amrani, A., Bensouici, C., Gül, F., Altun, M., Demirtas, Zama, D., Benayache, F., & Benayache, S. (2020). Chemical constituents, antioxidant, anticholinesterase and antiproliferative effects of Algerian Pistacia atlantica Desf. extracts. Recent patents on food, nutrition & agriculture, 11(3), 249-256.

Ahmed, I. A. M., Al-Juhaimi, F. Y., Özcan, M. M., Osman, M. A., Gassem, M. A., & Salih, H. A. (2019). Effects of cold-press and soxhlet extraction systems on antioxidant activity, total phenol contents, fatty acids, and tocopherol contents of walnut kernel oils. Journal of oleo science, 68(2), 167-173.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul M.H.A., Ghafoor K., Norulaini N.A.N., Omar A.K.M., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. Journal of food engineering, 117(4), 426-436.

Bellomo, M. G., & Fallico, B. (2007). Anthocyanins, chlorophylls and xanthophylls in pistachio nuts (Pistacia vera) of different geographic origin. Journal of Food Composition and Analysis, 20(3-4), 352-359.

Benhassaini, H., Bendahmane, M., & Benchalgo, N. (2007). The chemical composition of fruits of Pistacia atlantica desf. subsp. atlantica from Algeria. Chemistry of Natural Compounds, 43, 121-124.

Benlabiod, D., Sbabdji, M., Himrane, H., Chouicha, T., Hassiba, F., & Moulay, A. (2023). Diversification des espèces du barrage vert: Experimentation préliminaire. Annales de la Recherche Forestière en Algérie, 13(1), 54-61.

Benmohamed, M., Guenane, H., Messaoudi, M., Zahnit, W., Egbuna, C., Sharifi-Rad, M., Chouh, A., Ben Seghir, B., Rebiai, A., Boubekeur, S., Azli T., Harrat, M., Sawicka, B., Atanassova, M., & Yousfi, M. (2023). Mineral profile, antioxidant, anti-inflammatory, antibacterial, anti-urease and anti-α-amylase activities of the unripe fruit extracts of Pistacia atlantica. Molecules, 28(1), 349.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta, 76(5), 965-977.

Bhadange, Y. A., Carpenter, J., & Saharan, V. K. (2024). A comprehensive review on advanced extraction techniques for retrieving bioactive components from natural sources. ACS omega, 9(29), 31274-31297.

Bouterfas, K., Mehdadi, Z., Benmansour, D., Khaled, M. B., Bouterfas, M., & Latreche, A. (2014). Optimization of extraction conditions of some phenolic compounds from white horehound (Marrubium vulgare L.) leaves. International Journal of Organic Chemistry, 4(05), 292.

Corma, A., Abd Hamid, S. B., Iborra, S., & Velty, A. (2005). Lewis and Brönsted basic active sites on solid catalysts and their role in the synthesis of monoglycerides. Journal of Catalysis, 234(2), 340-347.

Demesa, A. G., Saavala, S., Pöysä, M., & Koiranen, T. (2024). Overview and toxicity assessment of ultrasound-assisted extraction of natural ingredients from plants. Foods, 13(19), 3066.

Dhobi, M., Mandal, V., & Hemalatha, S. (2009). Optimization of microwave assisted extraction of bioactive flavonolignan-silybinin. Journal of chemical metrology, 3(1), 13.

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., & Mérillon, J. M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. Journal of agricultural and food chemistry, 57(5), 1768-1774.

Dzah, C. S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G., & Ma, H. (2020). The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. Food bioscience, 35, 100547.

El Zerey-Belaskri, A., Belyagoubi-Benhammou, N., & Benhassaini, H. (2022). From traditional knowledge to modern formulation: potential and prospects of Pistacia atlantica desf. essential and fixed oils uses in cosmetics. Cosmetics, 9(6), 109.

Falahati, M., Sepahvand, A., Mahmoudvand, H., Baharvand, P., Jabbarnia, S., Ghojoghi, A., & Yarahmadi, M. (2015). Evaluation of the antifungal activities of various extracts from Pistacia atlantica Desf. Current medical mycology, 1(3), 25.

Fathollahi, M., Aminzare, M., Mohseni, M., & Hassanzadazar, H. (2019, December). Antioxidant capacity, antimicrobial activities and chemical composition of Pistacia atlantica subsp. kurdica essential oil. In Veterinary Research Forum (Vol. 10, No. 4, p. 299).

Ghalem, B. R., & Mohamed, B. (2009). Bactericidal activity of Pistacia atlantica. Desf mastic gum against certain pathogens. African Journal of Plant Science, 3(1), 013-015.

Ghalem, B., & Benhassaini, H. (2007). Etude des phytostérols et des acides gras de Pistachia atlantica. Afrique Science: Revue Internationale des Sciences et Technologie, 3(3).

Guenane, H., Bentireche, F., Bellakhdar, A., Ould Elhadj, M. D., & Yousfi, M. (2017). Total tocopherol content and antioxidant activity of fruit oil from Pistacia atlantica Desf. Growing wild in Algeria. Der Pharma Chem, 9(13), 153-7.

Guenane, H., Bombarda, I., OuldElhadj, M. D., & Yousfi, M. (2015). Effect of maturation degree on composition of fatty acids and tocopherols of fruit oil from Pistacia atlantica growing wild in Algeria. Natural Product Communications, 10(10), 1934578X1501001023.

Haminiuk, C. W., Maciel, G. M., Plata-Oviedo, M. S., & Peralta, R. M. (2012). Phenolic compounds in fruits–an overview. International journal of food science and technology, 47(10), 2023-2044.

Harfouche, A., Chebouti-Meziou, N., & Chebouti, Y. (2005). Comportement comparé de quelques provenances algériennes de pistachier de l'Atlas introduites en réserve naturelle de Mergueb (Algérie). Forêt méditerranéenne, 26(2), 135-142.

Hatamnia, A. A., Abbaspour, N., & Darvishzadeh, R. (2014). Antioxidant activity and phenolic profile of different parts of Bene (Pistacia atlantica subsp. kurdica) fruits. Food chemistry, 145, 306-311.

Herrera-Pool, E., Ramos-Díaz, A. L., Lizardi-Jiménez, M. A., Pech-Cohuo, S., Ayora-Talavera, T., Cuevas-Bernardino, J. C., García-Cruz, U., & Pacheco, N. (2021). Effect of

solvent polarity on the Ultrasound Assisted extraction and antioxidant activity of phenolic compounds from habanero pepper leaves (Capsicum chinense) and its identification by UPLC-PDA-ESI-MS/MS. Ultrasonics Sonochemistry, 76, 105658.

Iloki-Assanga, S. B., Lewis-Luján, L. M., Lara-Espinoza, C. L., Gil-Salido, A. A., Fernandez-Angulo, D., Rubio-Pino, J. L., & Haines, D. D. (2015). Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of Bucida buceras L. and Phoradendron californicum. BMC research notes, 8, 1-14.

Iqbal, A., Schulz, P., & Rizvi, S. S. (2021). Valorization of bioactive compounds in fruit pomace from agro-fruit industries: Present Insights and future challenges. Food Bioscience, 44, 101384.

Kakani, V. G., Reddy, K. R., Koti, S., Wallace, T. P., Prasad, P. V. V., Reddy, V. R., & Zhao, D. (2005). Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Annals of botany, 96(1), 59-67.

Karmakar, B., & Halder, G. (2019). Progress and future of biodiesel synthesis: Advancements in oil extraction and conversion technologies. Energy conversion and management, 182, 307-339.

Khaw, K. Y., Parat, M. O., Shaw, P. N., & Falconer, J. R. (2017). Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review. Molecules, 22(7), 1186.

Khot, M., Raut, G., Ghosh, D., Alarcón-Vivero, M., Contreras, D., & Ravikumar, A. (2020). Lipid recovery from oleaginous yeasts: perspectives and challenges for industrial applications. Fuel, 259, 116292.

Kim, J., Choi, K., & Chung, D. S. (2012). Sample preparation for capillary electrophoretic applications. Comprehensive sampling and sample preparation, 701-721.

Labdelli, A., Zemour, K., Simon, V., Cerny, M., Adda, A., & Merah, O. (2019). Pistacia atlantica Desf., a source of healthy vegetable oil. Applied Sciences, 9(12), 2552.

Lohvina, H., Sándor, M., & Wink, M. (2021). Effect of ethanol solvents on total phenolic content and antioxidant properties of seed extracts of fenugreek (Trigonella foenum-graecum L.) varieties and determination of phenolic composition by HPLC-ESI-MS. Diversity, 14(1), 7.

Mahjoub, F., Rezayat, K. A., Yousefi, M., Mohebbi, M., & Salari, R. (2018). Pistacia atlantica Desf. A review of its traditional uses, phytochemicals and pharmacology. Journal of medicine and life, 11(3), 180.

Mandal, S. C., Nayak, A. K., & Dhara, A. K. (Eds.). (2021). Herbal biomolecules in healthcare applications. Academic press.

Mattson, F. H., & Volpenhein, R. A. (1966). Enzymatic hydrolysis at an oil/water interface. Journal of the American Oil Chemists Society, 43(5), 286-289.

Medina-Torres, N., Ayora-Talavera, T., Espinosa-Andrews, H., Sánchez-Contreras, A., & Pacheco, N. (2017). Ultrasound assisted extraction for the recovery of phenolic compounds from vegetable sources. Agronomy, 7(3), 47.

Monjauze, A. (1980). Connaissance du bétoum Pistacia atlantica Desf. Revue forestière française, 32(4), 356-363.

Mungwari, C. P., King'ondu, C. K., Sigauke, P., & Obadele, B. A. (2024). Conventional and modern techniques for bioactive compounds recovery from plants. Scientific African, e02509.

Paull, R. (1999). Effect of temperature and relative humidity on fresh commodity quality. Postharvest biology and technology, 15(3), 263-277.

Pourreza, M., Shaw, J. D., & Zangeneh, H. (2008). Sustainability of wild pistachio (Pistacia atlantica Desf.) in Zagros forests, Iran. Forest Ecology and Management, 255(11), 3667-3671.

Rankou, H., M'sou, S., Babahmad, R. A., Ouhammou, A., Alifriqui, M., & Martin, G. (2018). Pistacia atlantica. The IUCN Red List of Threatened Species.

Singleton, V.L., Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144.

Taib, N. (2021). Etude de la variabilité de la variabilité biologique du Pistachier d'atlas (*Pistacia atlantica* Desf), (Défend d'une thèse de doctorat à l'université Moulay Tahar -Saida-).

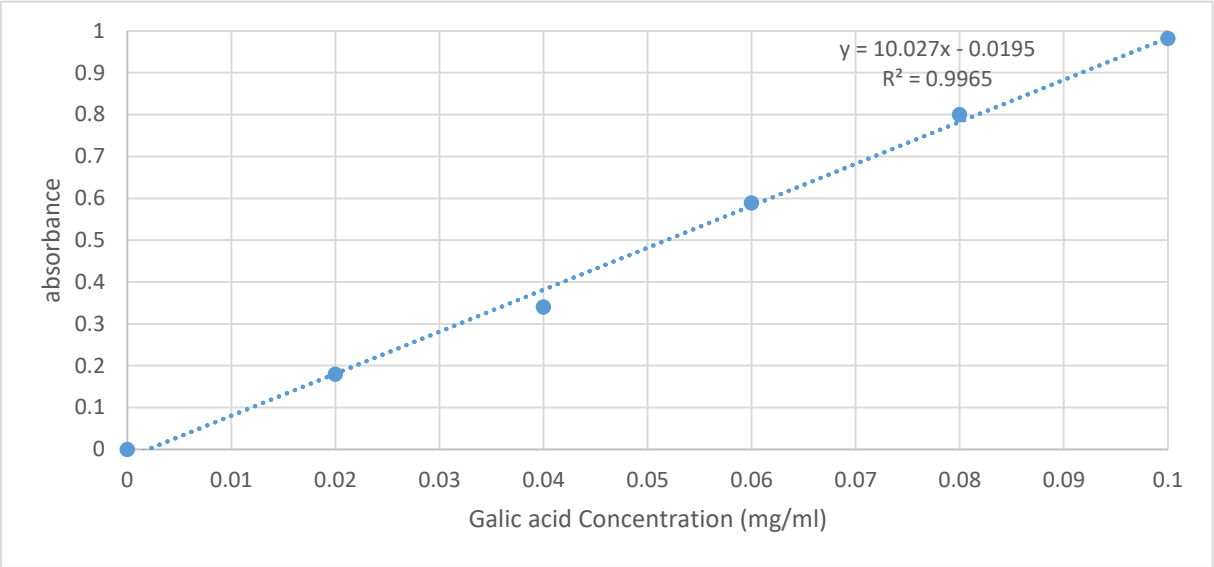
Tzakou, O., Bazos, I., & Yannitsaros, A. (2007). Volatile metabolites of Pistacia atlantica Desf. From Greece. Flavour and fragrance journal, 22(5), 358-362.

Zhu, J., Lu, Y., & He, Q. (2024). Recent advances on bioactive compounds, health benefits, and potential applications of jujube (Ziziphus Jujuba Mill.): A perspective of by-products valorization. Trends in Food Science & Technology, 145, 104368.

Zohary M. (1952). A monographical study of the genus Pistacia. J séries V. Palestine journ, Bot 4, p 187-228.

Annexes

Annex I



Title: Calibration curve of Galic acid

Abstract:

The main objective of this study was to optimize an ultrasound-assisted extraction (UAE) method for the recovery of phenolic compounds and antioxidant activity from delipidated powder. To this end, oil extraction from *Pistacia atlantica* fruits was carried out using solid-liquid extraction techniques, namely Soxhlet and maceration. The optimal extraction conditions were determined using response surface methodology (RSM). A central composite design was applied to evaluate the effects of three independent variables: ultrasound amplitude (40–100%), extraction time (0.5–12 min), and ethanol concentration (20–90%). The chosen ranges were identified through preliminary experiments. The oil yield extraction was 32.37 and 26%. The results of the UAE optimization demonstrated that ultrasound amplitude and extraction time had a statistically significant effect ($p < 0.05$) on the extraction yields of total phenolic content (TPC) and antioxidant activity. The optimized conditions 86% ultrasound amplitude, 12 minutes extraction time, and 53% ethanol concentration resulted in a TPC of 22.71 ± 0.41 mg GAE/g DW and an antioxidant activity of 63.44 ± 2.13%. The experimental results were not significant to the predicted values, confirming the validity of the optimization model.

Keywords: *Pistacia atlantica*, oil content, phenolic compound, antioxidant activity, UAE RSM optimization, central composite design.

Résumé :

L'objectif principal de cette étude était d'optimiser une méthode d'extraction assistée par ultrasons (UAE) pour la récupération des composés phénoliques et de l'activité antioxydante à partir de la poudre délipidée. Pour ce faire, une extraction de l'huile des fruits de *Pistacia atlantica* a été réalisée par extraction solide-liquide, en utilisant les techniques de Soxhlet et de macération. Les conditions optimales d'extraction ont été déterminées à l'aide de la méthodologie de surface de réponse (RSM). Un plan composite central a été appliqué pour évaluer les effets de trois variables indépendantes : l'amplitude des ultrasons (40–100 %), le temps d'extraction (0,5–12 min) et la concentration en éthanol (20–90 %). Les plages choisies ont été définies à travers des expériences préliminaires. Le rendement d'extraction d'huile était de 32,37 % par Soxhlet et de 26 % par macération. Les résultats de l'optimisation par UAE ont montré que l'amplitude des ultrasons et le temps d'extraction avaient un effet statistiquement significatif ($p < 0,05$) sur les rendements en contenu phénolique total (TPC) et en activité antioxydant. Les conditions optimales 86 % d'amplitude ultrasonore, 12 minutes de temps d'extraction et 53 % de concentration en éthanol ont permis d'obtenir un TPC de 22,71 ± 0,41 mg EAG/g MS et une activité antioxydant de 63,44 ± 2,13 %. Les résultats expérimentaux n'étaient pas significativement différents des valeurs prédites, confirmant ainsi la validité du modèle d'optimisation.

Mots-clés : *Pistacia atlantica*, teneur en huile, composés phénoliques, activité antioxydant, optimisation de l'extraction par l'ultrason assisté par RSM, plan composite central.