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# Phytochemical characterization and valorization of *Quercus ilex* (west of Algeria)

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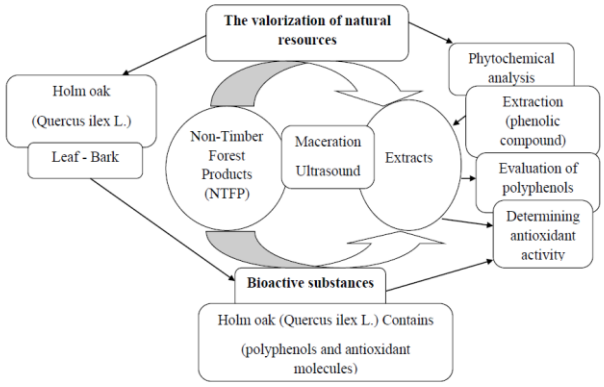
**Graphical abstract**




**Abstract**

This project is part of the promotion of natural resources such as Non-Timber Forest Products (NTFP). In order to assess the economic value of NTFP, this research focuses on the phytochemical analysis of holm oak (*Quercus ilex* L.) from the Terni region (Western Algeria). It focused on bioactive substances, particularly polyphenols. The method used consists of extracting the phenolic compounds by two methods which are maceration and ultrasound from the leaves and bark of this species. The quantity of these compounds is assessed spectrometrically and the antioxidant activity of the extracts in vitro using free radical scavenging and total antioxidant capacity assays. The results obtained indicate that maceration offers the best yield for extracting phenolic compounds from the leaves, with a yield of 10.74%. These results also showed that the extracts of holm oak leaves and bark are rich in phenolic compounds and have a content of total phenols (103,014±1,393 and 813.462±1.675 mg EAG/g DM), flavonoids (87.078±6.220 and 949.936±36.394 mg EC/g DM) and condensed tannins (57.194±12.447 and 940.195±20.417 mg EC/g DM). Regarding the antioxidant power, it is evident that all extracts have significant antioxidant capacity, and all have high free radical scavenging activity. The highest inhibition percents are 84.98±7.45% for the leaf extract and 83.15±9.27% for the bark extract. According to these results, holm oak contains a large quantity of polyphenols and antioxidant molecules. This information represents a natural source of antioxidants which remains to be exploited for future use in the agri-food and health sectors, within the broader framework of promoting the biodiversity of Algerian plants (NTFP).

**Keywords**: Holm oak, Valorization, NTFP, Polyphenols, Antioxidant activity.

## 1. Introduction

Non-timber forest products (NTFPs) have attracted major interest on a global scale in recent years due to the growing recognition of their role in the economy (Apema and *et al.* 2010). There are more than 150 non-timber forest products in international trade, with trade averaging between US$5 and US$10 billion in the 1990. The leaves, bark and fruits are NTFP, which are used for the production of phytotherapeutic products or other preparations such as perfumes, deodorants, food products or medicines.

The production of NTFP plays an important role in rural livelihoods and in the national and international economies of many African countries (FAO 2003). Interest in natural substances has grown in recent years in many fields, as they are increasingly reluctant to be used in products due to their bioactive chemical properties (Boissiere 2018).

Nowadays, more and more industries are moving towards incorporating these natural compounds into their products. Therefore, the exploitation of these natural resources offers significant economic potential. Thanks to the diversity of species, these substances are accessible. Many biologically active substances are present in the plant kingdom (Bahorun *et al.* 1996), which allows humans to have essential resources for their hygiene, health and nutrition (Cherif *et al.* 2015). Among these substances, we find polyphenols, which are natural

compounds very widespread in plants. They are all the more interesting today as they could be beneficial for the prevention of aging diseases. Indeed, their use as natural antioxidants has advantages for preventing and treating cancer (Chen *et al.* 2004), inflammatory diseases (Laughton *et al.* 1991) and neurodegenerative diseases (Orgogozo *et al.* 1997). According to Suhaj (2006), they are also used as additives in the food industry, pharmaceuticals and cosmetics. The antioxidant activity of plant phenols is similar to that of vitamins C and E (Rice-Evans *et al.* 1997). Many substances are currently being sought for plant extracts due to their biological properties. Current research mainly focuses on the study of natural antioxidant molecules.

The forests of the Tlemcen Mountains present a very diverse botanical landscape, depending on climatic conditions, soil and relief, from the coast to the steppe. They are characterized by mixed groups of holm oak and zeen oak. *Quercus* is one of the most widespread forest genera. It includes several hundred woody species from temperate and Mediterranean regions, America, Europe and Asia, some of which are economically important. In Algeria, the holm oak plays an essential role in soil preservation; it is present in the northwest of the country in a semi-arid state.

The holm oak, very widespread in the Mediterranean basin, has aroused the interest of numerous scientists who have explored very diverse aspects: botany, ecology, genetics, biochemistry (Rached-Kanouni *et al.* 2016). The holm oak is an evergreen species of the Fagaceae family. The acorns of this species are edible and the bark and leaves have therapeutic properties.

The present study focuses on the phytochemistry of leaves and bark of holm oak from the Terni national forest of the Monts de Tlemcen "West Algeria", in order to evaluate the economic value of NTFP of plant origin. The method used consists of extracting phenolic compounds (total phenols, tannins and flavonoids), quantifying them by spectrometry and evaluating their antioxidant activity using two different tests (DPPH and TAC). The objective is to find the best method for extracting phenolic compounds from holm oak by-products by testing different extraction methods and solvents and determine the antioxidant activity of these extracts.

## 2. Materials and methods

### *2.1. Plant material*

Professor Benabadji N., head of the Ecology and Management of Natural Ecosystems laboratory, Abou Bakr Belkaid-Tlemcen University (Alegria), identified the holm oak. Samples (leaves and bark) were taken from three sites located at different altitudes (1227-1338-1400m) in November 2022. The leaves and bark are first washed under running water before being processed and dried in the laboratory away from light. After drying, the samples were ground into a fine and homogeneous powder, and then stored in pill boxes in the dark for subsequent analyses.

### *2.2. Extraction of phenolic compounds*

Two different techniques were used for extraction: maceration and ultrasound with different solvents. According to (Hamia *et al.* 2014), maceration (solid-liquid extraction) involves letting the plant material (ground material) remain in aqueous methanol in order to extract the active ingredients such as phenolic and flavonoid compounds. Ultrasound-assisted extraction has been successfully employed to rapidly recover polyphenols and other bioactive compounds from various plant matrices in a short time (Orphanides *et al.* 2014).

### *2.3. Preparation of extracts*

Extraction by maceration consists of extracting the maximum of active substances contained in the samples by adding a quantity of water to increase its polarity.

A quantity of 1g of each dry sample (leaves or bark) and finely ground is macerated in 10 ml Methanol-water in a proportion (8:2) by volume, at room temperature for 24 hours. Manual stirring from time to time was carried out. Subsequently, the extracts were then filtered on filter paper. The filtrates were concentrated using a vacuum rotary evaporator at 45°C.

Regarding ultrasonic extraction, 1g of leaf and bark powder was added to 10 ml of a mixture (acetonitrile + distilled water + formic acid) at a temperature of 37°C. The optimal extraction time is 1 hour with a rotation frequency of 130 KHz, then leave to macerate for 24 hours in the dark.

Subsequently, the extracts were filtered through filter paper. The filtrates were concentrated using a vacuum rotary evaporator at 45°C. Finally, all filtrates are finally stored in the dark at 4°C (Falleh *et al.* 2013).

The following formula gives the extraction yield (R) of phenolic compounds:

$$R\,(\%) = 100*(Me/Mp) \qquad (1)$$

Me: mass of the extract (mg).

Mp: mass of the test portion (mg).

### *2.4. Analyzes of phenolic compounds*

#### *2.4.1. Dosage of total phenols*

Phenolic compounds are estimated using the Folin ciocalteu method (Brand *et al.* 1995; Tamert and Latreche 2015). This method is used to determine the polyphenol content of medicinal plants and foods (Abdel -Hameed 2009). A volume of 200 µl of extract, 1 ml of Folin-Ciocalteu reagent diluted 10 times with water and 800 µl of 7.5% sodium carbonate solution were added to a test tube. After stirring, 30 min later, the absorbance was measured by a JENWAY 7315 spectrophotometer at 765 nm. The tubes are shaken and kept for 30 min. A calibration curve was carried out in parallel under the same operating conditions with different concentrations of gallic acid (0 to 1000 µg/ml). Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DM) Hatami and al. (2014).

*2.4.2. Determination of total flavonoids*

The aluminum trichloride method of (Bahorun *et al.* 1996) is used to quantify the content of total flavonoids in the leaf and bark extract of *Quercus ilex*. One milliliter of the sample is added to 1 ml of the AlCl3 solution (2% in methanol), the mixture is vigorously shaken. After ten minutes of incubation, the absorbance is read at 510 nm. The concentration of flavonoids is deduced from a calibration range established with quercetin (0–40 µg/ml) and is expressed in milligram of quercetin equivalent per gram of extract (mg EC/g DM) (Bouayed *et al.* 2011).

*2.4.3. Dosage of condensed tannins*

Condensed tannins were evaluated using the method described by (Julkunen-Titto 1985) by vanillin assay. A volume of 50 µl of the diluted extract is added to 1500 µl of the vanillin/methanol solution (4%), and then mixed using a vortex. Then, 750 µl of concentrated hydrochloric acid (HCl) is added. The resulting mixture is left to react at room temperature for 20 minutes in the dark. The absorbance is measured at 550 nm using a JENWAY 7315 spectrophotometer. A calibration curve is produced in parallel under the same operating conditions using catechin as a positive control. The results are expressed in milligram (mg) equivalents of catechin per gram of dry matter (mg EC/g DM) (Naima *et al.* 2015).

*2.5. Evaluation of antioxidant activity*

The 2, 2-diphenyl-1-picrylhy-drazyl (DPPH) tests made it possible to measure the anti-radical activity according to the method developed by Sanchez-Moreno and al. (1998). The methanolic solution of DPPH (0.025 g/l) is prepared by adding 50 µl of each solution of the extracts at different concentrations (0.0625 to 1). The absorbance is measured at 515 nm, after incubation for 30 minutes in the dark and at room temperature. The following formula expresses the antioxidant activity associated with the scavenging effect of the DPPH radical˙ as a percentage of inhibition (I):

$$(I\,\%) = \left[(AC - AE) / AC\right] \times 100 \qquad (2)$$

AC: DPPH absorbance; AE: absorbance of the sample.

The concentration of extract providing an inhibitory concentration of 50% ($IC_{50}$) was calculated from the graphical curve representing the percentage of inhibition against the extract. Ascorbic acid was used as a positive control.

For each extract, the inhibitory concentration (IC50) was determined, the concentration of the extract which allows the reduction of DPPH by 50%. The $IC_{50s}$ are determined using Microsoft Office Excel 2016. They are calculated by the linear regression lines resulting from the graphs drawn from the percentages of antioxidant activity as a function of different concentrations of the extracts and the standard used (Brand *et al.* 1995; Tamert and Latreche, 2015).

Total antioxidant capacity (TAC) was quantified according to the method of Prieto et al. (1999). A volume of 300 µl of the diluted extract was mixed with 3 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. After 6 min at room temperature, the absorbance is measured at 695 nm using a JENWAY 7315 spectrophotometer. The total antioxidant activity is expressed in mg of gallic acid equivalent per g of dry matter (mg EAA /g DM).

*2.6. Statistical analysis*

Results are expressed as mean ± standard deviation. Statistical significance was determined using the one-way analysis of variance (ANOVA) test, which was used to compare the means of the parameters. A p value less than 0.05 were considered a significant difference. The statistical study was carried out using the XLStat 2016 software. The PCA is used to determine possible homogeneous groups for the three plots as well as to study the correlations between the variables and the holm oak organs (leaves and bark).

## 3. Results and Discussion

*3.1. Extraction yields*

The yield is defined by the ratio between the quantity of solute extracted and the quantity percolated. There are many authors who have examined the impact of various extraction conditions on the extraction performance of phenolic compounds from plant sources (Jokić, 2010; Bonnaillie, 2012). The results of two extractions (Maceration and Ultrasound) are presented in **Figure 1.**

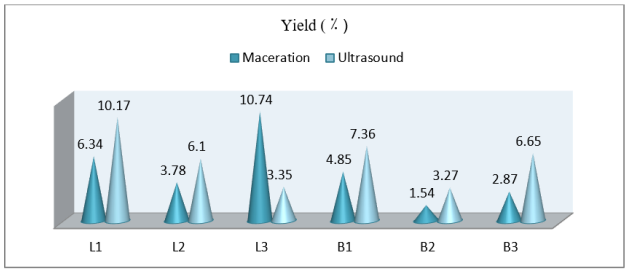



**Figure 1.** Yields of extracts obtained by maceration and ultrasound holm oak leaves and bark.

It is interesting to note that the differences in yields vary between leaves and bark. The best yield for extraction by maceration is recorded for the leaf samples from plot 3 (10.74 ±0.03); while the best yield of ultrasound extraction is obtained from the barks of plot 1 (7.36 ± 0.04% ). However, the difference remains non-significant (*p=0.14*) between the two extraction methods. Yield depends on several parameters (Penchev, 2012). Ultrasonic extraction for bark induced a significant increase in yield compared to extraction by maceration. On the other hand, the type of solvent strongly influenced the extraction yield. In this context, with the exception of the yield of the leaf extract from plot 3 having a better yield by maceration, all the yields of the other extracts obtained were higher by ultrasound. According to Mahmoudi and al. (2013), the presence of higher water content in the solvent system causes swelling of the plant material, which results in increased contact with the solvent, thus contributing to an improvement in extraction yield. In fact, the extraction yield and the

composition of the extracts vary depending on the nature of the solvent, the period, the place of harvest and the drying time, the temperature, the extraction time and the composition of the sample itself. (Penchev, 2012) ; according to Erdogan-Orhan and al. (2010), the extraction method can itself influence the extraction yield.

The results obtained clearly confirmed the influence of the solvent and the extraction method on the extractability of bioactive compounds. The results of the one-way ANOVA revealed the existence of a significant difference between the different extracts (B1, B2, B3, L1, L2, and L3) regarding performance (*p=0.001*). It is difficult to strictly compare these results with those carried out by other studies, because the yield is only relative and depends on the plant species studied, the geographical origin, the period of collection of the plant material, the part of the plant sampled, the content of each species in secondary metabolites, the nature of the solvent used in the extraction and the extraction method (Svoboda and Hampson, 1999).

**Table 1.** Total polyphenol contents (mg EAG/g DM) in holm oak leaves and bark.

| Extract | Maceration | U ltrasound |
|---|---|---|
| L1 | 414.432±2.708 | 813.462±1.675 |
| L2 | 541.320±0.911 | 719.369±75.249 |
| L3 | 225.547±2.098 | 549.770±3.567 |
| B1 | 514.154±6.196 | 787.667±3.858 |
| B2 | 103.014±1.393 | 446.179±2.728 |
| B3 | 572.623±3.427 | 648.581±2.110 |

*3.2. Phenolic compound contents*

Polyphenols are one of the most important groups of plant secondary metabolites. Studies have focused on the biological activities of phenolic compounds, known to be potential antioxidants and free radical scavengers (Zhang and Tsao, 2016). This is why, in this study, the concentrations of total polyphenols, flavonoids and tannins of the two aerial parts (leaves and bark) of the holm oak were measured.

The total polyphenol contents of the holm oak extracts were determined by the colorimetric method using the Folin-Ciocalteux reagent. This was chosen for its reliability and availability of the reagent.

All of the extracts in this study presented contents of phenolic compounds. The results mentioned in Table 1 show that the extracts of holm oak leaves and bark obtained by ultrasound are richer in polyphenols than those from maceration. Indeed, the highest levels are recorded in the leaves and bark of trees in plot 1 (813.462±1.675 / 787.667±3.858 mg EAG/g DM respectively); while the lowest levels are found in the leaves of plot 3 (225.547±2.098) and in the bark of plot 2 (103.014±1.393).

Total polyphenols were extracted by maceration and then ultrasound. The advantage of using the ultrasound technique is to improve the yields of total polyphenols (Bourgou *et al.* 2016; Boggia *et al*. 2016) . The results obtained are difficult to compare with those available in the literature because the structure and the proportion of extractables can vary depending on the species but also depending on the part of the tree considered (leaves or bark), the age of the tree , and environmental and seasonal conditions ( Chaouche *et al.* 2013 ; Thévenon, 2015). The extraction method and the solvent used also influence the polyphenol content (Bourgou *et al.* 2016). In the present case, the differences observed between the two methods used concern both the total polyphenol contents (higher contents for the ultrasound method) and the nature of these polyphenols (differences between the solvents used). These results are consistent with the work in the bibliography and fit into the range of data reported by Karioti (2010).

The flavonoid contents mentioned in Table 2 of all the extracts are between 87.078±6.220 and 949.936±36.394 mg EC/g DM; the best potentials are recorded respectively for the extracts of the leaves of plot 1 and the bark of plot 2 obtained by maceration (949.936±36.394 and 942.842±54.290 mg EC/g DM).

**Table 2.** Flavonoid contents in (mg EC/g DM) in the leaves and bark of the holm oak.

| Excerpt | Maceration | Ultrasoud |
|---|---|---|
| L1 | 949.936±36.394 | 248.320±70.766 |
| L2 | 485.755±2.588 | 213.248±6.345 |
| L3 | 245.399±2.733 | 97.815±36.601 |
| B1 | 431.009±10.676 | 591.070±8.794 |
| B2 | 942.842±54.290 | 87.078±6.220 |
| B3 | 151.577±9.020 | 122.339±3.823 |

These results corroborate those of several authors whose significant quantities of flavonoids are recorded in the leaves of the fabaceae *Uraria picta* and *Pericopsis laxiflora* with values of 334.48±0.49 and 210.27±0.70 mg EQ / 100g (Kouassi *et al.* 2020). Indeed, Koevi *et al.* (2015) and Koffi and al. (2015) indicated the presence of flavonoids in the leaves of *Pericopsis laxiflora* . The presence of flavonoids has also been shown in the methanolic extract of the leaves (Wink, 2013, Hari *et al.* 2014; Odubanjo *et al.* 2014). Flavonoids have the capacity to trap free radicals (Mpondo *et al.* 2012; Bakchiche and Gherib 2014) which promote cellular aging ( Burda and Oleszek, 2001 ). The phytochemical investigations of Karioti and colleagues (2010) on the methanolic extracts of *Quercus ilex* show that this species presents an important source of glycolyzed flavonoids, especially Kampferol (1.22%) which has been found with minimal quantities in other plants (0.5%).

The results showed that polyphenols (p= 0.035) and flavonoids (p= 0.017) are present in the different extracts obtained from the leaves and bark of this plant in a significant manner.

For the dosages of condensed tannins mentioned in Table 3, the contents are between 57.194±12.447 and 940.195±20.417 mg EC/g DM, the best values are recorded respectively for the extracts of leaves and bark collected from plot 3 obtained by ultrasound with contents of 940.195±20.417 and 366.770±4.075mg EC/g DM. The presence of condensed tannins would indicate

that there was no significant difference between the contents of the extracts obtained from holm oak including (p=0.18).

**Table 3.** Contents tannins (mg EC/g DM) condensed in the leaves and bark of holm oak.

| Excerpt | Maceration | Ultrasound |
|---|---|---|
| L1 | 284.966±19.743 | 154.554±18.461 |
| L2 | 235.793±2.759 | 137.272±11.715 |
| L3 | 57.194±12.447 | 940.195±20.417 |
| B1 | 124.170±11.380 | 184.105±0 |
| B2 | 82.122±0 | 72.930±6.630 |
| B3 | 113.311±50.360 | 366.770±4.075 |

According to Mahmoudi and al. (2013), the extraction of condensed tannins depends on their chemical nature, the solvent used and the operating conditions. However, the contents of condensed tannins can also be variable due to several factors such as: the sensitivity of tannins to several degradation pathways (oxidation, light, etc.), the stage of maturity, cultural conditions, climatic, pedological or predation stress.

In plants, tannins are located in various organs. The content and nature of tannins in a plant will also vary depending on the species, tissue and phenological stage (Schweitzer *et al.* 2008), as well as environmental conditions: increase in content with thermal stress and/or water (Tharayil *et al.* 2011; Bunglavan and Dutta 2013).

Indeed, these phenolic compounds are present in all parts of plants but with a quantitative distribution that varies between different tissues. Our results corroborate other research on the phytochemical study carried out on aqueous and hydromethanolic extracts which determines the contents of polyphenols, flavonoids and tannins. For the extracts obtained from *Griffonia simplicifolia,* the concentrations of polyphenols, flavonoids and tannins are variable in the aqueous and hydro-methanolic extracts (Akakpo *et al.* 2023). They are respectively of the order (8 - 14 mgEGA/g, 5.66 - 10.08 mgEQ/g and 10.89 - 14.39 mgECat/g).

These secondary metabolites are rather involved in defense mechanisms, they protect the plant against attacks by pathogenic microorganisms (fungi and bacteria) (Rira, 2006). Tannins are also a defense against attacks due to predators such as insects but also herbivores (Mueller-Harvey, 2006).

Tannins prevent the rapid degradation of plants in the soil, thus preserving a stock of nutrients for future growing periods. Tannins also play a physiological role as regulatory factors for plant growth; their presence in cells and the increase in their concentration in the presence of light provide a protective function against stress caused by the sun (Rira, 2006). As with all flavonoids, the presence of aromatic nuclei gives tannins an antioxidant function which protects the resins from enzymatic degradation and delays the autoxidation of ascorbic acid contained in certain plant substances. Tannins are among the main constituents of wood. Thanks to their helical structure, they prevent the degradation of wood cells caused by water deficiency.

*3.3. Antioxidant activity of holm oak extracts*

The antioxidant effect of holm oak leaf and bark extracts was evaluated in vitro by the DPPH test. The antioxidant capacity of the different extracts was determined from the inhibitory concentration ($IC_{50}$), this is the concentration necessary to reduce 50% of radicals. Note that a low $IC_{50\ value}$ implies a strong antioxidant activity of a compound (Locatelli *et al.* 2009; Salhi, 2020). The results obtained are represented in **Table 4**. The best extracts which have a very high reducing power are the bark extracts from plot 1 and the leaf extracts from plot 2 obtained by ultrasound with $IC_{50}$ equal to 0.019±0.0005 and 0.116±0.002 mg/ml respectively. For the hydro-methanolic extracts obtained by maceration, the best extracts are those of the leaves of plot 2 and of the bark of plot 1 which have an $IC_{50}$ equal to 0.181±0.001 mg/ml and 0.361±0.001 mg/ml respectively. This strong antioxidant activity of the extracts is due to the contents of the phenolic compounds that are the basis of this activity.

**Table 4.** Antioxidant activity of the extracts tested by DPPH.

| | | $IC_{50}$ (mg/ml) | | |
|---|---|---|---|---|
| Ultrasound | Plots | P1 | P2 | P3 |
| | Leaves | 0.151±0.001 | 0.116±0.002 | 0.227±0.001 |
| | Barks | 0.019±0.0005 | 0.145±0.002 | 0.183±0.002 |
| Maceration | Leaves | 0.259±0.001 | 0.181±0.001 | 0.330±0.001 |
| | Barks | 0.361±0.001 | 0.470±0.001 | 0.437±0.032 |

The inhibition rate of DPPH indicates that the most active extracts by ultrasound are the bark extracts from plot 1 with an inhibition rate equal to 47.55% for an extract concentration of 0.062 mmol/ml which inhibits half of DPPH and extracts of P2 leaves with an inhibition rate equal to 93.46% for an extract concentration of 1mmol/ml; whereas for hydro-methanolic extracts obtained by maceration; the best extract is recorded in the leaves of P2 with an inhibition rate equal to 91.96 %for an extract concentration of 1 mmol/ml and the bark extract of P1 with an inhibition rate equal to 34.98 for an initial concentration of 0.062 mmol/ml. Through these results the antioxidant power, extracts have strong anti-radical activity. This power is inversely proportional to the $IC_{50}$ (Prakash *et al.* 2007). The antioxidant power linked to phenolic compounds presents a wide range of physiological properties, including antiallergenic, antiartherogenic, anti-inflammatory, antimicrobial (Pietta *et al.* 1998; Yanishlieva *et al.* 2006; Kirka and Arslan 2008).

Antioxidant activity is generally assessed using a limited number of tests, namely in vitro tests. The DPPH radical trapping power of holm oak extracts revealed a very significant antioxidant activity of the leaves and bark with an inhibition percent of 84.98±7.45 and 83.15±9.27% respectively; which corroborate studies on leaf extracts and stem bark of *Pericopsis laxiflora* with a percent of inhibition 95.86±9.27 and 99.99±0.0% respectively (Kouassi *et al.* 2020).

The total antioxidant capacity (TAC) of the extracts obtained from holm oak bark leaves was estimated using a calibration curve, carried out with a reference solution of ascorbic acid at different concentrations. TAC values are expressed in milligram equivalents of ascorbic acid per gram of dry matter (mg EAA/g DM). The calibration curve is established with a correlation coefficient $R^2 = 0.954$. The results of the total antioxidant capacity reveal that the highest capacity contents are in the bark extracts of plot 2 obtained by maceration (909.2±9.43 mg EAA/g DM) and in plot 1 obtained by ultrasound (711.9 ±12.20 (mg EAA/g DM) (**Figure 2**). While the leaves have a capacity of around 639.51±19.12 (mg EAA/g DM) for the leaf extract from plot 1 (ultrasound), followed by the leaf extract from plot 2 with a rate of 489.22±4.88 mg EAA/g DM (maceration). These results confirm that holm oak has a high antioxidant capacity for these barks and leaves (**Figure 2**).

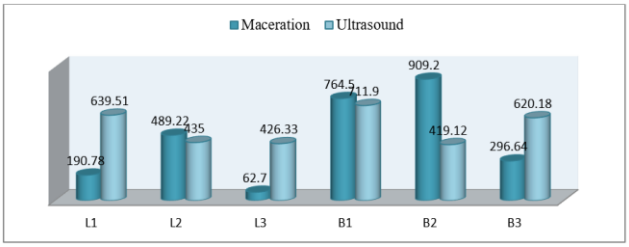



**Figure 2.** Total antioxidant capacity in (mg EAA/g DM) extracts obtained by ultrasound and maceration of the leaves and bark of holm oak.

A one-way analysis of variance (ANOVA) confirms that there is a significant difference between the TAC values of the different extracts ($p < 0.05$); and a non-significant difference between the two extraction techniques. For the DPPH test, the results indicate that there is no significant difference between the extracts ($p=0.053$), but there is a significant difference between the two extraction methods ($p=0.01$). It is possible that this effect is caused by the chemicals present in these extracts and their powerful antioxidant activity. In fact, several authors have reported a positive and significant relationship between antioxidant components, including phenol acids, flavonoids and tannins, respectively with DPPH radical scavenging capacity (Trabelsi *et al.* 2010).

In addition, several studies have demonstrated a strong correlation between the reducing power of extracts and the presence of antioxidant compounds (Lesjak *et al.* 2011; Taviano et al., 2013; Keskes *et al.* 2014). Generally, it is impossible to assess the antioxidant activity of plant extracts using a single method due to the complexity of phytochemicals. Also, environmental and agricultural conditions, such as soil and climate, have a significant effect on the accumulation of phenolic compounds and antioxidant activity.

Principal component analysis (PCA) using the means of the variables studied shows that **Figure 3** PCA Biplot shows the distribution of leaf (L) and bark (B) samples according to the two extraction techniques used: maceration (blue) and ultrasound (red). The first two principal dimensions explain 57.6% of the total variance. The ellipses show the dispersion of the samples for each technique. Variables such as DPPH and Flavonoids are strongly associated with maceration, while Polyphenols and condensed tannin are more correlated with ultrasound. Leaf and bark samples cluster distinctly according to the extraction techniques used.

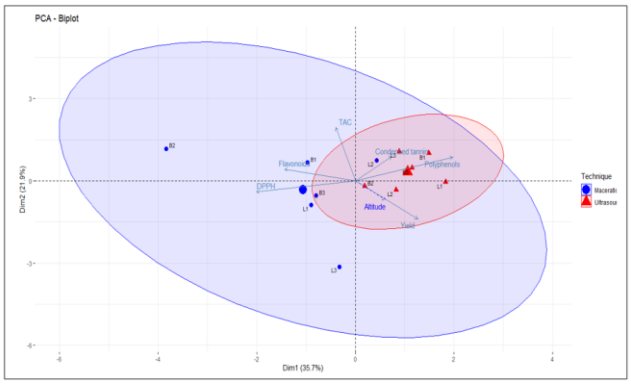



**Figure 3.** Principal component analysis (PCA) using the means of the variables studied by maceration and ultrasound.

Holm oak, through their antioxidant activity and their phenolic composition, can play a role in inhibiting free radicals and this plant can therefore have a protective role against several diseases. It will need to be further explored and exploited in research in several fields (pharmaceuticals, cosmetics and the food industry).

## 4. Conclusion

The results obtained are a first step towards the search for biologically active compounds from holm oak (*Quercus ilex*). This plant could be used in the manufacture of new products. The study of phenolic compounds (total polyphenols, flavonoids and tannins) was carried out on the leaves and trunk bark of *Quercus ilex*. Through the measurement of these compounds and the analysis of the antioxidant activity by spectrophotometry in leaf and bark extracts, it was concluded that the recurrent use of this plant would be associated with their relative concentration of polyphenolic compounds. . The findings mentioned above suggest that the leaves and bark of the trunk of *Quercus ilex* contain bioactive substances which could be used in various sectors (pharmaceuticals, cosmetics and food industry) with the aim of developing and adding value to non-wood forest products of Algerian forest resources. Additional in vivo studies should be carried out to evaluate the effects of holm oak.

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