

# Effect of Growing Area on Total Polyphenols, Flavonoids, Tannins and Antimicrobial Activity in *Quercus suber* L. Acorn Oil

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## Abstract

The investigation was conducted on oil extracted from mature acorns of *Quercus suber* L. harvested from three different Tunisian sites. The content of total polyphenols, tannins and flavonoides were determined. Antimicrobial activities were also evaluated. Antimicrobial activity of this oil was tested against: *Escherichia Coli*, *Bacillus subtilis* and *Candida albicans*. Gentamicin and amphotericin B were respectively used as a positive reference for bacteria and fungi. Discs without samples were used as a negative control. Fixed oil of cork oak acorn was extracted by the soxhlet apparatus. The oil content, expressed as dry weight, ranged from 0.9 to 2.02%. Fixed oil extracted from acorns of cork oak showed a high amount of total polyphenols 18.93 mg GAE/g. Amounts of flavonoids and tannins were also important. The results showed a significant antimicrobial effect against *B. subtilis* and *C. albicans* with inhibition diameter of 10.6 mm and 9.8 mm, respectively.

## Keywords

*Quercus suber* L., Acorn oil, Growing area, Polyphenols, Antioxydants, Antimicrobial activity.

## Introduction

Within Mediterranean basin, the forests of cork oak (*Quercus suber* L.) are typical ecosystems because of the high ecological value and the cork bark giving the cork oak its economic importance worldwide [1]. The cork oak spreads in the western Mediterranean areas of southern Europe and North Africa, mostly integrating multifunctional agro-forestry systems usually combining the production of cork with grazing, hunting, and other non-wood productions: acorns, medicinal plants, mushrooms, etc [2, 3]. Commonly used in reforestation and in animal feed, several studies highlighted the nutritional value of cork oak acorns. This non wood forest product is considered as a nutritionally rich product; containing high amounts of starch, proteins, minerals and fat [4-7]. Acorn oil has been getting rising consideration due to its nutritional potentials. In some countries, acorn oil has been used for cooking and as a wound healing product [8, 9] showed that cork oak acorn oil is a source of unsaturated fatty acids and has various biological active compounds such as tannins, phenolic acids, and flavonoids. The most abundant fatty acids were oleic and linoleic acids which have valuable effects such as regulating blood lipid profile [10].

To our knowledge, few studies were investigated on antimicrobial effect of cork oak acorns. The aim of this study is to determine the effect of growing area on

phytochemical composition of cork oak oil and to highlight the antimicrobial activity of this product.

## Material and methods

### Plant material and oil extraction

Cork oak acorns (*Quercus suber* L.) were collected from three Tunisian sites (Fernana, Majen Essef and Kef Rand) in November 2019. The geographical coordinates of each site are summarized in table 1. Acorns were kept in a Cold room at 4 °C until subsequent analyses. Thus, they were shelled and then dried at 105 °C for two days. Finally, dried acorns were ground using an electric grinder (1 mm screen, RetschR300). 20g of the resulting powder was then introduced into a soxhlet apparatus for oil extraction. Petroleum ether was used as solvent [11].

The extracted oil was weighed and then stored at 4 °C. Oil yield was calculated according to the following formula:

$$R(\%) = \frac{Mo}{Mp} \times 100$$

where R: yield expressed in %, Mo: mass in grams of the oil and Mp: mass in grams of the DM.

**Table 1:** Geographical coordinates of the cork oak harvesting sites.

Site	Latitude (N)	Longitude (E)	Altitude (m)
Fernana	36°39'39.38"N	8°41'4.00"E	286
Kef Rand	36°49'26.00"N	10°46'11.00"E	101
Majen Essef	36°46'7.76"N	8°47'47.76"E	538

### Phytochemical properties

#### Total polyphenol content

Total phenols were determined by Folin Ciocalteu reagent [12]. A volume of 150 µl of each sample was mixed with Folin Ciocalteu reagent (500 µl, 1:10 diluted with distilled water) and aqueous Na<sub>2</sub>CO<sub>3</sub> (2 ml, 2%). The mixtures were allowed to stand for 30 min and the total phenols were determined by colorimetric method against a blank without sample at 755 nm. The standard curve was prepared using 0, 0.03, 0.06, 0.12, 0.25, 0.5 g/L solutions of gallic acid in water. Total phenol values are expressed in terms of gallic acid equivalent (mg/g DM), which is a common reference compound.

#### Total flavonoids content

The total flavonoids content of the crude extract was determined by the aluminum chloride colorimetric method as described by Quettier Deleu et al [13]. Briefly, 1 ml of diluted sample was mixed with 1 ml of 2% methanolic solution of aluminum chloride. The mixture was incubated for 15 minutes and the absorbance was measured at 430 nm against a blank without sample. The total flavonoid content was calculated from a calibration curve and the result was expressed in mg Quercetin equivalent per g of dry weight (mg QE / g DM).

#### Total tannins content

The determination of the total tannins was performed with the Folin-Denis colorimetric reagent. In this; 150µl of the oil was dissolved in 150 µl of hexane. The whole was stirred every 10 minutes for 30 minutes then centrifuged for at 4000 rpm / 15 min. The supernatant was collected and diluted in 1ml of hexane. To 50 µl of this solution, 10 µl of Folin-Denis reagent and 25 µl of saturated Na<sub>2</sub>CO<sub>3</sub> solution were added; the final volume was adjusted to 500 µl with distilled water. After 90 minutes of incubation at room temperature, the absorbance was measured at 760 nm. Result was expressed in mg Catechin equivalent per g of dry weight (mg CE / g dry mass).

#### Antimicrobial activity

##### Microbial strains

The bactericidal effect was tested against *Escherichia coli* (ICP54126), *Candida albicans* (CIP 5262) and *Bacillus subtilis* (CIP 5262). Culture was performed on a tryptic soy agar medium (TSA). All microbial strains were provided by Pasteur Institute of Tunis, Tunisia.

##### Disc diffusion assay

The disc diffusion method was used according to Choi et al, sterile filter paper discs (6 mm diameter) were impregnated with 15 µl of fixed oils from cork oak acorns and then placed on the surface of petri dishes each containing 15 ml of TSA medium previously inoculated with 1 ml of the suspension of the selected germ (*E. coli*, *B. subtilis* and *C. albicans*) [14]. For each strain, three replication were performed.

Before incubation, all Petri dishes were kept in the refrigerator (4 °C) for 2 h and then incubated for 18 h at 37 °C for the two bacteria and for 48 h for the fungal strain *C. albicans*. Gentamicin and amphotericin B were respectively used as a positive reference for bacteria and fungi. Discs without samples were used as a negative control. Antimicrobial activity was assessed by measuring the diameter of the growth-inhibition zone in millimetres (including disc diameter of 6 mm) for the test organisms and compared to the controls.

##### Statistical analysis

Data were processed using one-factor analysis of variance, according to the General Linear Models (GLM) procedure of Statistical Analytic System (SAS, 2002) AS program. The model included only the region effect and the comparison between the 3 regions were performed using LSMEANS test.

## Results and discussion

### Oil yield

Results on oil yield from the different regions are presented in table 2.

Significant (P < 0.001) variation in yield between harvest sites was found. Indeed, the highest yield was recorded in acorns from the region of Majen Essef (2.02% DM), while acorns from Kef rand exhibited the lowest value (0.89% DM).

**Table 2:** Fixed oil yield of cork oak acorns.

Site	Yield (%DM)
Kef Rand	0.89 <sup>a</sup> ± 0.06
Fernana	1.41 <sup>b</sup> ± 0.02
Majen Essef	2.02 <sup>a</sup> ± 0.79

These results are different from those found by Charef et al, which reported that the total fat content of *Q. suber* acorns was estimated to 9%. This yield was lower than that determined for other *Quercus* species, such as *Q. ilex* (9%) [15].

### Total polyphenols, flavonoids and tannins content

The results relative to secondary compounds are summarized in table 3. The total phenols content of acorn oils ranged from 17.26 mg GAE/g to 18.93 mg GAE/g with no significant differences between the regions. This finding is similar to that found by Charef et al for the fixed oil from *Quercus ilex* acorn (2 mg GAE/g) [16]. Phenols are secondary metabolites synthesized by plants during their development but also as a response to stress conditions. These molecules are known by their beneficial action on human health, especially as antioxidant substances and in the cancer treatment [17, 18]. Significant differences between regions were found for both total flavonoids ( $P < 0.01$ ) and tannin contents ( $P < 0.01$ ).

The highest total flavonoids content was reached by oils from Majen Essef (5.76 mg QE/g), while the lowest rate was recorded for Fernana oil (2.21 mg QE/g).

**Table 3:** Total polyphenols, flavonoids and tannins content in cork oak acorn oils.

Site	Polyphenols (mg GAE/g)	Polyphenols (mg GAE/g)	Tannins (mg CE/g)
Fernana	18.93 <sup>a</sup> ± 0.02	4.88 <sup>b</sup> ± 3.5	14.12 <sup>c</sup> ± 0.65
Kef Rand	18.73 <sup>a</sup> ± 0.19	2.21 <sup>c</sup> ± 4.9	15.98 <sup>b</sup> ± 0.43
Majen Essef	17.26 <sup>a</sup> ± 0.03	5.76 <sup>a</sup> ± 4.5	21.71 <sup>a</sup> ± 0.21

The means of the same column with different letters are significantly different ( $p < 0.05$ ).

Flavonoids are recognized as the most substantial group in the human diet [19]. These components were reported by several studies for their important antioxidant, antiinflammatory, antidiabetic, anticancer, and cardioprotective activities [20-22].

Oil from Majen Essef location exhibited the highest total tannin rates (21.71 mg QE/g) and oil from Ken Rand showed the lowest value (14.12 mg QE/g).

In literature, tannins were cited for their anticancer, antimutagenic and antibacterial activities [23]. The significant differences observed between the three studied sites for flavonoids and tannins content could be related to differences in climatic and edaphic conditions.

### Antimicrobial activity

Results relative to antimicrobial activity of oils from cork oak acorns are summarized in table 4. No significant differences

were observed between the three studied sites. Diameters of inhibitions ranged from 9 to 10.8 mm, indicating that all the tested strains are sensitive to the fixed oil of cork oak acorns.

The bactericidal and fungicidal powers of the studied oils can be related to the presence of phenols, flavonoids and tan-

**Table 4:** Diameters of inhibition (mm) of oils extracted from cork oak acorns.

Strains	Majen Essef	Kef Rand	Fernana	Gentamicin	Amphotericin B
<i>C. albicans</i>	10 <sup>a</sup> ± 0.1	9 <sup>a</sup> ± 0.2	10.5 <sup>a</sup> ± 0.08	-	15
<i>E. coli</i>	10.8 <sup>a</sup> ± 0.2	9.3 <sup>a</sup> ± 0.05	10.8 <sup>a</sup> ± 0.02	24	-
<i>B. subtilis</i>	10.3 <sup>a</sup> ± 0.07	10.6 <sup>a</sup> ± 0.07	10.6 <sup>a</sup> ± 0.07	21	-

The means of the same column with different letters are significantly different ( $p < 0.05$ ).

nins. All these components were reported to have important antimicrobial activities [18, 23, 24]. In addition, this result can be associated to the inhibitory effect of fatty acids on microorganisms. Several investigations reported that oleic and linoleic acids, which are the major constituents of this oil [9], have potential antibacterial activity, mainly attributable to its unsaturated long chain lengths [25].

### Conclusion

In this study we investigated the total phenols, flavonoids and tannins content and antimicrobial activity of oils from cork oak acorns. Oils showed important amounts of flavonoids and tannins. It exhibited a considerable antimicrobial activity.

The findings of this work suggest that cork oak acorns could be considered as a valuable phytochemical source likely for preventing several human diseases and that could be used as an antioxidant ingredient in food industry.

### Conflict of Interest Statement

There is no conflict of interest.

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