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Oak acorns in Tunisia : Evaluation and current status

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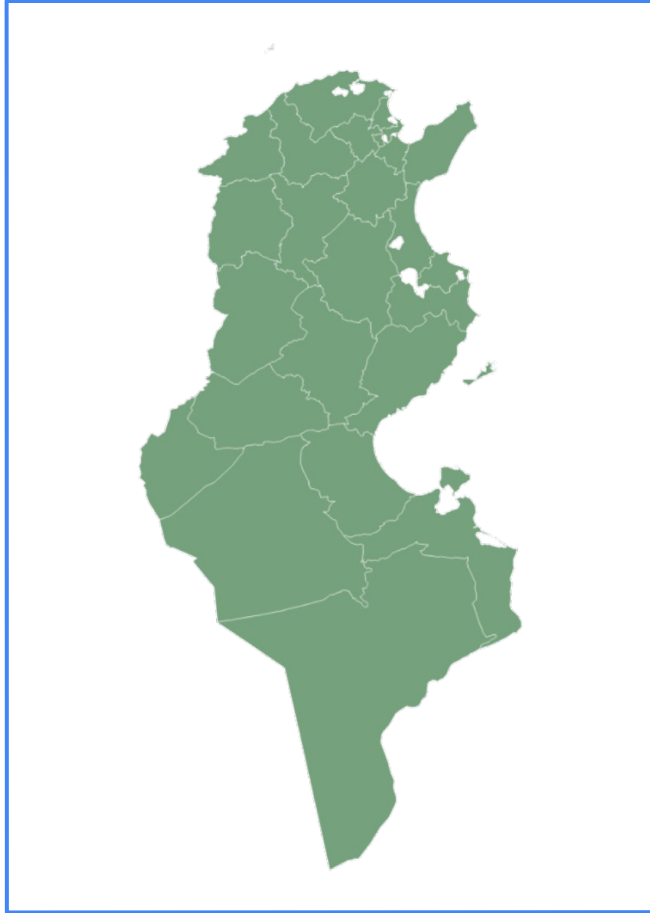
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01

Oak geographic distribution in Tunisia

Q. canariensis Willd. (canary oak)



Q. suber L. (cork oak)



Q. coccifera L. (Kermes oak)



deciduous

evergreens

Q. afares Pomel (pomel oak)



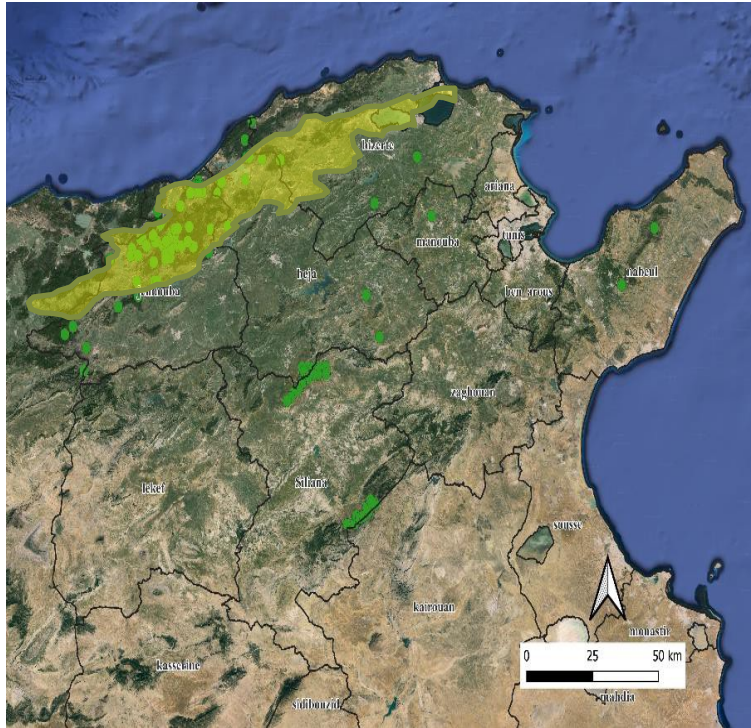
**In Tunisian
flora, *Quercus*
L genus is
represented by 5
species**

Q. ilex L. (holm oak)



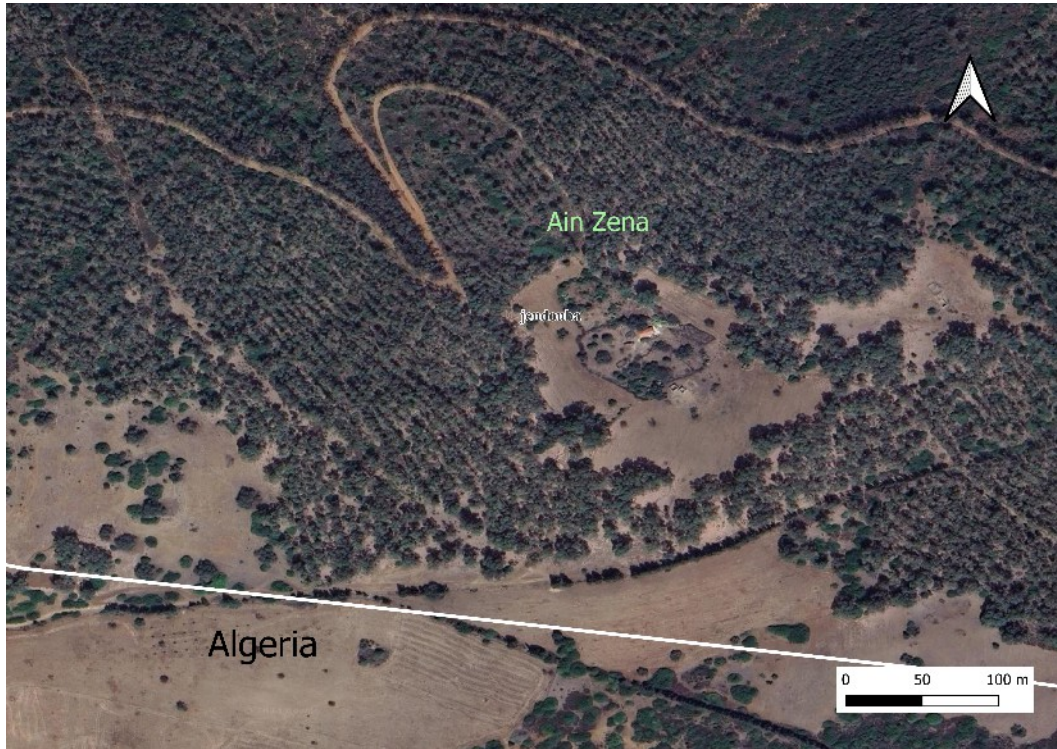
Q. Suber + *Q. canariensis*

confined especially to the Kroumirie and the Mogods



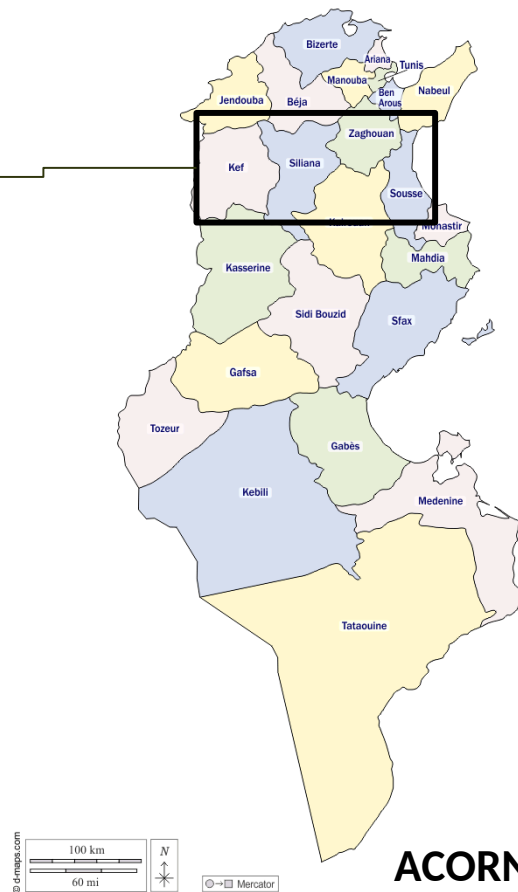
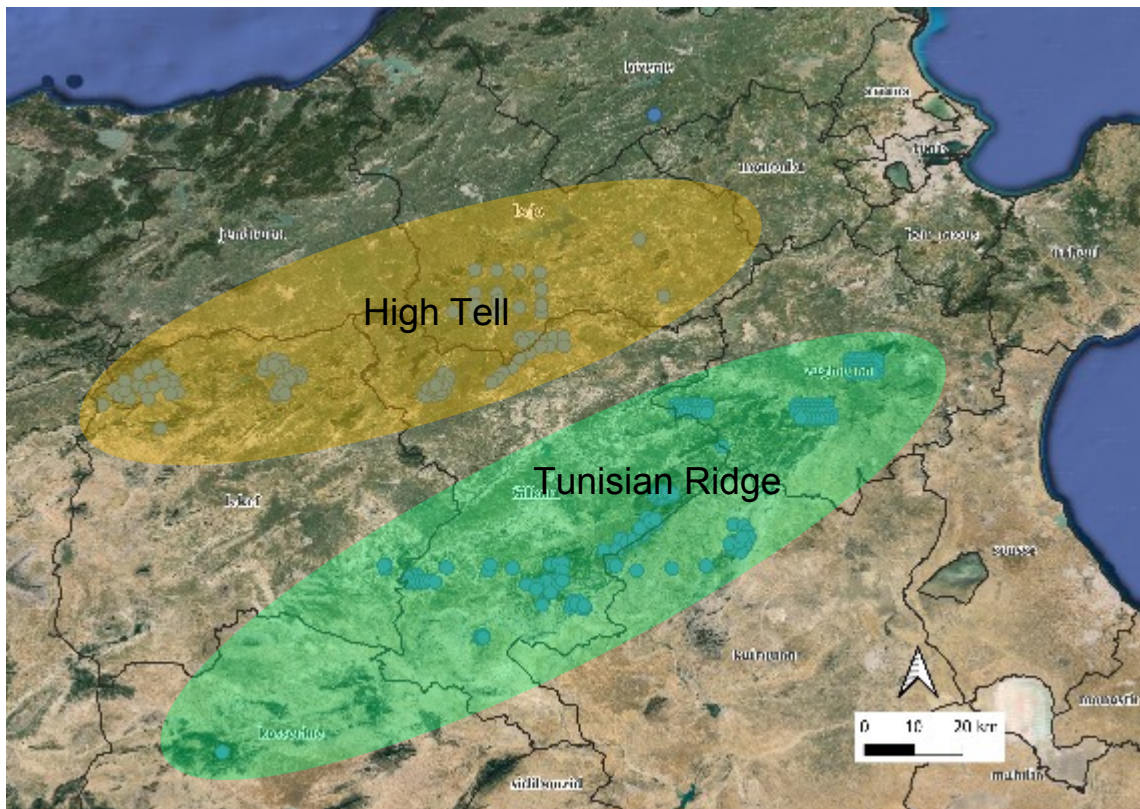
Q. afares

currently restricted to a unique population in the forest of Ain Zena located in Ain Drahem, Kroumirie with a population of approximately 750 individuals



Q. ilex

mainly distributed across two orographic regions in Northern Tunisia: the High-Tell and the Tunisian Ridge



Q. coccifera

encountered along the entire coastline, from Tabarka to Hammamet.





02

Harvesting sites
per species

Quercus suber

Dar Fatma,
Ain Drahem
36°49'16"N
8°46'42"E
792 m

Sidi Mechreg,
Bizerte
37°08'51"N
9°05'20"E
306 m



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Quercus canariensis

Oued Ezzen,
Ain Drahem
36°49'37"N
8°49'43"E
400 m

Ain Soltane,
Ghardimaou
36°31'37.5"N
8°19'50.9"E
832 m



(Tunisian-Algerian
border)



Quercus ilex

Oued Tricha,
Bargou
36°09'16"N
9°45'14"E
528 m



Jebel Serj
35°59'32"N
9°36'13"E
668 m



Quercus coccifera

Sidi Mechreg,
Bizerte

37.16766N

9.14525E



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03

Morphological description of acorns



Quercus suber

The fruit is enclosed in a tomentos cupule



Quercus canariensis

The fruit is partially enclosed in an almost sessile cupule



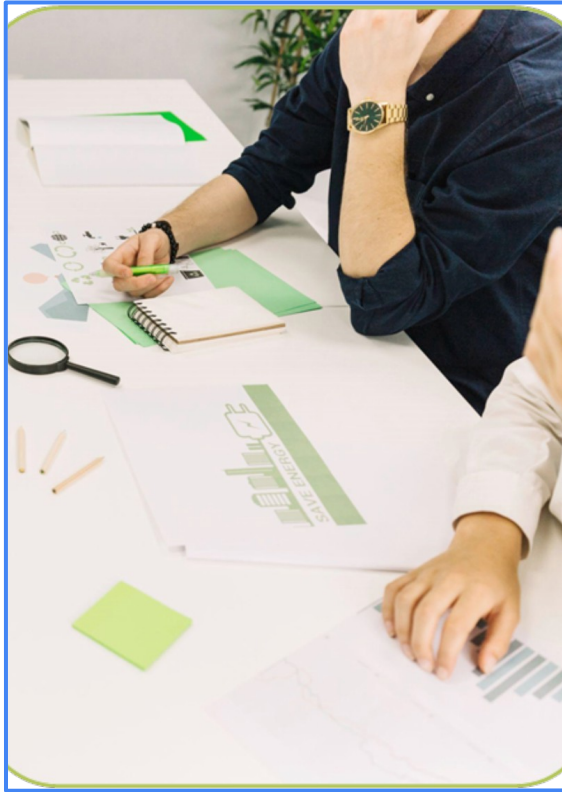
Quercus ilex

The fruit is enclosed in grey, very tomentose, and soft cupule typically lanceolate to ovate



Quercus coccifera

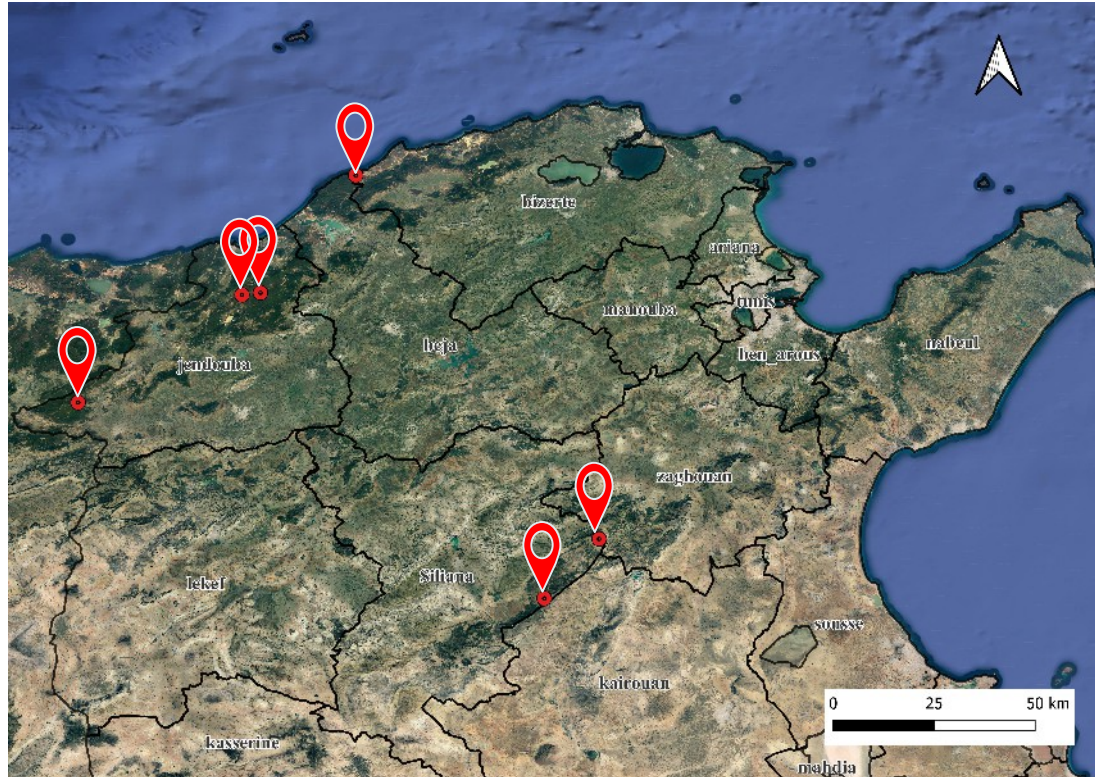
The fruit is partially enclosed in a scaly cupule



04

Ethnobotanical survey and current
common uses of acorn

Field survey conducted at 6 sites with 10 participants each





Acorn based dishes in Tunisia



Grilled acorn



Porridge



Bsissa



Boiled acorn



Fried acorn



05

Biochemical and
Biological
Characterization of
acorns

ACORN-FLOUR



Shells



Kernels



Oven at 45°C

Milled using



Disk miller



Fine flour

Polyphenolic compounds

(Folin–Ciocalteu+Aluminum and chloride colorimetric)

Secondary metabolites analysis	<i>Q. ilex</i>		<i>Q. suber</i>		<i>Q. canariensis</i>		<i>Q. coccifera</i>
	Site 1: Bargou	Site 2: Jebel Serj	Site 1: Dar Fatma	Site 2: Sidi Mechreg	Site 1: Oued Ezzen	Site 2: Ain Soltane	Sidi Mechreg
Total Phenol Content (mg GAE /g dry ext)	154.78 ± 7.1	159.90 ± 15.69	120.02 ± 15.24	119.06 ± 8.15	180.17 ± 2.4	147.32 ± 4,84	257,84 ± 39,25
Total flavonoid Content (mg QE/g dry ext)	2.93 ± 0.16	4.15 ± 0.48	2.89 ± 0.03	2.11 ± 0.08	5.87 ± 0.32	7.75 ± 0.27	10,56± 0,38

GAE:gallicacidequivalent;CTE:catechinequivalent
 QE: quercetine

Chemical composition

Chemical composition (%)	<i>Q. suber</i>		<i>Q. canariensis</i>		<i>Q. ilex</i>	
	Site 1: Dar Fatma	Site 2: Sidi Mechreg	Site 1: Oued Ezzen	Site 2 : Ain Soltane	Site 1: Bargou	Site 2: Jebel Serj
Water content	31.72 ± 0.36	39.21± 0.47	39.25 ± 0.64	38.42 ± 0.55	17,34 ± 0.48	15,93 ± 0.39
Ash content	2.3 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.0 ± 0.1	1.7 ± 0.1	1.9 ± 0.1
Protein content	5.19 ± 0.08	6.67 ± 0.08	7.43 ± 0.1	4.59 ± 0.06	3.4 ± 0.05	3.34 ± 0.05
Total lipids	5.47	5.20	3.65	4.73	7.97	9.33
Carbohydrates	87.04	85.43	86.23	88.68	86.93	85.43
Crude fiber content	1.9 ± 0.5	2.5 ± 0.7	2.6 ± 0.7	2.4 ± 0.6	2.6 ± 0.7	3.1 ± 0.5
Energy (kcal/100 g)	418,15	415,2	407,425	415,65	433,075	439,05

Fatty acid profile of acorn flour

Fatty acids %	<i>Quercus suber</i>	<i>Quercus canariensis</i>	<i>Quercus ilex</i>
C14:0	0.11±0.01	0.14±0.01	0.06±0.00
C16:0	13.18±0.06	14.74±0.02	14.75±0.34
C16:1	0.16±0.03	0.41±0.09	0.15±0.00
C17:0	0.09±0.00	0.09±0.00	0.11±0.00
C17:1	0.09±0.00	0.09±0.02	0.06±0.00
C18:0	1.19±0.00	1.72±0.24	3.06±0.21
C18:1	63.64±1.64	56.05±0.80	64.36±0.40
C18:2	19.01±1.57	23.88±0.20	15.64±0.24
C18:3 (n-3)	1.54±0.05	1.69±0.23	0.71±0.03
C20:0	0.20±0.01	0.43±0.02	0.37±0.04
C20:1	0.53±0.02	0.42±0.02	0.55±0.02
C22:0	0.16±0.01	0.22±0.02	0.13±0.00
C24:0	0.11±0.01	0.11±0.00	0.04±0.00

%	<i>Quercus suber</i>	<i>Quercus canariensis</i>	<i>Quercus ilex</i>
Σ MUFA	64.42±1.59	56.98±0.68	65.12±0.38
Σ PUFA	20.54±1.61	25.56±0.42	16.35±0.21
Σ SFA	15.04±0.03	17.45±0.25	18.53±0.59

Σ SFA. sum of saturated fatty acids; Σ MUFA. sum of monounsaturated fatty acids; Σ PUFA. sum of polyunsaturated fatty acids.

Vitamin content

Vitamins (UI/kg)	<i>Q. suber</i>		<i>Q. canariensis</i>		<i>Q. ilex</i>	
	Site 1: Dar Fatma	Site 2: Sidi Mechreg	Site 1: Oued Ezzen	Site 2 : Ain Soltane	Site 1: Bargou	Site 2: Jebel Serj
Vitamin E	24.00	38.00	12.00	18.00	21.00	39.00
Vitamin A	Below the limit of quantification					

Mineral Elements Content

Elemental analysis (mg/100g)		<i>Q. suber</i>		<i>Q. canariensis</i>		<i>Q. ilex</i>	
		Site 1: Dar Fatma	Site 2: Sidi Mechreg	Site 1: Oued Ezzen	Site 2: Ain Soltane	Site 1: Bargou	Site 2: Jebel Serj
Microelements	Fe	6.1 ± 0.1	8.7 ± 0.1	7.7 ± 0.2	4.2 ± 0.1	2.5 ± 0.0	3.0 ± 0.0
	Cu	0.50 ± 0.01	0.60 ± 0.01	0.70 ± 0.01	0.30 ± 0.01	0.40 ± 0.01	0.40 ± 0.01
	Zn	1.10 ± 0.01	1.30 ± 0.01	0.90 ± 0.01	0.50 ± 0.01	0.80 ± 0.01	0.90 ± 0.01
	Mn	0.80 ± 0.02	1.80 ± 0.05	4.00 ± 0.17	1.80 ± 0.03	0.60 ± 0.01	0.50 ± 0.01
Macroelements	Ca	30.21 ± 0.2	30.20 ± 0.1	90.0 ± 1.1	80.0 ± 1.0	60.0 ± 1.0	60.0 ± 1.0
	P	80.0 ± 0.9	120.5 ± 0.5	120.5 ± 0.5	80.0 ± 1.1	60.0 ± 1.0	80.0 ± 0.7
	Na	10.3 ± 0.3	10.3 ± 0.1	10.0 ± 0.1	10.4 ± 0.1	10.3 ± 0.2	9.9 ± 0.3
	K	900.0 ± 10.0	1150.0 ± 6.2	1120.0 ± 7.1	710.0 ± 12.1	650.0 ± 11.3	870 ± 9.7
	Mg	50.0 ± 1.0	70.0 ± 1.2	50.0 ± 2.0	40.0 ± 1.4	60.0 ± 0.9	60.0 ± 1.3

Techno-functional properties of acorn flour

Parameter	<i>Quercus suber</i>		<i>Quercus canariensis</i>		<i>Quercus ilex</i>	
	Qs1(Site1)	Qs2(Site2)	Qc1(Site1)	Qc2(Site2)	Qi1(Site1)	Qi2(Site2)
WAC (g/g of flour)	1.32±0.32	1.41±0.00	1.43±0.01	1.24±0.00	1.06±0.02	1.15±0.01
OAC (g/g of flour)	1.41±0.31	1.67±0.02	1.50±0.04	1.52±0.04	1.18±0.05	1.16±0.03
WSRC (%)	124.22±1.83	131.58±1.34	131.95±0.58	115.33±0.70	94.66±2.32	106.58±1.36
SCSRC (%)	129.86±3.48d	140.95±1.82	147.24±1.30	119.98±0.09	115.43±1.33	112.98±0.37
LASRC (%)	135.16±1.02	140.41±0.11	141.57±0.86	123.41±0.02	96.41±0.09	107.70±0.18
SuSRC (%)	152.51±0.46	168.35±3.47	172.76±0.82	151.85±0.42	124.90±0.03	137.96±2.51

WAC = Water Absorption Capacity; OAC = Oil Absorption Capacity; Solvent Retention Capacity (SRC) [Water Retention Capacity (WRC). 5% (w/w) Sodium Carbonate SRC (SCSRC). 50% (w/w) Sucrose SRC (SuSRC) and 5% (w/w) Lactic Acid SRC (LASRC).

- The *in vitro* antioxidant activity (DPPH, ABTS and FRAP)**

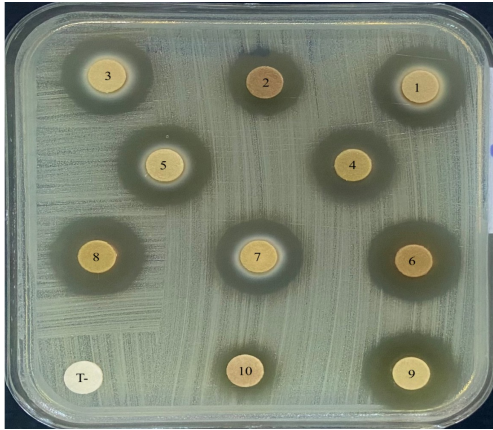
Antioxidant activity	<i>Q. ilex</i>		<i>Q. suber</i>		<i>Q. canariensis</i>		<i>Q. coccifera</i>
	Site 1: Bargou	Site 2: Jebel Serj	Site 1: Dar Fatma	Site 2: Sidi Mechreg	Site 1: Oued Ezzen	Site 2 : Ain Soltane	Sidi Mechreg
DPPH ($\mu\text{g TE/ mg dry ext}$)	356.65 \pm 27.24	309.83 \pm 6.68	338.92 \pm 26.18	284.75 \pm 6.68	346.62 \pm 18.07	383.07 \pm 7.6	536,76 \pm 18,74
ABTS ($\mu\text{g TE/ mg dry ext}$)	0.35 \pm 0.02	0.37 \pm 0.02	1.43 \pm 0.03	0.40 \pm 0.03	1.38 \pm 0.01	1.31 \pm 0.05	-
FRAP ($\mu\text{g TE/ mg dry ext}$)	317.74 \pm 1.3	311.51 \pm 9.89	351.56 \pm 7.22	302.74 \pm 13.4	521.42 \pm 14.03	590.48 \pm 14.11	-

Trolox was used as a standard solution and the results were expressed as $\mu\text{g trolox equivalents per mg of dry extract}$ ($\mu\text{g TE/mg dry ext}$).

DPPH:2,2-diphenyl-1-picrylhydrazyl assay;

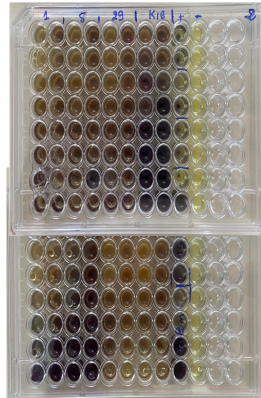
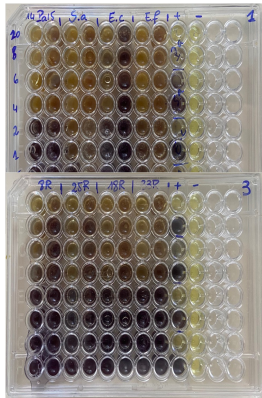
ABTS:2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid)radicalcation-basedassay;

Antibacterial activity
(Dish diffusion method + Microdilution method)



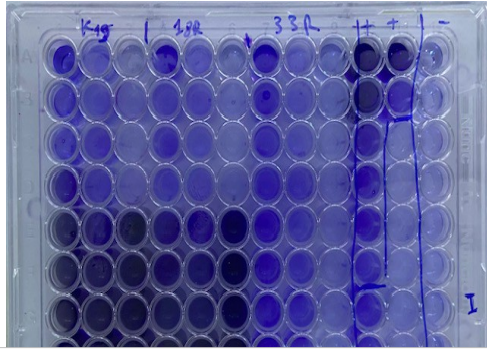
- **Gram+:** Inhibition of *Staphylococcus aureus* and *Enterococcus faecalis* with MIC=2mg/ml of kernel hydro-ethanolic extract and 0,25mg/mL of shell extract

Gram- : *Klebsiella pneumoniae*, *E. coli* and *Pseudomonas aeruginosa* were inhibited mainly with 4mg/mL of kernel extract.



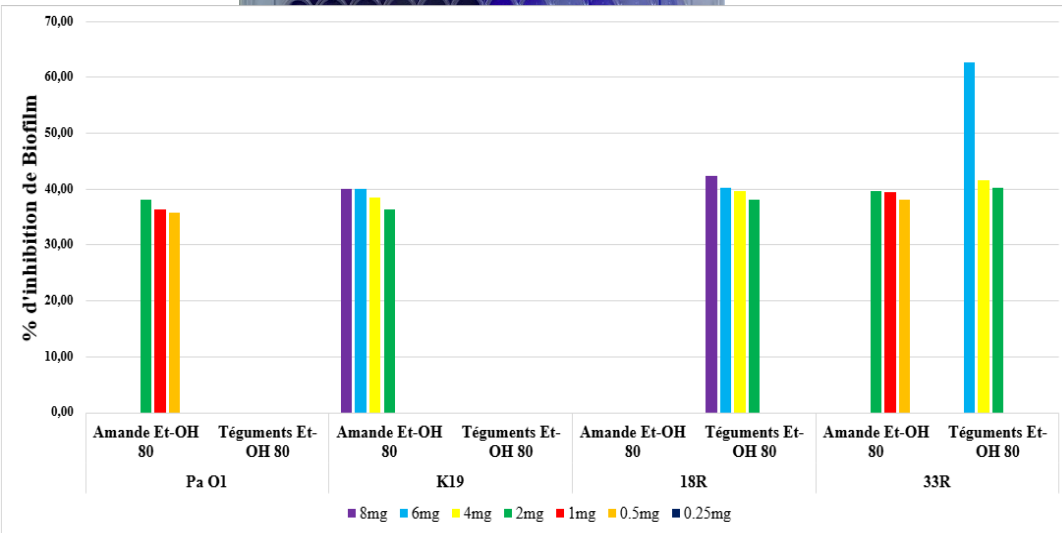
The anti-bacterial activity depended on the concentration of the extract and its solvent of extraction

Antibiofilm activity (Cristal violet Method)

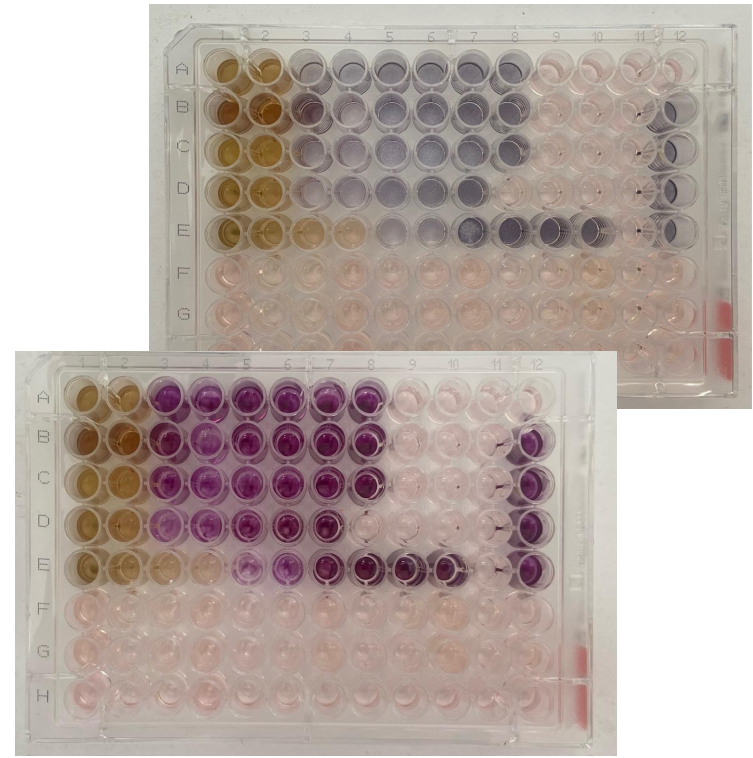
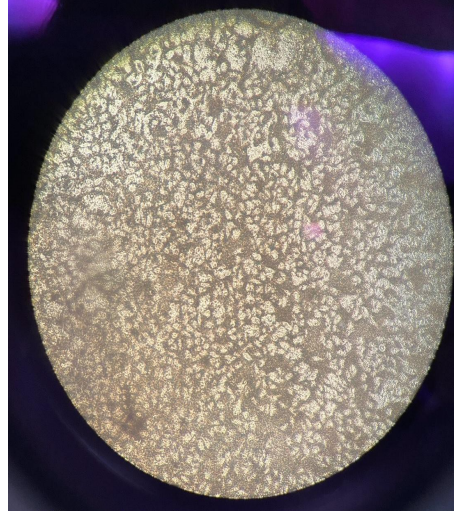
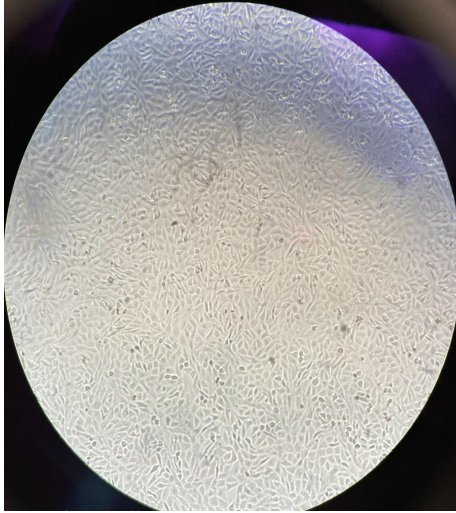


Both of extracts of kernel and shell inhibited 40% of preformed biofilm of different strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*

The Biofilm inhibition is concentration-independent



Cytotoxicity (Vero Cell Lines)

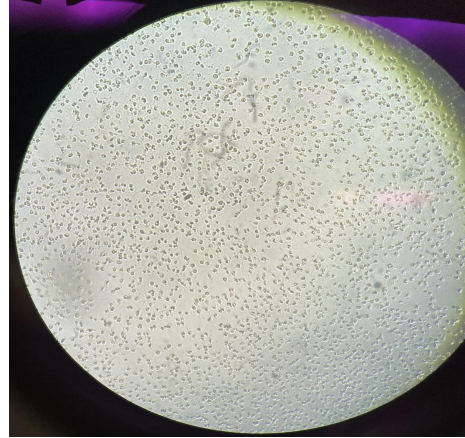


The extracts of kernel and shell showed reduction in cell growth with CC50 812,83 μ g/ml and 432,64 μ g/ml respectively

Antiviral Acitivity



Vero cells infected by HSV-2



Vero cells infected by CVB-3

Quercus coccifera extracts have an effect on herpes virus HSV-2 at concentrations of 12.94 $\mu\text{g/mL}$ and 11.44 $\mu\text{g/mL}$ for kernel and shell extracts (CC50/64).

Conclusion

Thanks to its nutritive value (being **gluten-free**, higher in lipids, vitamin E, proteins, carbohydrate, rich in ions, fibers and polyphenols; Acorns play a crucial role as a **healthy functional food**.

 **medacornet** : the valorization of acorns from our native forests

Oak acorns are an excellent source of natural antioxidants and antimicrobial compounds which could be exploited in the pharmaceutical, agriculture and food sectors.

Thank you for your attention

