Phytochemical Analysis, GC–MS Profile and Determination of Biological Activities of *Quercus canariensis* L. Leaf Extracts

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ABSTRACT

The current study was conducted to characterize the chemical composition of *Quercus canariensis* L. leaf extracts from Algeria and to evaluate their biological activities. The extraction was performed using solid-liquid extraction with two solvents: acetone and ethanol. The chemical profile was determined by GC-MS (Gas Chromatography-Mass Spectrometry) analysis. Antibacterial activity was assessed using the agar diffusion method, while antifungal activity was tested using the direct contact method. Antioxidant activity was evaluated by DPPH radical scavenging. The total polyphenol content of the extracts was measured using the Folin-Ciocalteu reagent, yielding the following values: ethanol (9.90 ± 0.05 mg GAE/g) and acetone (9.55 ± 0.11 mg GAE/g). Phytochemical analysis revealed that *Quercus canariensis* L. contains a mixture of diterpenes, fatty acids, cyclopentones, and quinic acid. In the acetonic extract (AcE), 16 compounds were detected, making up 97% of the total extract. The dominant compounds were 2-Pentanone, 4-hydroxy-4-methyl (25%), Neophytadiene (22%), and n-Hexadecanoic acid (10%). The ethanolic extract (EtE) contained 58% of identified compounds, with 6-oxabicyclo[3.1.0]hexane-2,4-diol (28%) as the major constituent. The antibacterial activity study demonstrated that the acetone extract had a substantial inhibitory effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with inhibition zones exceeding 21 mm. The evaluation of antifungal activity indicated that both the acetone and ethanol extracts exhibited high activity against *Aspergillus brasiliensis*, with inhibition rates of 57.47% and 54.02%, respectively. All extracts demonstrated a DPPH scavenging ability. The ethanolic extract had the lowest IC50, hence demonstrating the strongest antioxidant activity with an inverse IC50 of 2.63 mg/mL compared to the acetone extract. These findings suggest that the

extracts of *Quercus canariensis* L. possess strong antimicrobial properties and may serve as promising sources for future pharmaceutical applications.

Keywords: *Quercus canariensis*, polyphenols, antibacterial activity, antifungal activity, Gas Chromatography-Mass Spectrometry.

Introduction

Despite the fact that plants are considered one of the primary suppliers, only 15% of the 300,000 identified plant species have been researched for phytochemicals, with the remaining 6% studied for biological activity **(Verpoorte, 2002).** A large class of secondary metabolites, phenolic compounds is present in many higher plant organs, such as fruits, vegetables, cereals, legumes, nuts, and spices. These substances are essential for a variety of physiological functions, including assessing the quality of plants, influencing their color and flavor, and strengthening their resilience to stress **(Zhang *et al.*, 2022; Tyagi *et al.*, 2020).** In the context of a circular economy, the utilization of plant-derived resources like phenolic compounds can contribute to more sustainable industrial processes by reducing waste and fostering renewable materials. This methodology corresponds with the values of minimizing environmental impact, as evidenced by research on digital inclusive finance, which underscores the significance of sustainable practices in reducing carbon emissions **(Wang and Ma, 2024).**

Around the world, plants in the genus Quercus L. (*Fagaceae*) have long been utilized as traditional medicines as hemostatics, antiseptics, and to treat diarrhea, hemorrhoid **(Söhretoglu *et al.*, 2014)**. Oakwood plays a significant part in the maturing of wine in oak barrels and in the wood industry due to its pigmentation, durability, and resistance to fungal degradation **(Martínez-Gil *et al.*, 2022).** Additionally, its leaves have recognized ethnopharmacological value, traditionally used for treating gastrointestinal and microbial disorders **(Söhretoglu *et al.*, 2014).** These uses align with bioeconomic principles that promote the sustainable use of local biodiversity to improve health and support rural economies **(Wen *et al.*, 2025).**

With roughly 65,000 hectares in Algeria, *Quercus canariensis* **(Figure 1)** is one of the most significant trees in the Mediterranean region **(Zerizer & Mansseri, 2003).** It is a small, less than two-meter-tall evergreen shrub that bears acorns with stings. In accordance with **Welter *et al.* (2012)** this tree is also a phytogenic source of highly reactive volatile organic compounds. It exhibits significant polymorphism, which may be due to the high degree of genetic diversitythat results from the interaction of several factors, both internal and external. Various phenolic compounds was isolated from this species included 2,6-bis(hydroxymethyl)-4-methoxyphenyl-β-glucopyranoside (kermesoside), 5,6-dihydro- β -ionone 3-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (cocciferoside) and ((-)-8-chlorocatechin **(Söhretoglu *et al.*, 2014).** The investigation of such various phytochemicals can be considered as a fundamental component of the bioeconomy, as natural resources are exploited innovatively to manufacture valuable chemicals and medications. This correlates with broader trends in technology adoption and innovation, where trust and behavioral models play a key role in the acceptance of new technologies **(Wu *et al.*, 2025; Shen *et al.*, 2025).**

According to **Molina-García et *al.* (2018)** forty-one compounds in all were identified or given preliminary characterizations. Condensed tannins and gallic acid derivatives formed the majority of the chemicals, despite the identification of certain flavonoids, primarily kaemp ferol and quercetinde derivatives. Extracts were especially rich in allotannins, which is consistent with findings from other species of Quercus. Though (epi) catechin dimers were only found in leaves and hexahydroxydiphenyl-digalloyl-glucose isomers were only found in acorns, the phytochemical profiles of acorns and leaves were similar.

Our extensive study investigation revealed a lack of sufficient scientific data on Algerian varieties of this plant, particularly concerning its phytochemical profile, antimicrobial and

antioxidant properties. Therefore, the current study aims to analyze the chemical composition of ethanol and acetone extracts from *Quercus canariensis* L. leaves and to highlight the importance of this species by evaluating its inhibitory effects on selected bacterial and fungal strains. Additionally, the total polyphenol content was quantified, and the phenolic profile of the extracts was determined using Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

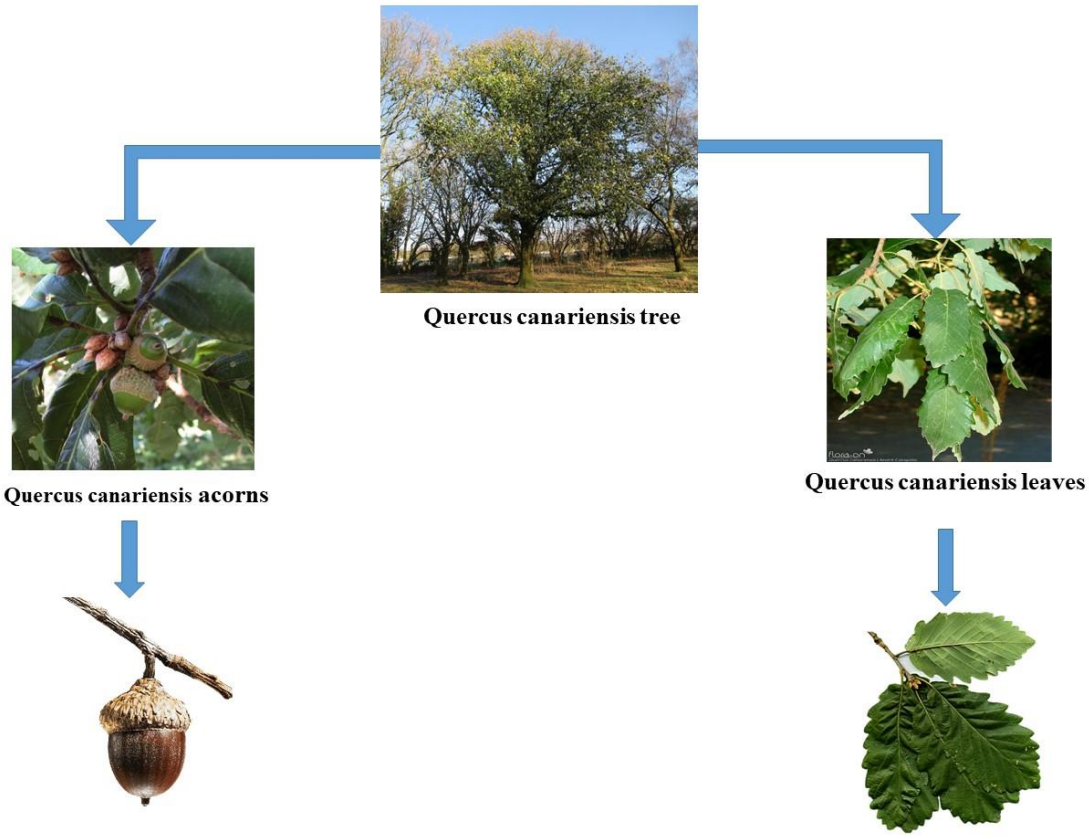

Figure 1: *Quercus canariensis* plant.

Material and methods

Plant Material

The *Quercus canariensis* plant species was selected based on a thorough bibliographic research, which revealed that it has received a comparatively insignificant attention. The leaves of this plant used to conduct this study were harvested in the CHREA- ALGERIA (36° 25′ 32″ North, 2° 52′ 36″ East) region in March 2021. The plant sample was identified by the forestry conservation experts of the Tipaza province. The voucher number BC-07-T36.252 was assigned and deposited in the herbarium of the Laboratory for Research on Medicinal and Aromatic Plants (LRPMA), Faculty of Natural and Life Sciences, University of Blida 1, Soumaa, Blida, Algeria.

For optimum preservation of heat-sensitive molecules, the collected leaves are rinsed and then dried in the open air at room temperature, away from light. Once the leaves have dried, they are ground into a fine powder with the use of a mortar and stored under dry conditions away from moisture and light until required.

Extraction of Polyphenols

A 25 g sample of *Quercus canariensis* leaves were separately macerated in 250 ml of ethanol and acetone, each diluted to 80% / 20% ; (solvent/H2O). The mixture was continuously stirred for 48 hours at room temperature and in the dark and then filtered using Wattman N°5 filter paper. Resulting filtrates were concentrated under vacuum at 40 °C using a rotary evaporator (BÜCHI Rotavapor R-200) and then preserved at 4°C until use.

Calculation of yield

The yield is the amount of extract obtained from the vegetable powder. The ratio of the extract's mass to that of the vegetable powder was calculated in practice and multiplied by 100. This leads to the formula provided by Harbone[0].

$$R = \frac{Mass\ of\ obtained\ extract}{Mass\ of\ the\ sample}\ x100$$

Total Phenolic Content

The Folin-Ciocalteu technique, as modified by **Hayouni *et al.* (2007)**, was used to determine the total phenolic content of the extracts. Briefly, 100µl of Folin-Ciocalteau reagent (1:10) with 800µl of Na_2CO_3 (7.5%) were added to 100µl of the extract, respectively. After incubation for 2 hours at room temperature and under limited lighting conditions, the absorbance was measured at 765 nm using a Perkin Elmer Enspire microplate reader. The content of total polyphenol was determined from a calibration curve established using the acid gallic solution at concentrations of 0, 12.5, 25, 50, 100, and 200 mg/ml and the values were expressed in milligram of gallic acid equivalents per gram of extract (mg GAE/g extract).

Phytochemical screening of Quercus canariensis extracts

Phytochemical screening is the systematic execution of a sequence of standard tests on natural substances obtained from plants. This screening can identify the existence of different categories of bio-phytochemically active compounds, which are collectively referred to as secondary metabolites. The present study aims to provide first colorimetric, solubility, and precipitation data in order to establish the existence of several categories of secondary organic compounds.

The analysis of phytochemicals in this work was conducted following Harborne's protocols with some modifications **(Harborne, 1998).**

Alkaloids

In order to identify the presence of alkaloids 1ml of the extract was added to 5 ml HCL 1%, and then was heated on boiled water bath. After the cooling process, the mixture was subsequently separated into two equal halves. One portion was subjected to a few drops of Mayer's reagent, while the other portion was treated with equal quantities of Wagner's reagent.

Flavonoids

The amount of 1ml of the extract was treated with a few drops of concentrated HCL and 0.1g magnesium turnings (Mg). We left it to be operating for 3 minutes. The reddish-orange coloration signifies the existence of flavonoids.

Tannins

To detect the presence of tannins, we add 100 µl of a 1% $FeCl_3$ solution to 1 ml of each extract obtained from *Quercus canariensis*. The existence of a hue informs us about the type of tannin encountered.

Quinones

We add a few drops of 1% NaOH to 1 ml of each extract. The appearance of yellow, red, or purple suggests the existence of released quinones.

Terpenoids

A test tube was used to combine 5 ml of extract with 2 ml of chloroform. A layer was formed by carefully putting 3 ml of concentrated sulfuric acid along the wall of the test tube. Terpenoids are recognized by a reddish-brown interface.

GC-MS analysis.

***Sample's preparation*.**

Prior to being placed into a GC vial for analysis, the solution's volume is adjusted to 1 ml using hexane.

Extracts analysis by GC / FID and GC-MS/MS

The analysis of the constituents of Acetone and Ethanol extracts was conducted using Gas Chromatography Coupled to tandem Mass Spectrometry (GC/MS-MS) with an Agilent 7000 Triple Quadrupole instrument. This instrument consists of a GC 7890A for separation and a triple quadrupole mass spectrometer (QqQ) operating at 70V for tandem mass spectrometry.

The relied-on GC instrument incorporates the Agilent 7890A Gas Chromatograph, which is equipped with an auto-sampler and an air cool multimode inlet. The temperature of the vaporizer is adjusted to 280 °C.

The injection volume was 1 µL in split mode and the split ratio was set to 10:1, the concentration of the injected oil is 1% in hexane. A HP-5MS capillary column (30 m length; 0.25 mm inner diameter; 0.25 µm film thickness) was put to use. Helium of high purity (N60) was employed as carrier gas at a 1 mL/min flow rate. The temperature oven was held at 50 °C for 1 min, raised by 9 °C/min up to 280 °C. The ultimate temperature was held for 5 min. The proportion of the components of the essential oil was determined according to the area of the chromatographic.

The extract's components are identified using tandem mass spectrometry (GC/MS-MS) on an Agilent 7000 Triple Quadrupole instrument. Constituent's identification was found with MassHunter (MH) Workstation Software Qualitative Analysis Workflows (version B.10.00, Agilent Technologies Inc., Santa Clara, CA, USA). It was operated with the following parameters: compounds were discovered by chromatogram deconvolution with the default settings; substances were identified using MS library (NIST17) search.

Antimicrobial Activity

Microorganisms Test

The following microorganisms were used as test organisms in the screening: two-Gram positive bacteria, namely, *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212); two-Gram negative bacteria, *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922); one yeast *Candida albicans* (clinically isolated); and two fungi *Aspergillus brasiliensis* and *Fusarium oxysporum f. sp. albedinis*. The microorganisms were acquired from the Algerian Pasteur Institute's laboratory of microbiology.While *C. albicans* was obtained from the laboratory of microbiology of Yessad Khaled Hospital, Mascara University, Algeria.

Antibacterial and anticandidal Activities

Antimicrobial activity against *S. aureus, E. faecalis, P. aeruginosa, E. coli* and *Candida albicans* was determined by the agar disc diffusion assay according to the method of Performance Standards for Antimicrobial Susceptibility Testing (M100) of NCCLS (National Committee of Clinical and Laboratory Standards) **(CLSI, 2021).** Petri plates were prepared with 20 mL of sterile Mueller-Hinton agar (Sigma, Paris, France). The surface of the medium was inoculated with a 200 µL cell suspension adjusted to a concentration of 10^6

CFU/mL using the McFarland 0.5 standard. Sterile filter paper discs, 6 mm in diameter were deposited and impregnated with 20µl of extracts dissolved in Diméthyl sulfoxyde (DMSO). After 20 min of diffusion, the dishes were incubated at 37°C for 24hours. The Colistin (10µl) and vancomycin (30µl) discs were used as a positive control while those impregnated with DMSO as a negative control. The antimicrobial activity was estimated by measuring the diameter of the growth inhibition zones surrounding the discs. To reduce test error, all tests were done three times. High antibacterial activity was defined as an inhibitory zone of 14 mm or higher.

Antifungal Activity Assay

The antifungal activity of *Quercus canariensis* leaf extracts was evaluated using the direct contact method. Acetonic extract is incorporated at varying concentrations in the agar culture medium. After solidification, the medium is seeded and incubated.

1mL of extract was pipetted onto empty petri dishes, followed by 9ml of culture medium: Sabouraud agar (liquefied and cooled). The mixture is then poured and left at room temperature allowing the medium to solidify.

The seeding takes place by depositing the fragments of 1cm² of diameter of each species of the evaluated fungus, taken from the periphery of a mycelial carpet from a 6-day culture in the Sabouraud medium. The boxes were incubated for 6 days at 25°C.

After the requisite incubation period, the diameters of the various fungus colonies are estimated. For each fungus colony, the results are given as a percentage inhibition of mycelium growth (I%). It is calculated using the following formula **(Slougui *et al.*, 2023)**:

$$\text{Inhibition of the mycelial growth (\%)} = \frac{dc-dt}{dc} \times 100$$

Where *dc* is mean diameter of colony in the control sample, and *dt* is mean diameter of colony in the treated sample.

Antioxidant capacity determination by 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) assay

The DPPH method of determining antioxidant capacity was performed in accordance with **Silva *et al*. (2024)** with some modifications. Briefly, a 100 µM DPPH (Sigma-Aldrich, Munich, Germany) solution was prepared in 100% methanol. The assay was carried out by adding 100 µL of DPPH solution to each sample and standard, followed by a 30-minute incubation period. The absorbance was measured in a plate reader (TECAN, Temecula, United States), at an optical density of 512 nm. Formula (1) was used to determine the antioxidant activity as a percentage of radical scavenging activity (RSA%):

$$\text{DPPH radical scavenging activity (RSA \%)} = \frac{(\text{Abs blank} - \text{Abs sample})}{\text{A blank}} \text{ x } 100.$$

Abs_{Blank}- Absorbance of the control sample (methanol 100 % + DPPH in methanol 100 %)

Abs_{Sample}- Absorbance of the sample (experimental samples in methanol 100 % + DPPH in methanol 100 %).

Ascorbic acid and butylated hydroxy toluene (BHT)) were used as positive controls IC50 values were determined graphically from the sigmoidal shaped curve of antioxidant concentration (mg/mL) versus %inhibition. For comparison purposes the reciprocal 1/IC50 values were used **(Debib *et al.*, 2014)**.

Statistical Analysis

The variables were represented numerically and as a percentage. SPSS 21.0.0 software for Windows™ was adopted for calculating means and standard deviations. Thedifference between the variables was determined using a single factor analysis of variance (ANOVA), the post-hoc Tukey's test and student's t-test. For a $p \leq 0.05$ difference, the variables were regarded to demonstrate a statistically significant difference.

Results

Yield of extraction and Total Phenolic Content

The average yield (%) of *Quercus canariensis* extracts is shown in **Table 1**. The resultsindicate that extraction with acetone (12%) was relatively similar to that obtained with ethanol (11.2%). Additionally, the total phenolic content in the ethanol extract (EtE) and acetone extract (AcE) was 9.90 ± 0.05 mg GAE/g and 9.55 ± 0.11 mg GAE/g, respectively. Statistically, no significant difference was observed between the two solvents.

Table 1. Extraction yield and total phenolic content of *Quercus canariensis* leaves extracts.

Extract	EtE	AcE
Extraction yield	11.2 %	12 %
Total Phenolic Content	9.90 ± 0. 05	9.55 ± 0. 11

Phytochemical screening

Based on the results shown in the Table 2, it can be stated that *Quercus canariensis* is an extremely rich source of flavonoids, particularly in the EtE extract. Similarly, both the AcE and the EtE showed a significant content of Alkaloids and Terpenoids. Flavonoid has a moderately

positive effect on AcE with low concentrations. Tannins have a strongly positive appearance in both extracts EtE and AcE. An abundance of Flavonoids can be detected in various *Quercus canariensis* extracts. Conversely, Quinones were not detected and marked an absence in *Q.canariensis* fractions. All our extracts were positive for the presence of alkaloids.

Table 2. Phytochemical screening of *Quercus canariensis* leaves extracts.

Extract	**EtE**	**AcE**
Alkaloids	+++	+++
Flavonoids	++	+
Tannins	+++	+
Terpenoids	++	+++
Quinones	-	-

+++: Strongly Positive result; ++: Moderately Positive result; +: Positive result and -: negative result.

GC-MS analysis

The compounds identified in the acetonic and ethanolic extracts of *Quercus canariensis* were established using Gas Chromatography coupled to Mass Spectrometry (GC-MS analysis). The chemical compositions of the extracts are listed in the order of their column elution time **(Table 3 and 4).** In AcE 16 compounds were detected, which constitute 97 % of the whole extract.

The most dominant of all the identified compounds were 2-Pentanone, 4-hydroxy-4-methyl (25 %), Neophytadiene (22 %), n-Hexadecanoic acid (10%), Neophytadiene (13%), while the most representative compounds identified were trans-13-Octadecenoic acid (6 %), Methyl 8,11,14-

heptadecatrienoate (6%), 9,12-Octadecadienoic acid (Z,Z)- (3%). The other constituents present in appreciable amounts.

Chemical composition of EtE allowed us to identify 58 % **(Table 4).** A high proportion of 6-oxabicyclo[3.1.0]hexane-2,4-diol **(Table 4, Figure 3)** characterized EtE. 6-oxabicyclo[3.1.0]hexane-2,4-diol is the major constituent of EtE with respectively 28%. On the other hand, Quinic acid is a characteristic of *Quercus canariensis* with an amount of 10%, Neophytadiene (3 %) and Phytol (3 %) are characteristic of the ethanolic extract of *Quercus canariensis*.

The presence of newly identified compounds especially Neophytadiene (13 %) or their combination with other secondary metabolites might contribute to the specific antimicrobial effect.

Table 3. Retention time (RT) and area % of the bioactive compounds detected in the Acetone *Quercus canariensis* L. leaf extracts analyzed using GC/MS.

Nº	Compounds	Area %	Retention time (min)
		Acetone extract	
1	**4-hydroxy-4-methyl-2-Pentanone**	**25**	**4.225**
2	2-propanone, 1-(4-acetylphenoxy)-	1	11.52
3	2-Dodecene, (Z)-	1	13.06
4	Propanoic acid, 3-(trimethylsilyl)-	1	13.59
5	β-D-Glucopyranose, 1,6-anhydro	1	14.28
6	Ethanethioic acid, S-(tetrahydro-2H-pyran-3-yl) ester	1	15.93
7	1-Tetradecanol	1	18.29
8	**Neophytadiene**	**13**	**19.33**
9	**n-Hexadecanoic acid**	**10**	**20.17**
10	Dibutyl phthalate	1	20.28
11	**Neophytadiene**	**22**	**21.82**
12	9,12-Octadecadienoic acid (Z,Z)-	3	22
13	trans-13-Octadecenoic acid	6	22.06
14	Methyl 8,11,14-heptadecatrienoate	6	22.07
15	9,12-Octadecadien-1-ol, (Z,Z)-	3	22.35
16	Diisooctylphthalate	1	25.9

Table 4. Retention time (RT) and area % of the bioactive compounds detected in the ethanolic *Quercus canariensis* L. leaf extracts analyzed using GC/MS.

Nº	Compounds	Area %	Retention time (min)
		Ethanolic extract	
1	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1	9.219
2	Methyl-β-D-arabinopyranoside	1	13.68
3	beta.-D-Glucopyranose, 1,6-anhydro-	2	14.33
4	(1'R,5'R,6'R,7'R)-(3',3'-dimethyl-6',7'-epoxy-2',4'dioxabicyclo[3.3.0]oct-6'-el)acetic acid	1	14.65
5	1,1,4,5,6-Pentamethyl-2,3-dihydro-1H-indene	2	14.87
6	**Quinic acid**	**10**	**16.02**
7	Ethyl α-d-glucopyranoside	1	16.03
8	**6-oxabicyclo[3.1.0]hexane-2,4-diol**	**28**	**16.29**
9	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	1	18.13
10	Neophytadiene	3	18.84
11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2	19.33
12	n-Hexadecanoic acid	3	20.16
13	Phytol	2	21.81
14	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	1	22.05

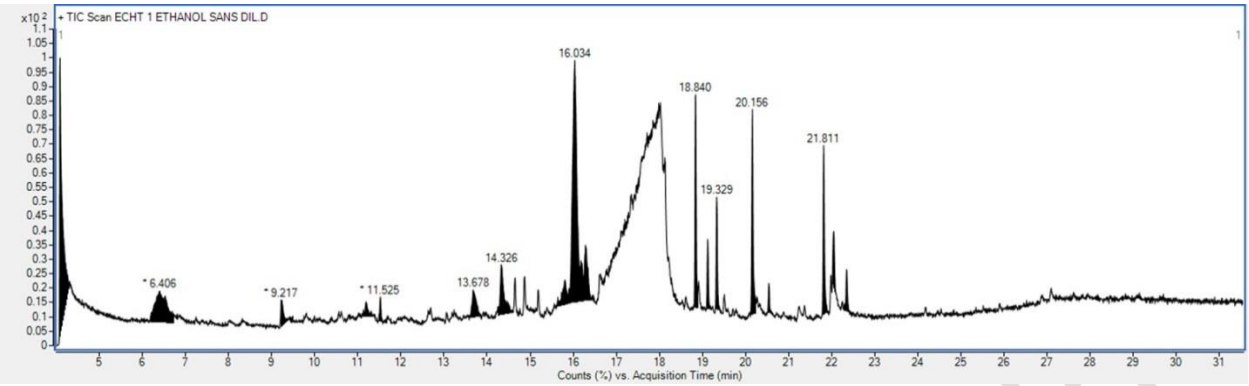

Figure 2: GC-MS analysis chromatogram of the ethanol's extract of *Quercus canariensi*s leaves.

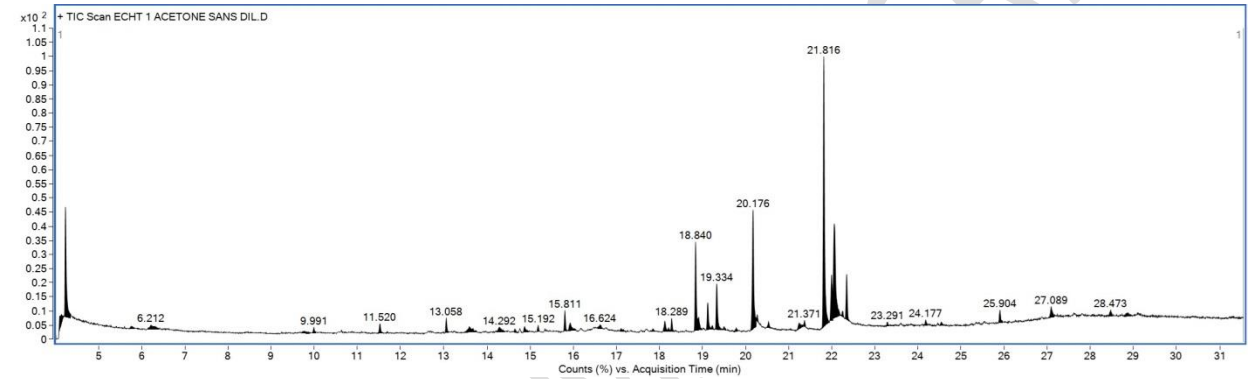

Figure 3: GC-MS analysis chromatogram of the acetone's extract of *Quercus canariensi*s leaves.

Antibacterial Activity

Results presented in Table 5 showed the antibacterial test, performed with EtE and AcE extracts of *Q. canariensis* against Gram-positive and Gram-negative bacteria.

The result of this test revealed that the extracts had a highly noteworthy effect against all the tested strains, showing clear zones of growth inhibition around the discs. No effect was exhibited by DMSO, used as the negative control.

The sensitivity of examined bacteria to the inhibitory activity of *Quercus canariensis* varied from sensitive to very sensitive, giving diameters ranging from 12.66 ± 1.52 to 23.33 ± 3.51 mm. The developed extracts are significantly more active than the two positive controls (Colistin and

Vancomycin), which elicit relatively mild inhibition, while the negative control indicates no inhibitory efficacy.

The acetone extract of *Q. canariensis* leaves promotes a remarkable growth inhibition on the tested bacterial strains. Compared to *E. feacalis*, the acetone extract seemed to act effectively on *E. coli* (18 mm), *P. aeruginosa* (21 ± 3.00 mm) and *S. aureus* (23 ± 3.51mm). The latter two strains, showed also significant unchangeable inhibitions towards the ethanolic extract with respective diameters of 18 ±2.00 and 19 ±2.64 mm, unlike *E. coli*, which showed significant resistance to this extract. The *E. feacalis* strain reacted in the same way, whether with the ethanolic (14 ± 0.00mm) or acetone extract (13.5 ± 1.50mm). This strain showed slight resistance to both extracts compared to other strains.

Table 5. Antibacterial potential for the ethanol and aceton *Quercus canariensis* L. leaf extracts.

Stains **Treatments**	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. feacalis*
EtE	12.66±1.52 ^{b}	18 ±2.00 ^{a}	19 ±2.64^{a}	14 ±0.00 ^{a}
AcE	18 ±2.00 ^{a}	21 ± 3.00 ^{a}	23.33 ±3.51^{a}	13.5±1.50 ^{a}
DMSO	-	-	-	-
Colistin	-	-	13.5 ± 2.12^{b}	-
Vancomycin	-	10 ± 2.82 ^{b}	19	-

Each value is expressed as mean ± standard deviation (n=3). Differences were analyzed by one-way student's t-test. Means with different letters within the same column are significantly different ($p<0.01$). EtE: Ethanolic extract, AcE: Acetone extract, (-): No activity ÷

Where; "-" : negative result .

Antifungal activity.

Q. canariensis extracts were investigated for their antifungal efficacy. The result of the anticandidal test of *Q. canariensis* AcE and EtE extracts, carried out by the agar disc diffusion assay, revealed a notable inhibitory effect on the growth of *C. albicans* **(Table 6).** The sensitivity of this strain towards these extracts appeared identical, showing a non-significant difference in the inhibition zones, with diameters of 15.66 and 16.66 mm, respectively. No positive effect was observed for DMSO.

For the two molds, the evaluation of the antifungal effect of EtE and AcE extracts, carried out using the impregnation method, revealed a statistically significant sensitivity ($p < 0.01$) to the EtE extract, with *Fusarium oxysporum f. sp. albedinis* showing an inhibition percentage of 57.47%, compared to 45.97% for the acetone extract (table 6). Similarly, for *Aspergillus brasiliensis*, statistically significant results ($p < 0.01$) were found, with inhibition values of 54.02% and 57.47% for the EtE and AcE extracts, respectively.

Table 6. Effect of *Quercus canariensis* extracts on the growth of fungal strains.

Inhibitions zones (mm)		Inhibition rate (%)	
Stains **Treatments**	*C.albicans*	*F. oxysporum Albanis*	*A. brasiliensis*
EtE	17.25 ±0.25 [a]	57.47%	54.02%
AcE	16.25 ±0.25 [a]	45.97%	57.47%
DMSO	-	-	-
Hexaconazole	ND	40.77%	35%
Amphotericin	10 ±0.25	ND	ND

-: negative result ; ND; not determined.

Antioxidant Activity

The DPPH is a synthetic and stable free radical, is commonly used to assess the antioxidant properties of natural products. The results indicated that all extracts demonstrated DPPH scavenging activity in a concentration-dependent manner. The ethanolic extract had the lowest IC50, and consequently, the highest antioxidant activity with $1/IC_{50}$ of 2.63 mg/mL^{-1} compared to the acetone extract **(Table 7).** However, the antioxidants standard, ascorbic acid and BHT, exhibited significantly higher scavenging activity, with values of 14.7% and 83.33%, respectively.

Table 7. IC50 and 1/IC50 values obtained in DPPH radical scavenging assay

		IC50 (mg/mL)	**1/IC50 $(mg/mL)^{-}$**
Extract	EtE	0.38	2.63
	AcE	3.84	0.26
Controls	Ascorbic acid	0.068	14.7
	BHT	0.012	83.33

Discussion

The traditional use of the Quercus plant has allowed several research studies to be conducted in order to validate the therapeutic use of species of the genus Quercus. However, the work devoted to the species *Q. canariensis* focused on other aspects, namely growth, physical characteristics, natural regeneration, diversity of terpene emissions and the influence of environmental conditions on dieback **(Messaoudene, 1991; Welter *et al.*, 2012; Mechergui *et al.*, 2022; Bouandas *et al.*, 2024).** To our knowledge, this plant has not been the subject of any previous research relating to biological activities, in particular antimicrobial power. Current work focuses on examining the range of phytochemicals and biological characteristics of *Quercus canariensis* extracts cultivated in Algeria.

In our study, two extracts were prepared from the leaves of *Quercus canariensis* species using two solvents of different polarities. Since most parts of the plant are recognized for their numerous medicinal properties, as confirmed by several previous studies, we therefore chose to focus our work on the leaves **(Taib *et al.*, 2020)**.

The dry weight yield of the Ethanolic extract was found to be the same as that of the Acetone extract. It is difficult to compare the findings of extraction yield of *Q. canariensis* to other research since the output is only relative and may be due to different chemical composition. The latter depends on several factors, including the genetic properties of plants, geographical origin, the part of the plant used, the drying conditions, and the extraction procedure and solvent **(Fkiri *et al.*, 2024)**.

Qualitative phytochemical screening is an important step that serves to provide information on the composition of the extracts and thus to generate a large library of bioactive phytochemical constituents of plants with medicinal significance (**Trease, 1989; Yusof & Abdullah, 2020).** Studies have demonstrated that plant extracts include different phytochemicals with antioxidant, antibacterial, and other therapeutic activities **(Kumar *et al.*, 2021)**. The phytochemical evaluation of EtE and AcE extracts revealed the presence of tannins, alkaloids, terpenoids, flavonoids, except quinones which were absent in both extracts **(Table 2).** These compounds are involved in a number of medicinal properties of the plants **(Yoo *et al.*, 2018).**

Our findings are in agreement with previous studies that reported the presence of various compounds, such as flavonoids, steroids, triterpenes, tannins, saponins, alkaloids, carbohydrates, phenolic compounds, and glycosides in different extracts obtained from Quercus species **(Şöhretoğlu & Renda, 2020)**.

Phenolic compounds represent the main targets of research carried out on the genus "oak" which

are found in all organs; leaves, bark, and corn **(Morales, 2021).** Previous work carried out on the genus Quercus has shown variations in the composition of phenolic compounds. Indeed, our results are in good agreement with other studies which have shown that *Quercus canarienris L.* was found to contain strong phenolic compounds, particularly in its leaves **(Romussi *et al.*, 1992).** According to **Vinha *et al.* (2016)**, the total polyphenols concentration in the gland tissues of different species of Quercus ranges from 18 to 32 mg GAE/g extract.

Anlas *et al.* (2019) reported that total phenolic contents of aqueous and methanolic extracts in *Quercus coccifera L*. stem were 67.58 and 165.88 mg GAE/g, respectively. For the same species, ethanolic and aqueous extracts prepared from the shelled acorn parts contained 100.14 mg GAE/g and 67.98 mg GAE/g, respectively **(Gezici and Sekeroglu, 2019).** In a previous study, the use of methanol for extraction from *Q. suber L.* leaves showed the highest phenolic compounds (211.0 mg GAE/g) compared to those extracted in water (61.2 mg GAE/g) and hexane (2.2 mg GAE/g **(Custodio *et al.*, 2015).** This reflects that the choice of solvent is a very important parameter during extractions **(Hadj Rabia *et al.*, 2024)**, as reported in several works on the capacity of methanol to extract polar and semi-polar compounds belonging to various chemical classes **(Sunil *et al.*, 2019).**

Furthermore, contents of phenolic compounds, extracted from different Algerian species have been found to be high compared to *Q. canariensis*. A content of 490.81 µg GAE/mg was observed in the extract of *Quercus ilex* (root bark) cultivated in eastern Algeria **(Meziti *et al.*, 2019). Touati *et al.* (2015)** obtained contents of the order of 79.80 g GAE/ kg in cork of *Q. suber*. Another study carried out on Algerian acorn oil, extracted from three species, *Quercus ilex* , *Quercus suber* and *Quercus coccifera*, showed values varying between 121- 299 mg /kg **(Makhlouf *et al.*, 2018).**

These fluctuations in phytochemical content could be attributed to variations in genetic factors, climate and soil conditions, extraction procedure, geographical origin, and distribution of phenolic compounds within the different parts of the plant **(Touati *et al.*, 2015; Ahmadi *et al.*, 2019).**

The phytochemical examination revealed that the composition of diterpenes, fatty acids, cyclopentone, and quinic acid of *Quercus canariensis* grown in Algeria from Algiers differs from Quercus that is grown in France, Spain, and America due to the presence of linoleic acid and its derivatives **(Fernández de Simón *et al.*, 2006; Khodadoust *et al.*, 2014).** This characteristic composition could allow us to consider that the *Q. canariensis* cultivated in North of Algeria would be a new chemotype for Algeria with linalool and its derivatives as the majority compound.

The studies carried out on the ethanolic and acetone extracts showed a great diversity in its chemical composition. Indeed, the existence of specific chemotypes of *Quercus brantii* has been demonstrated in Iran, chemotype of oleic acid (52.99–66.14%), linoleic acid (10.80–11.11%) and palmitic acid (8.08–10.06%) **(Khodadoust *et al.*, 2014)** and in Turkey of *Quercus cerris* chemotype of Guaiacol (18.6 %) and 4-Vinylguaiacol (39.6 %) **(Marques, A.V. and H. Pereira, 2013).** Thus, these different chemotypes may be due to ecological factors and the difference in geographical positions **(Maffei M. and M. Mucciarelli, 2003; Liu *et al.*, 2016).**

Many studies have been widely conducted on extracts from several medicinal plants including the genus Quercus in order to identify different phytochemical groups with antimicrobial power. So, extracts prepared from different parts of the plant such as leaves have been tested against bacteria (Gram- and Gram+) and fungi, particularly multi-drug resistant pathogenic strains **(Taib**

***et al.*, 2020).**

In the present work, ethanolic and acetone extracts prepared from the leaves of *Q. canariensis* showed a positive effect on all strains tested (bacteria and fungi). This activity could be attributed to the bioactive compounds, identified by the qualitative and quantitative analysis, which are responsible for a variety of biological functions. As a result, much emphasis has been dedicated to natural compounds produced mostly from plants **(Chaudhary *et al.*, 2012; Chaitra *et al.*, 2015).**

Comparing the two extracts, the antimicrobial activity revealed a higher efficiency of acetone extract against the microbial strains, *E. coli* ($P < 0.05$) and *A. brasiliensis* ($P < 0.01$) with the exception of *F. oxysporum Albanis* whose effect was more active for the ethanolic extract. The other strains, *S. aureus, P. aeruginosa, E. feacalis* and *C.albicans* seem to react in the same way to both extracts, showing no significant difference in microbial growth inhibition ($P > 0.05$).

The antimicrobial difference observed in our study, can be explained by a difference in composition or concentration of active substances in the prepared extracts, which largely depends on the solvent used. The antimicrobial effect of the genus Q. has been supported by several results cited in the literature.

Extracts of *Q. crassifolia, Q. persica* and *Q. castaneifolia* have been reported to be effective against *E.coli* **(Valencia-Aviles *et al.*, 2019; Taib *et al.*, 2020**) and that of *Q. brantii*, which showed an inhibition on *S. aureus* and *C. albicans* growth **(Tahmouzi, 2014).**

S. aureus biofilm formation was found to be limited by both the leaf and stem/fruit butanolic extracts of *Q.cerris*, giving the most effect at a dose of 200ug/ml.

Sarwar *et al.* (2017) emphasized that an enhanced antibacterial activity against bacterial

pathogens has been observed using gold nanoparticles prepared from the leaves of *Q. incana*. The effect was found to be more active against *Aspergillus ssp.*

Taib *et al.* (2020) reported the evident efficacy of *Q. infectoria* extracts for the decontamination of eggshells against different strains including *S. aureus, E.coli, P. aeruginosa* and *Candida albicans* **(Tayel *et al.*, 2018).** They also noted the antimicrobial efficacy of the species *Q. suber*, cultivated in Algeria against *S.aureus* and *P. aeruginosa* unlike the *E.coli* strain, which was resistant **(Touati, 2015).**

Several studies reported the antibacterial effect of certain phytochemical compounds, namely saponins, tannins, anthraquinone, flavonoids, alkaloids, terpenoids, steroids, etc., known by their versatile pharmacological properties **(Saddiq *et al.*, 2019; Shamsudin *et al.*, 2022; Abdellatif *et al.*, 2023; Elouafy *et al.*, 2023).** These compounds constitute an excellent source for clinical applications and food preservation due to their inhibitory power on a multitude of pathogens (Morales, 2020). This in fact explains the satisfactory antibacterial effect observed by our extracts whose composition in bioactive molecules was confirmed by phytochemical analysis, which highlighted several classes of secondary metabolites.

Touati, (2015) reported that phenolic-rich extracts may involve multiple modes of antibacterial action, either by acting on membrane proteins via their hydroxyl groups, thereby disrupting membrane permeability, or by causing coagulation of cellular contents once penetrated.

Flavonoids are also found to be important bioactive compounds of Quercus leaves **(Taib *et al.*, 2020)** which have been widely evaluated for their antimicrobial potency on a wide range of gram-negative and gram-positive bacteria, and fungi **(Zheng *et al.*, 2019; Shamsudin *et al.*, 2022; Zhou *et al.*, 2023).**

Interestingly, the acetone extract showed a notable effect on *E. coli* compared to that of ethanol. This is probably related to the lipophilicity of the existing bioactive compounds which is a very important indicator of membrane permeation **(Liñán-Atero *et al.*, 2024; Yan *et al.*, 2024).**

The observed effect is due to the predominance of lipophilic bioactive compounds, compared to those of the ethanolic extract. These compounds were able to easily penetrate through the inner membrane (lipid bilayer) of Gram-negative bacteria, knowing that the cell envelope of the latter, contains two membranes, a hydrophilic external membrane and another internal lipophilic. However, their penetration through the first hydrophilic membrane is probably facilitated by the action of other molecules having good affinity with the membrane. Therefore, our suggestion can be supported by that of previous studies, reporting that antibacterial activity is linked to a synergistic effect between the different phytochemical groups present in the extracts **(Ganfon *et al.*, 2019).**

It is also interesting to note that although *P. aeruginosa* is a Gram-negative bacterium like *E. coli*, a notable sensitivity was observed for both extracts, especially that of ethanol which is supposed to predominate hydrophilic molecules. This is probably explained by the synergistic effect mentioned above or by other unknown mechanisms of action **(Yan *et al.*, 2024)** that have favored the penetration of the molecules through the two membranes.

For Gram+ bacteria, a remarkable effect was observed for *S. aureus* unlike *E. faecalis*, which showed a certain resistance to both extracts despite also having a single membrane (lipid bilayer) unlike Gram-negative bacteria. This could be due to the requirement of a certain concentration to amplify the effect without however neglecting the lipophilic parameter **(Yan *et al.*, 2024).**

Our result is in agreement with previous study, which showed that S. aureus was more sensitive to the flavonoids and the effect resulted from the deterioration of the bacterial cell wall and the cytoplasmic membrane **(Zhou *et al.*, 2023).**

Yan *et al.* (2024) and **Yuan *et al.* (2022)** also reported that the cell membrane is the major action site of plant flavonoids against Gram-positive bacteria, acting on the phospholipid bilayers and respiratory chain.

The antimicrobial effect observed in our study could also be due to the presence of tannins, terpenoids and alkaloids **(Sharma *et al.*, 2016; Wang *et al.*, 2020; Adhikari *et al.*, 2021).** The latter appear to cause cell membrane disruption with loss of cytoplasmic content **(Cushnie *et al.*, 2014; Ngobeni *et al.*, 2020).** The chelating properties of tannins on metals essential for microbial growth confer them this inhibitory power, by forming complexes with enzyme substrates or inhibiting enzymes **(Hadj Rabia *et al.*, 2024).**

The long-standing knowledge of fatty acids' antibacterial capabilities is intriguingly attributed to their production by algal and plant cells as a defence mechanism against infections, including bacteria that are resistant to several drugs **(Kabara, 1984; P Desbois, 2012).** Also a review provides a comprehensive summary of 229 recently discovered diterpenoids over the past 5 years. These compounds have demonstrated potential antimicrobial capabilities, including antibacterial, antiviral, and antifungal activities **(Saha *et al.*, 2022)**.

Some phytochemicals such as Neophytadiene and quinic acid…., identified in our study have been previously reported for their antimicrobial effect **(Venkata *et al.*, 2012 and Ma, 2015; Mustapa *et al.*, 2015; Ceyhan-Güvensen *et al.*, 2016; Chang *et al.*, 2018; Lu *et al.*, 2021).**

The presence of Quinic acid in the ethanolic extract with a noticeable rate compared to other identified compounds, could probably explain the important inhibitory effect observed *against S. aureus and P. aeruginosa,* especially *E. coli*. This suggestion is supported by the work of **Heena *et al.* (2024)** who found that Quinic acid was effective against certain pathogenic bacteria, such as E. coli and S. aureus. This compound caused an antibiofilm activity, a significant increase in cell constituent release with irreversible damage to the cytoplasmic membrane, as well as a synergistic effect, enhancing antibacterial and anti-biofilm efficacy. Furthermore, this compound has shown to be active on *P. aeruginosa* by exerting in vitro an inhibitory effect on mobility, toxin release as well as on biofilm formation **(Lu *et al.*, 2021).** This latter action, which is assured by interfering with the secretion of extra polymeric substances (EPS), appears to improve antibacterial resistance, making it difficult to treat infections **(Wang *et al.*, 2023).**

As for the fungus, the antifungal role of quinic acid was demonstrated by **Muthamil *et al.* (2018),** reporting that a synergistic combination of this compound with undecanoic acid significantly inhibited virulence factors of candida spp such as the biofilm formation, production of extracellular enzymes and biosynthesis of essential compounds, filamentous growth and yeast-to-hyphal transition.

In contrast to some reports in the literature, there has been conflicting evidence about the antifungal properties of Quercus species. **Sridhar (2019)** stated that *Quercus infectoria* gall extracts exhibited antifungal activity against Penicillium and Aspergillus species. **Elansary (2019)** found phenolic acids in Quercus bark that displayed antibacterial and antifungal properties. **Bhardwaj (2012)** studied the antifungal effectiveness of plant extracts against wood rotting fungi, but did not explicitly focus on Quercus species. Previous work showed that Neophytadiene (diterpenoid) presents several functionalized agents, such as antimicrobial

properties **(Mustapa *et al.*, 2015; Ceyhan-Güvensen *et al.*, 2016); Swamy *et al.*, 2017; Ngobeni *et al.*, 2020); Alrajhi *et al.*, 2022).** This compound, identified in our study, was found to exert inhibitory effect against some strains such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*, as well as yeast *Candida albica* **(Alrajhi *et al.*, 2022).** Microscopic examination of *S. aureus* and *P. aeruginosa* revealed severe alterations in the internal and external structure of these bacteria, leading to leakage of intracellular contents, deformation and death of cells. These authors attributed this effect to the occurrence of the natural ingredients of the plant, including Neophytadiene and other constituents. Likewise, **Ceyhan-Güvensen *et al.* (2016)** reported that the high content of Neophytadiene obtained in their study explained the observed antimicrobial effect.

n-Hexadecanoic (palmitic acid) which is a saturated fatty acid is also found to have antibacterial and antifungal properties. This compound has proven its action on pathogenic bacterial strains by acting on the hydroxyl group of membrane lipopolysaccharides. This caused disturbances in the balance of the structure of these membranes as well as on their integrity **(Nadhila *et al.*, 2022).** Its anti-biofilm and antifungal power against mycelial growth and spore production has also been revealed on Candida ssp **(Hrichi *et al.*, 2022).** In addition to its interaction with the cell wall surface of *Fusarium oxysporum* and *Aspergillus flavus*, causing a fungistatic effect **(Sjafaraenan *et al.*, 2021; Nadhila *et al.*, 2022).**

The antioxidant activity of *Quercus canariensis* leaves, assessed via DPPH radical scavenging assays, offers significant insights in comparison to analogous research on other species within the Quercus genus. The leaves and bark of *Quercus robur, Quercus salicina, Quercus serrata, Quercus durifolia, Quercus resinosa, Quercus eduardii, Quercus sideroxyla,* and *Quercus ilex* exhibit notable antioxidant properties **(Benyagoub *et al.*, 2022; Tuyến *et al.*, 2016; Gamboa-**

Gómez *et al.*, 2013; Hadidi *et al.*, 2017). These activities were tested utilizing methods such as DPPH radical scavenging, ABTS, reducing power, and β-carotene bleaching tests. The antioxidant capability varied among species and plant sections, with *Q. salicina* bark extract having the highest potential **(Tuyến *et al.*, 2016). Boucif *et al.* (2024)** observed that the Q. ilex leaves have a capacity of roughly 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 demonstrate that holm oak has a high antioxidant capacity for the barks and leaves. Phenolic chemicals, particularly ellagic, chlorogenic, and benzoic acids, were discovered as substantial contributions to the antioxidant capabilities **(Tuyến *et al.*, 2016)**. Extraction methods and solvents influenced the antioxidant activity, with acetone extracts generally performing better than methanol extracts **(Gamboa-Gómez *et al.*, 2013)**. Holm oak, through their antioxidant activity and their phenolic composition, can play a function in inhibiting free radicals and this plant can therefore have a preventive role against numerous diseases. It will need to be further investigated and used in research in numerous domains (pharmaceuticals, cosmetics and the food business) (**Boucif *et al.*, 2024).**

Conclusion

Based on our results, it can be concluded that the phytochemical properties and antimicrobial activity of the organic solvents used in the extraction of *Quercus canariensis* leaves indicated that, following a solid-liquid extraction with these organic solvents, the resultant yields were nearly equivalent. This similarity was also observed in the polyphenol assay results. While the antibacterial activity was determined using the disc diffusion method, and the extracts indicated notable activity against the bacteria examined. Acetonic extract had the strongest inhibitory efficacy against *P. aeruginosa* and *S. aureus*. However, the antifungal activity assessment

findings show that the tested fungi are sensitive to acetonic and ethanolic extracts of our researched plant. *Quercus canariensis* leaf extracts demonstrate significant antioxidant properties, particularly in ethanolic form, additional investigations are necessary to fully understand their potential applications and improve their effectiveness.

According to the GC-MS analysis, for the two extracts, numerous compounds relative to the class of diterpenes, which are known for their diverse therapeutic potential, were identified. These molecules exhibit a wide range of biological activities, including anti-inflammatory, antioxidant, antidiabetic, anticancer, antimicrobial, and antiviral effects, thus chemically validating the bioactivity of the extracts.

This study offers valuable insights into the phytochemical profile and biological activities of *Quercus canariensis* leaf extracts; however, it is not without limitations. The biological assays were conducted solely *in vitro*, and further *in vivo* validation is needed to confirm the antimicrobial and antioxidant activities Moreover, while GC–MS identified key compounds, the isolation and structural characterization of individual bioactives were not performed. Environmental influences on phytochemical composition were also not explored.

Future research should focus on investigating the molecular mechanisms behind the observed activities, isolating and testing pure compounds, and developing advanced delivery systems to improve efficacy. These steps would enhance the pharmacological understanding and practical applications of *Q. canariensis* in medicine and nutrition.

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Ethical approval

Not applicable in this study

Author's Contributions

Conceptualization: N.T., A.D., A.F., and S.H; Investigation: N.T., W.Z. and N.M Methodology: N.T., W.Z. and C.C.; Supervision: A.D.; Roles/Writing - original draft: N.T., W.Z., S.H. and A.D. Reviewing, and editing: A.D and S.H.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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