ORIGINAL PAPERS

THE *IN VITRO* EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF METHANOLIC AND AQUEOUS LEAVES' EXTRACTS OF *QUERCUS ROBUR* L., FROM THE ALGERIAN HIGH PLATEAUS AGAINST SOME UROPATHOGENIC MICROBIAL STRAINS

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Key words: *Quercus robur L., Macerates, phytochemical analysis, antimicrobial activity, uropathogenic strains*

INTRODUCTION

Antimicrobials, such as antibiotics, are essential to treat infections that are caused by pathogenic microorganisms. However, their abusive or excessive use in veterinary and human medicine should be correlated with the urgency and spread of resistant bacteria, which render the treatment of infectious diseases ineffective on animals and humans.

Because of this problem, researchers are turning increasingly to traditional medicine as a source of new drugs [1]. Herbal medicines are an essential part of healthcare around the world. Medicinal plants are important for pharmacological research and drug development, when not only the plant constituents are directly used as therapeutic agents, but also as raw materials for drug synthesis or as models for pharmacologically active compounds.

In order to ensure the conservation and availability of these plants for the future, the regulation of their exploitation and export is essential, as is the cooperation and coordination at an international level [2].

Among these medicinal plants, we are interested in the plant *Quercus robur* L. which is defined as one of the species of *Fagaceae* family, whose leaves are used against hemorrhages, diarrhea and incontinence.

In this context, the objective of this work is to highlight the antimicrobial activity of two methanolic and aqueous macerates of *Quercus robur* L leaves on some uropathogenic microbial strains.

MATERIALS AND METHODS

Our work has focused on the characterization, on the one hand, of the phytochemical properties of pedunculate oak leaves of "*Quercus robur* L.", and on the other hand, the antimicrobial activity of two macerates on some pathogenic microbial strains.

The isolation and identification of the microbial strains were carried out at the level of Dr. Benchaib S. Md's medical analysis laboratory (Bechar-Algeria) while the phytochemical analysis and the various antimicrobial tests were carried out at the level of Dr. Hamid KADI's biological laboratory at *Tahri Mohammed* University of Bechar (Algeria).

Vegetable material

Harvesting the plant

The pedunculate oak leaves (*Quercus robur* L.) were collected at the level of the Mezi mountain of Djeniene Bourezg in the region of Ain-Sefra (Province of NAAMA-Algeria) during the month of October 2017 (Figure 1).

Figure 1. Geographical location of collection site of the plant in Djeniene Bourezg (Province of NAAMA-Algeria) [3].

Preparation of plant material

The pedunculate oak leaves (*Q. robur* L.) are washed and dried at room temperature and protected from light. Then the dry plant is coarsely ground in an electric mixer (IKA® Werke M20, Germany) and stored at room temperature in a closed jar to preserve their original quality.

Qualitative phytochemical analysis

Regarding the tri-phytochemical study, three extractions were performed according to the protocol developed by Georgievskii et al., [4]. The crude extracts were obtained by successive extractions with solvents of increasing polarities. In this order, petroleum ether, methanol and distilled water were used. The preparation of the extracts made it possible to carry out a qualitative phytochemical analysis.

Extraction processes

The aqueous and methanolic macerates of the studied plant were extracted by maceration, the method is described below:

Preparation of macerates

A test sample of 10 g of the dried plant was mixed with 100 mL of distilled water. The mixture is stirred for 24 hours. After filtration through a filter paper, the filtrate is evaporated by steam evaporation in rotary flask evaporator (Buchi Rotavapor R-210, Switzerland), dried under reduced pressure at 100 °C to obtain the aqueous macerate residue.

However, for the methanolic macerate, a test sample of 5 g of the dried plant was mixed with 85 mL of methanol. The mixture is stirred for 24 hours. After filtration, the filtrate is evaporated, dried under reduced pressure at 65 °C.

The obtained residues are determined by weight to calculate the yield of aqueous, methanolic and etheric macerates [5].

Yield of macerates

The yield of the macerate was determined after the total evaporation of the solvent. The yield of the extract is defined as the ratio between the mass of the plant material to be treated and the mass of the obtained extract [6]. This yield is calculated according to the following formula:

$$\mathbf{R = (M / M_0) \times 100}$$

R: Yield expressed in %.

M: Mass in a gram of the obtained dry extract.

$\mathbf{M_0}$: Mass in a gram of the plant material to be processed.

Microbial strains

The evaluation of the extracts' antimicrobial activity was carried out in accordance with official methods. However, the tested microorganisms were isolated from the pathological samples (urine and vaginal discharge) by cyto-bacteriological exam of urine and vaginal discharge in women. These analyzes were carried out at the level of Dr. Benchaib's medical analysis laboratory (Bechar-Algeria) and *Boudjemaa TOURABI* 240 beds' public Hospital (Bechar-Algeria), where we focused more on hospitalized patients.

For this study, we selected urine samples with significantly bacteriuria greater than or equal to 10^5 CFU/mL.

The isolated strains were firstly subjected to macroscopic identification of colonies on nutrient agar phenotypic and microscopic characterization by fresh state observation between slide and coverslip, and differential Gram staining; secondly, identification of biochemical characteristics (classical gallery using IMVIC test, oxidase test, catalase test, miniaturized gallery API 20E, staphylocoagulase test and API Staph). The fungal strain *Candida albicans* is isolated on Sabouraud-chloramphenicol medium and undergoes the blastosis test (filamentation test). The isolated and identified microorganisms were eleven (11) strains in total, distributed as follows: Three (03) strains of *Escherichia coli*, three (03) strains of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Salmonella sp* and *Pseudomonas aeruginosa*.

Antimicrobial tests

The colonies that have been isolated from young cultures on nutrient agar medium (Fluka, India) incubated at 37 °C for 18 to 24 hours are transferred into tubes containing sterile physiological saline water (0.9 % NaCl), in order to prepare bacterial suspensions having an equivalent turbidity of 0.5 McFarland. Then, the bacterial suspension, previously prepared, was seeded with a sterile swab on the entire surface of a Mueller-Hinton agar medium (Himedia, India) by tight property streaks.

Agar diffusion method (disc and well):

The antimicrobial resistance profile study towards antibiotics was carried using the MH agar diffusion method, with loaded antibiotic discs as recommended by the National Committee on Clinical Laboratory Standards (NCCLS) [7].

Antibiotic discs, used for disc diffusion method, were the first rank for *Escherichia coli*, *Klebseilla pneumoniae* and *Salmonella sp* as follows: cefoxitin, cefazoline, chloramphenicol, amoxicillin-clavulanic acid, ampicillin, ofloxacin, imipenem, and cefotaxime; secondly for *Staphylococcus aureus*: penicillin, oxacillin, vancomycin, fosfomycin, fusidic acid, erythromicin, amikacin, gentamycin; thirdly for *Pseudomonas aeruginosa*: ofloxacin, tobramycin, imipenem, rifampin, ticarcillin, fosfomycin, amikacin and cefotaxime; and finally for *Enterococcus faecalis*: vancomycin, tetracycline, gentamicin, erythromicin and ampicillin.

The antimicrobial activity of the extracts was determined by the disc diffusion method on agar medium and well diffusion method [9,10]. The first method consists in substituting the antibiotic discs with other discs confined from Wattman paper impregnated with the extract which concentrations were prepared as follows (0.18g/mL, 0.15 g/mL, 0.1 g/mL, 0.05 g/mL; 0.03 g/mL, 10 mg/mL, 5 mg/mL and 1 mg/mL) for the methanolic macerate, and two

concentrations for the aqueous macerate (0.15 g/mL, 0.06 g/mL).

Each inoculated Petri dish contains the impregnated discs with dimethyl sulphoxide (DMSO), methanol and diethyl ether which they used as a control. Finally, the dishes were incubated at 37 °C for 24 h

The second method was described by Vlietinck and Vanden Berghe [11] which we applied to confirm the action of the tested extracts. This method consists of cutting a circular hole in the MH agar; the solution of each extract is poured with a volume of 10 µl into the well. The radially diffused of the extract gives a circular inhibition zone on the surface of the agar seeded with the bacterial suspension by streak swabbing.

Broth dilution method

The MIC is the lowest concentration of antimicrobial agent that completely inhibits the growth of the microorganisms in tubes visible to the unaided eye.

Broth macro-dilution method is one of the basic methods for antimicrobial susceptibility testing. The procedure involves the preparation of different dilutions of the tested antimicrobial agent in a liquid growth medium dispensed into tubes containing a minimum volume of 2 ml (macro-dilution). Then, each tube is inoculated with a microbial inoculum prepared in the same medium after standardization of the microbial suspension to 0.5 McFarland [12,13].

Statistical Analysis

In this study, the inhibitory zones means of three experimental tests of antimicrobial activity as well as the standard deviation were calculated using Excel software, from which we are going to release graphical presentations as a histogram for antibiotic susceptibility and antimicrobial testing.

RESULTS AND DISCUSSIONS

Yields of macerates

The extraction of the compounds obtained from the leaves by maceration, allowed us to obtain two raw macerates: aqueous macerate and methanolic macerate. The extraction yield of the leaves is shown in Figure 2 below.

The yield of macerates is variable for leaves from one macerate to another. It ranges from 8.5 to 22.4 %. The yield of the methanolic macerates was higher compared to the aqueous macerates.

Qualitative phytochemical analysis

Phytochemical tests consist in detecting the different chemical families in the leaves of *Quercus robur* L. This detection is based on the constituent solubility test, precipitation reactions, turbidity and a specific color change. The results of the phytochemical analysis are given in Table 1 below:

The carried out phytochemical analysis revealed the presence of seven major chemical groups in the leaves: tannins, coumarins, fatty acids, reducing compounds, sterol glycosides, saponins and alkaloid salts (Figure 3), with absence of starch, anthocyanins, anthracenosides, flavonoids, free quinones, terpenoids, sterols or triterpenes, emodols and basis alkaloids.

Table 1 (a, b, c**):** Qualitative phytochemical analysis of *Quercus robur* L leaves

(a) Etheric extract

Components	**leaves**	**CN (Petroleum ether)**
Coumarins	+	-
Fatty acids	+	-
Starch	-	-
Basis alkaloids	-	-
Emodols	-	-
Sterols or triterpenes	-	-
Free quinones	-	-
Terpenoids	-	-

(b) Aqueous extract

Components	**leaves**	**NC (Distilled water)**
Alkaloid salts	+	-
Reducing compounds	+	-
Tannins	+++	-
Saponins	+	-
Starch	-	-

(c) Methanolic extract

Components	**leaves**	**NC (Methanol)**
Alkaloid salts	+	-
Tannins	+++	-
Reducing compounds	+	-
Flavonoids	-	-
Sterol glycosides	+	-
Coumarins	+	-
Anthocyanins	-	-
Anthracenosides	-	-

(-): Negative test; (+): Positive test; (+++): strongly positive; (NC): Negative Control

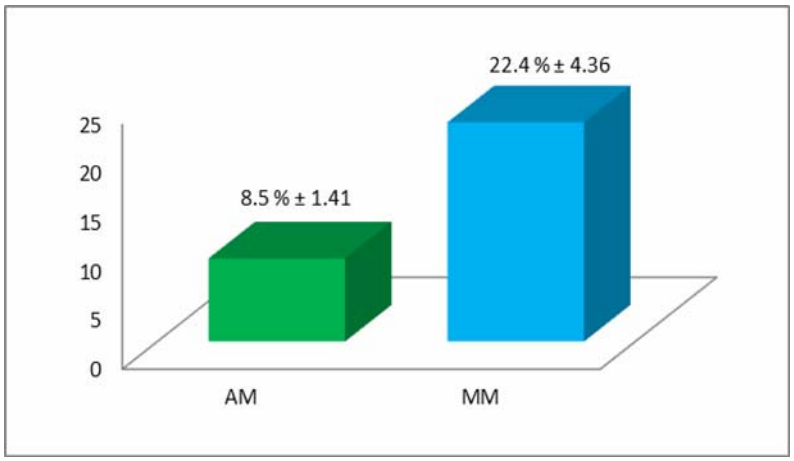

Figure 2. Yields of leaves macerates of *Q. robur* L. AM: Aqueous macerate; MM: Methanolic macerate
Results of three tests (mean ± standard deviation)

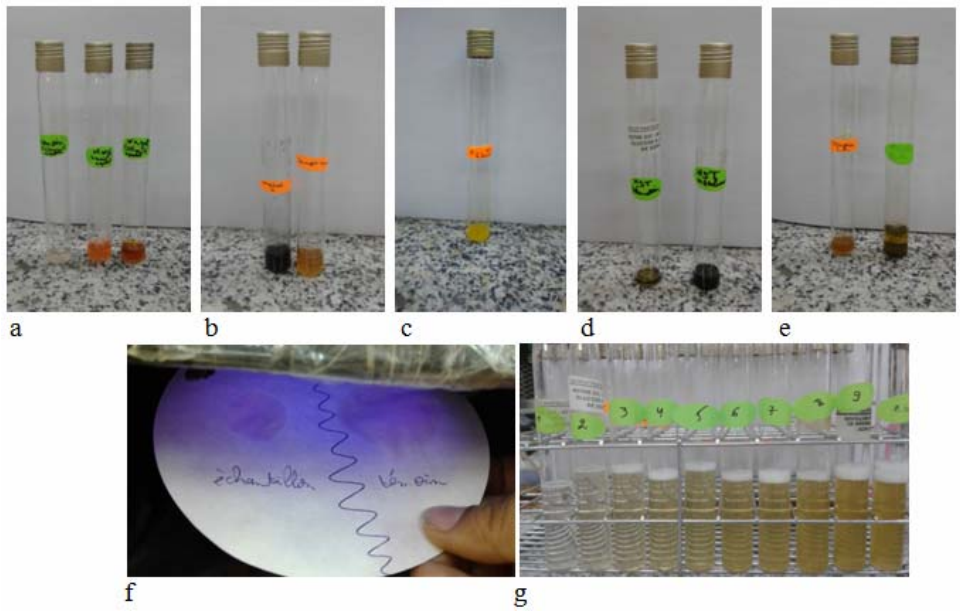

Figure 3. Photographic illustration of qualitative phytochemical tests of *Quercus robur* L leaves
(a): Alkaloids salts; (b): Tannins; (c): fatty acid; (d): Triterpene steroid heterosides;
(e): Reducing compounds; (f) Coumarines; (g) Saponins.

Tested uro-bacterial strains

The antimicrobial resistance profile results of bacterial strains carried out by diffusion method on Mueller-Hinton agar are reported in Table 2 (a, b), and Figure 4.

These results reveal that the bacterial resistance is relatively important, with several antibiotics namely ampicillin, amoxicillin+clavulanic acid for enterobacteria (the three strains of *Escherichia coli* and *Salmonella sp*); in addition to these two antibiotics, *Klebsiella pneumoniae* strain was resistant to amoxicillin. The three strains of *Staphylococcus aureus* were resistant to fusidic acid, penicillin and oxacillin. While *Enterococcus faecalis* was resistant to fusidic acid, penicillin, oxacillin and ticarcillin. *Pseudomonas aeruginosa* strain of iatrogenic origin was resistant to ticarcillin, ceftazidime and cefotaxime.

The results of the antimicrobial testing by disc and well diffusion method for both macerates of *Q. robur* L. are presented in Tables 3 and 4, and Figure 5, 6 and 7.

Table 2 (a, b). Diameters and percent inhibition values of inhibition zones (I %) of antibiotics against tested uro-bacterial strains

(a)

Bacterial strains / ATBs	*E. coli* 1			*E.coli* 2			*E. coli* 3			*K. pneumoniae*			*Sal. sp*		
	D (mm)	I (%)	P. ATB	D (mm	I (%)	P. ATB	D (mm)	I (%)	P. ATB	D (mm)	I (%)	P. ATB	D (mm)	I (%)	P. ATB
Amoxicillin	32	35.56	S	30	33.33	S	22	24.44	S	6	6.66	**R**	39	43.33	S
Amoxicillin + clavulanic acid	6	6.66	**R**	6	6.66	**R**	6	6.66	**R**	6	6.66	**R**	6	6.66	**R**
Cefalexin	22	24.44	S	25	27.78	S	19	21.11	S	20	22.22	S	24	26.67	S
Ofloxacin	30	33.33	S	52	57.78	S	30	33.33	S	42	46.67	S	42	46.67	S
Ampicillin	6	6.66	**R**	6	6.66	**R**	6	6.66	**R**	6	6.66	**R**	6	6.66	**R**
Imipenem	36	40	S	34	37.78	S	39	43.33	S	26	28.89	S	39	43.33	S
Cefazolin	32	35.56	S	25	27.78	S	35	38.89	S	29	32.22	S	37	41.11	S
Chloramphenicol	38	42.22	S	28	31.11	S	38	42.22	S	21	23.33	S	45	50	S
Cefoxitin	27	30	S	26	27.89	S	25	27.78	S	29	32.22	S	18	20	S
Cefotaxime	27	30	S	30	33.33	S	25	27.78	S	22	24.44	S	30	33.33	S

(b)

Bacterial strains / ATBs	*S. aureus* 1			*S. aureus* 2			*S. aureus* 3			*E. faecalis*			*P. aeruginosa*		
	D (mm)	I (%)	P. ATB	D (mm)	I (%)	P. ATB	D (mm)	I (%)	P. ATB	D (mm)	I (%)	P. ATB	D (mm)	I (%)	P. ATB
Fusidic acid	14	15.56	**R**	12	13.33	**R**	18	20	**R**	6	6.66	**R**	-	-	-
Fosfomycin	6	6.66	**R**	6	6.66	**R**	6	6.66	**R**	6	6.66	**R**	-	-	-
Penicillin	6	6.66	**R**	6	6.66	**R**	6	6.66	**R**	6	6.66	**R**	-	-	-
Oxacillin	30	33.33	S	28	31.11	S	6	6.66	**R**	6	6.66	**R**	-	-	-
Vancomycin	25	27.78	S	24	26.67	S	14	15.55	S	15	16.67	S	-	-	-
Cefalexin	25	27.78	**S**	24	26.67	S	25	27.77	S	26	28.89	**S**	17	18.89	S
Amikacin	24	26.67	S	22	24.44	S	31	34.44	S	32	35.56	S	18	20	S
Ticarcillin	-	-	-	-	-	-	-		-	6	6.66	**R**	6	6.66	**R**
Imipenem	-	-	-	-	-	-	-	-	-	-	-	-	24	26.67	S
Ciprofloxacin	-	-	-	-	-	-	-	-	-	-	-	-	25	27.78	S
Ceftazidime	-	-	-	-	-	-	-	-	-	-	-	-	16	17.78	**R**
Cefotaxime	-	-	-	-	-	-	-	-	-	-	-	-	19	21.11	**R**

Table 3. Diameters values of inhibition zones by disc diffusion method of methanolic and aqueous macerates towards the tested uro-microbial strains

	Methanolic macerate								Aqueous macerate	
Concentration / MS	1 mg/mL	5 mg/mL	10 mg/mL	0.03 g/mL	0.05 g/mL	0.1 g/mL	0.15 g/mL	0.18 g/mL	0.06 g/mL	0.15 g/mL
***E.coli* 1**	-	-	8.5±2.12	10±1.14	15±0.0	15.5±0.7	18.5±0.7	18.5±0.7	12.5±0.7	15.5±0.7
***E.coli* 2**	-	-	7±1.41	12±4.24	17±1.41	17.5±3.53	20±0.0	20±0.0	11.5±0.70	23.5±2.12
***E.coli* 3**	-	-	9.5±0.7	9.5±2.12	14.5±0.7	15.5±0.7	17±2.82	15.5±0.7	13.5±0.70	17±1.4
***S.aureus* 1**	-	-	-	-	9.5±0.70	15±0.0	20.5±2.12	22.5±3.53	-	-
***S. aureus* 2**	-	-	-	-	8.24±0.0	9±0.0	10.5±0.0	22±3.53	-	-
***S. aureus* 3**	-	-	10±1.41	13±1.41	16.5±2.12	21±1.41	25±1.41	24.5±3.53	13±1.41	17±1.41
E. faecalis	-	-	7.5±2.12	8.5±0.70	14±2.82	16.5±0.70	18±1.41	20±1.41	-	16.5±0.70
C. albicans	-	-	-	-	-	13.5±2.12	13.5±0.70	12.5±2.12	10±0.0	16±0.0
K. p	-	-	-	9.5±2.12	14±1.41	15.5±3.53	18.5±0.70	21±1.41	-	-
P. a	-	-	-	-	-	-	-	-	-	-
Sal. sp	-	-	-	12.5±2.12	16.5±2.12	16±4.24	21±1.41	22±0.0	11.5±2.12	18.5±0.70

(MS): Microbial strains; **(D)**: Diameter of inhibition zones in (mm), **I (%)**: Percent inhibition, **(P.ATB)**: Antibiotic resistance profile, **(S)**: Susceptible, **(R)**: Resistant, **(I)**: Intermediate, **ATBs**: antibiotics, **E. coli**: *Escherichia coli*, **P. a**: *Pseudomonas aeruginosa*, **E. faecalis**: *Enterococcus faecalis*; **Sal. sp**: *Salmonella sp*; **S. aureus**: *Staphylococcus aureus*, **K. p**: *Klebseilla pneumoniae*. **Ac.**: Acid

Table 4. Diameters values of inhibition zones by well diffusion method of methanolic and aqueous macerates against the tested uro-microbial strains

	Methanolic macerate								Aqueous macerate	
Concentrations / MS	**1 mg/mL**	**5 mg/mL**	**10 mg/mL**	**0.03 g/mL**	**0.05 g/mL**	**0.1 g/mL**	**0.15 g/mL**	**0.18 g/mL**	**0.06 g/mL**	**0.15 g/mL**
***E.coli* 1**	-	-	-	-	13±0.0	16±0.0	17±0.0	19±0.0	13±0.0	18±0.0
***E.coli* 2**	-	-	-	10±0.0	12±0.0	17±0.0	20±0.0	19±0.0	12.5±0.70	18.5±0.70
***E.coli* 3**	-	-	-	14±0.0	14±0.0	17±0.0	20±0.0	23±0.0	12±0.0	15.5±0.70
***S.aureus* 1**	-	-	-	10±0.0	12±0.0	19±0.0	20±0.0	19±0.0	14.5±0.70	18±1.41
***S. aureus* 2**	-	-	-	11±0.0	15±0.0	19±0.0	20±0.0	10±0.0	14±1.41	19±1.41
***S. aureus* 3**	-	-	-	10±0.0	13.5±2.12	16.5±4.94	20.5±3.53	22.5±3.53	13±0.0	16±0.0
E. faecalis	-	-	-	9±2.82	14.5±0.70	18±2.80	20.5±2.12	24±0.0	14.5±0.70	19.5±0.70
C. albicans	-	-	-	12±0.0	15±0	17±0	20±0.0	24±0.0	17±1.41	18±2.82
K. p	-	-	-	9.5±2.12	14±1.41	15.5±3.53	18.5±0.70	21±1.41	13.5±0.70	12±0.70
P. a	-	-	-	12±0.0	13±1.41	15±2.82	19.5±0.70	22±0.0	19±0	20±0.0
Sal. sp	-	-	-	10±0.0	12±0.0	17±0.0	21±0.0	24±0.0	14.5±0.70	18.5±2.12

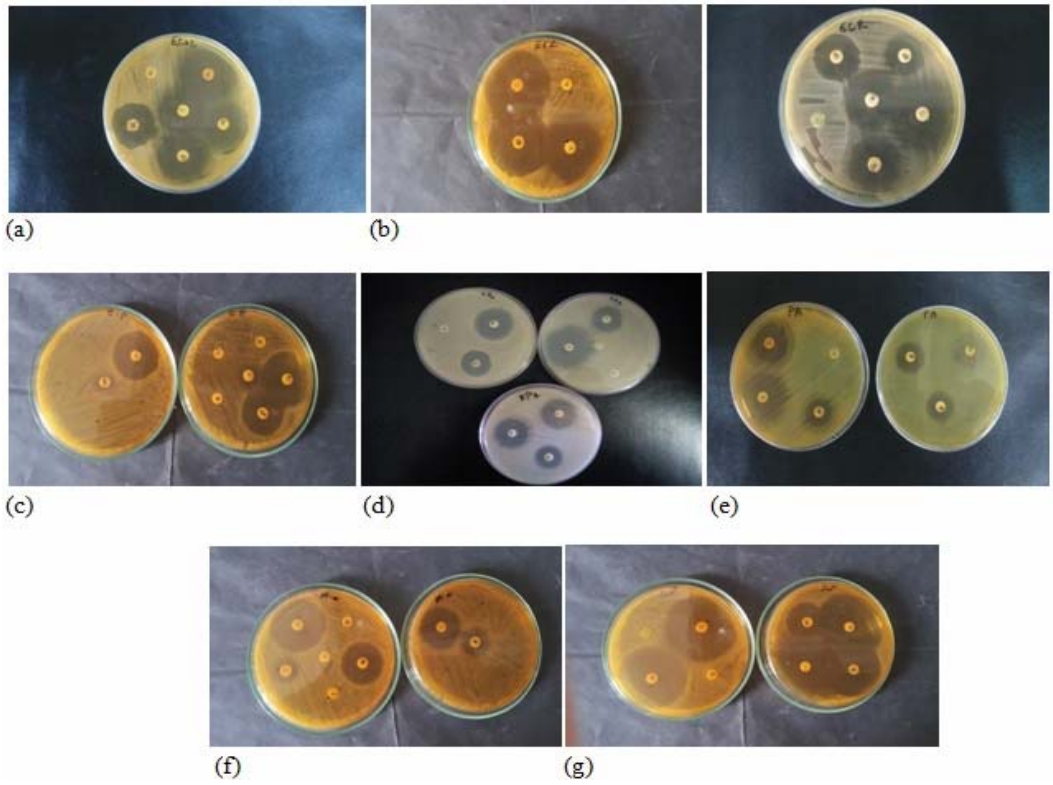

Figure 4. Antibiogram assay of tested uro-bacterial strains on Mueller-Hinton agar
(a) *E.coli* 2 ; (b) *E. coli* 3 ; (c) : *E. faecalis* ; (d) : *K. pneumoniae* ; (e) : *P. aeruginosa* ; (f) : *S. aureus*; (g) : *Salmonella sp*.

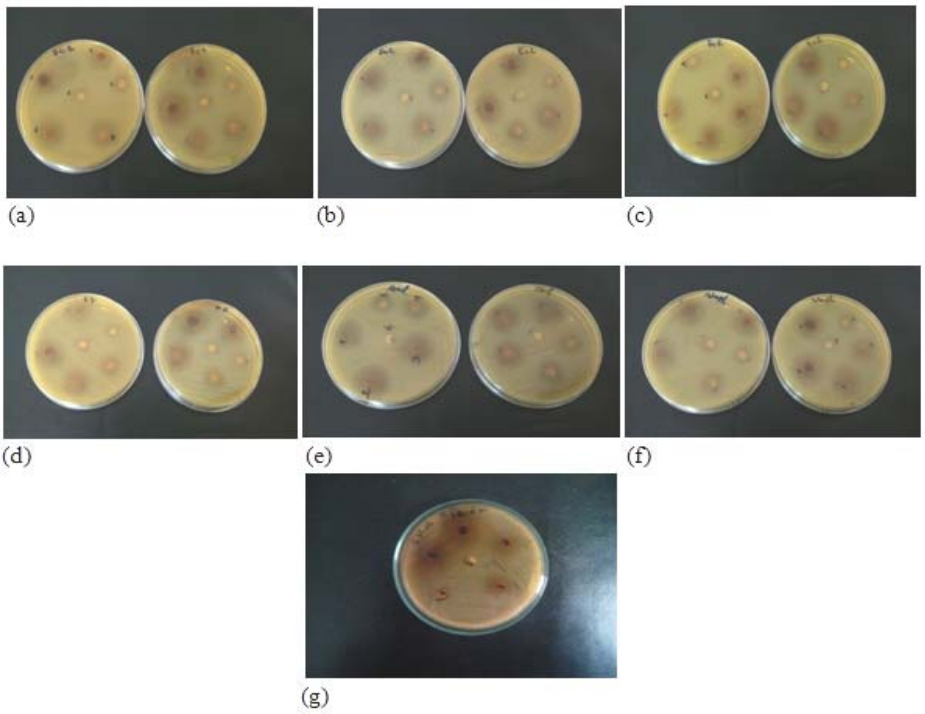

Figure 5. Antimicrobial activity of the methanolic macerate against tested uro-bacterial strains on Mueller-Hinton agar (disc diffusion method). (a) *E.coli* 1 ; (b) *E. coli* 2 ; (c) : *E. coli* 3 ; (d) : *E. faecalis* ; (e) : *Salmonella sp* ; (f) : *S. aureus P. aeruginosa* ; (g) : *K.pneumoniae.*

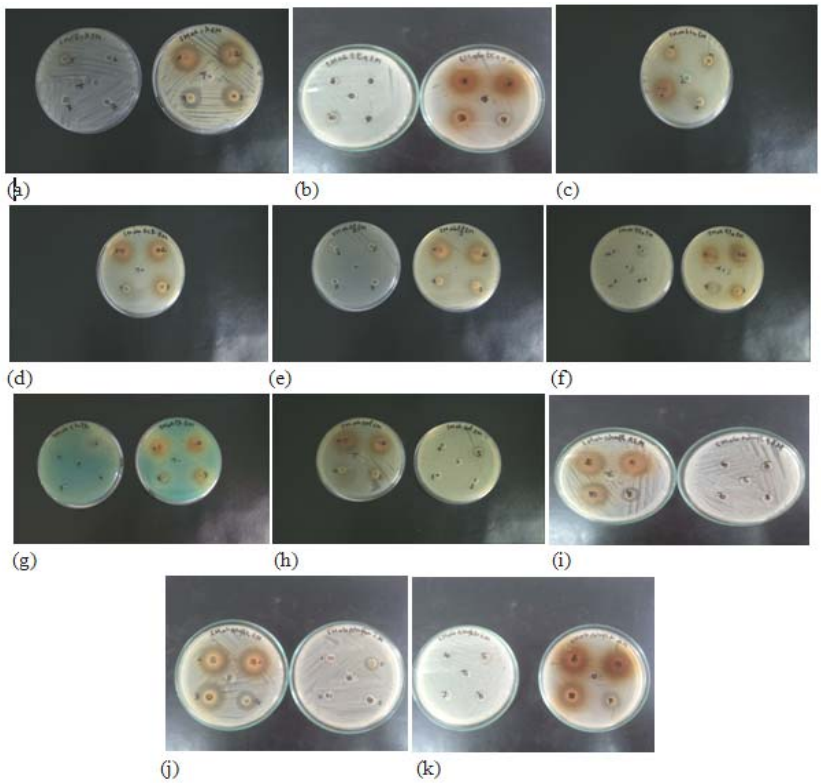

Figure 6. Antimicrobial activity of methanolic macerate against tested uro-microbial strains on Mueller-Hinton agar (well diffusion method). (a): *C. albicans*; (b) *E. coli* 1; (c) : *E. coli* 2; (d) : *E. coli* 3; (e) : *E. faecalis*; (f) : *K.pneumoniae* ; (g) : *P. aeruginosa* ; (h) : *Salmonella sp* ; (i) : *S. aureus* 1; (j) : *S. aureus* 2 ; (k) : *S. aureus* 3.

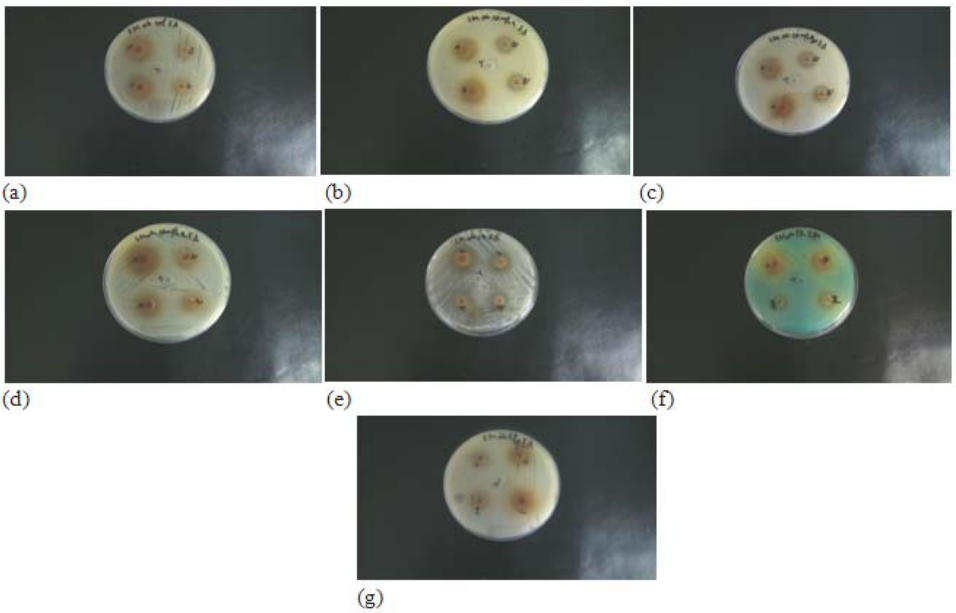

Figure 7. Antimicrobial activity of the aqueous macerate against tested uro-microbial strains on Mueller-Hinton agar (well diffusion method). (a) : *Salmonella sp*; (b) : *S. aureus* 1; (c) : *S. aureus* 2; (d) : *S.aureus* 3; (e) : *C. albicans*; (f) : *P. aeruginosa;* (g) *: K.pneumoniae.*

Discussion

The search for new antimicrobial agents becomes an obligation before the development of many diseases and resistance of microorganisms to antibacterial and antifungal molecules.

According to the extraction yield results, the yield of the methanolic extract is greater than the aqueous extract. This result proves that the methanolic extract contains more phytochemical compounds of analyzed oak leaves [14].

The phytochemical results are similar with those given by Uddin and Rauf [15] where the species has a high concentration of phenolic compounds, coumarin, tannins, fatty acid; triterpene steroid heteroside and alkaloids [16-18].

The antibiotic resistance profile results of *E. coli* strains showed resistance to amoxicillin +clavulanic acid and ampicillin. These results reveal the sensitivity of isolated *E. coli* uropathogenic strains, not ESBL producing, compared to several authors who sound the alarm on the risk of the multi-resistance of strains involved in urinary tract infection and which also considerably limits the therapeutic options and constitutes a real public health problem.

We note the work of Elbouamri et al., [19] where they isolated 1472 uropathogenic *Enterobacteriaceae* where 924 non-repetitive of *E. coli* strains had an overall isolation frequency of 63%.

The antimicrobial resistance of isolated E. coli strains revealed variable levels of resistance to amoxicillin, sulfamethoxazole - trimethroprim, amoxicillin + clavulanic acid, ciprofloxacin, gentamicin, nitrofuran, amikacin and fosfomycin. In addition, the work of El Bakkouri et al., [20] showed a significant increase in resistance to ampicillin and norfloxacin by the analysis of 799 *E. coli* uropathogenic community strains where the consumption of antibiotics - a non-negligible risk factor favoring the evolution of these resistances- is a real problem.

K. pneumoniae strain was resistant to amoxicillin, amoxicillin+clavulanic acid and ampicillin. Nori and Ziadi Chibane's study [21] on the antibiotic resistance of *K. pneumoniae* isolated in the hospital's environment, where isolated strains from urine were more than 50 % of the other pathological samples where all the studied strains were resistant to amoxicillin and ticarcillin (natural resistance).

However, these strains had variable resistance to amoxicillin+clavulanic acid, to cefazolin and to cefotaxime. In addition, imipenem, cefoxitin and colistin remain the most active molecules. Moreover, the emergence of multi-resistant pathogenic strains requires effective therapeutic strategies that must be defined with preservation of molecules that seem more active.

Urinary infection with non-typhoid *Salmonella* occurs on a predisposed terrain, such as a state of cellular immune-depression or acquired uropathy (lithiasis, schistosomiasis) or congenital uropathy. Moreover, this type of infections has no specificity in

their clinical presentation compared to other Gram-negative infections [22]. The isolated *Salmonella* sp was resistant to the combination of amoxicillin+clavulanic acid and ampicillin, the work of Weill et al. [23] showed resistance to amoxicillin and to ampicillin.

Isolated *Staphylococcus aureus* strains were resistant to oxacillin, fusidic acid and penicillin. The resistance to the latter molecule is probably due to the acquisition of a plasmid penicillinase where this resistance was initially restricted to the hospital environment which spread quickly in the community and currently affects more than 90 % of *S. aureus* strains. During the 1950's, those *S. aureus* multi-resistant strains appeared [24].

Enterococcus faecalis strain was resistant to fusidic acid, penicillin, oxacillin, and ticarcillin. According to studies by Stucki et al., [25] because of their natural resistance to several classes of antibiotics and the increase in resistance to penicillin, *E. faecalis* should be recognized, including prior treatment with antibiotics such as cephalosporins or quinolones. A restriction in the empirical use of cephalosporins or quinolones and antibiogram-targeted antibiotic therapy are essential measures to prevent the emergence of resistant enterococci strains, particularly resistant to vancomycin.

In the studies of Hernández et al., [26], which concern the molecular characterization of *P. aeruginosa* strains isolated from patients at Del Niño Hospital, Republic of Panama, where the percentage of resistant isolates to ceftazidime, to imipenem and to gentamicin was 14; 16 and 16 % respectively. Only one strain presented a multi-resistance.

According to the results of disc diffusion method, *S. aureus* 3 strain was very sensitive to the methanolic extract with an average inhibition diameter of 10 mm at a concentration of 10 mg/mL. Then for *E. coli* 3, the inhibition zone was 9.5 mm at the same concentration, and for the tested aqueous extract, *E.coli* 3 was even more sensitive with an average inhibition diameter of 13.5 mm at the concentration of 0.06 g/mL.

According to our results, the absence of the antibacterial activity of the aqueous extract of *Q.robur* leaves against *K. pneumoniae* strain is noted. In contrast, according to the work of Mladenovic et al., [27], the aqueous extract of *Q. robur* showed a good antimicrobial activity against *K. pneumoniae* strain with an inhibition diameter of 15.4 mm at a concentration of 30 mg/disc. This difference is probably due to various conditions including temperature, location and drying time, genotype and geographical origin.

The results of the antibacterial activity of the aqueous extract against the tested strains *S. aureus*, *Escherichia coli* and *E. faecalis* are similar to those of Mladenovic et al., [27]. However, methanolic macerates have a broad activity against both Gram-positive and Gram-negative bacteria.

These results corroborate the work of Satish et al., [28] where the antibacterial activity of the methanolic extract of *Q. robur* was greater against *Staphylococcus aureus*, *Streptococcus faecalis*, *Salmonella paratyphi* A and *Salmonella paratyphi* B strains with inhibitory zones of 15.2; 14.3; 13.3 and 12.8 mm respectively compared to the aqueous extract that has a low activity.

Studies by Andrensek et al., [29] confirm the antimicrobial activity of the methanolic extract against the species of *Staphylococcus aureus*, *Enterobacter aerogenes* and *Candida albicans* that was moderate and variable bactericidal, fungicide to bacteriostatic fungistatic. This antimicrobial activity is probably attributed to phenolic compounds such as tannins, terpenoids and flavonoids whose studies have shown the antibacterial activity of tannins. These molecules have been reported as bacteriostatic or bactericidal against several bacterial strains namely: *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* ... etc.

However, many tannin molecules have been reported for their actions to reduce the mutagenicity of several mutagens. The tannins also have anti-carcinogenic activity and as an inhibitory effect on fungal, bacterial and viral species. Tannins in food plants serve as a natural defense mechanism against microbial infections. Thus, tannins can theoretically serve as natural regulators of the microbial population in different habitats, including the human gastrointestinal tract [30].

According to Bouhadjera et al., [31], flavonoids that have a very broad and diverse antibacterial activity depending on the microorganism and the ecosystem they are able to inhibit the growth of different types of bacteria namely *Staphylococcus aureus, Escherichia coli* and *Enterococcus faecalis*. Terpenoids also showed strong activity against *Staphylococcus aureus*, and low effect against Gram-negative bacteria.

CONCLUSION

Medicinal plants are an inexhaustible source of bioactive natural substances and compounds. Among these plants we have selected *Q. robur* L., which belongs to the family of *Fagaceae*. The methanolic macerate of its leaves was found to be rich in phytochemical compounds with a higher extraction yield compared to aqueous macerate.

The antibio-resistance profile of the tested strains showed different degrees of sensitivity where the resistance rate of bacterial strains is relatively important to several antibiotics namely amoxicillin+clavulanic acid and ampicillin for Enterobacteriaceae (*E. coli* and *Salmonella sp*), while *S. aureus* strains exhibited resistance to fusidic acid, penicillin and oxacillin. However, *E. faecalis*

exhibited resistance, in addition to the latter two antibiotics, to ticarcillin.

The antibacterial activity of aqueous and methanolic macerates was more active against Gram-positive bacteria than against Gram-negative bacteria, with the particularity that the methanolic extract has a broad activity spectrum compared to the aqueous extract where *S. aureus* strains showed high sensitivity from a concentration of 10 mg/mL of the methanolic extract.

ABSTRACT

The medicinal value of the plants is due to their chemical components that bring a definite physiological action on the human body to prevent the diseases.

In this work, we investigated the antimicrobial activity of leaves' extracts of *Quercus robur* L., collected from the Algerian upper highlands, on ten bacterial strains and one fungal strain known to be pathogenic.

Firstly, we performed a qualitative phytochemical analysis, and secondly, antimicrobial activity tests performed by agar diffusion method (disc and well) with the determination of MIC by broth macro-dilution method.

Given the results, it appears that obtained macerates of *Q. robur* L. were rich in bioactive phytoconstituents such as alkaloids, anthraquinones, saponins, tannins and other components. The yield of aqueous and methanolic macerates of leaves was 8.5±1.41 and 22.4±4.36 % respectively.

The bacterial resistance was relatively important to several antibiotics namely ampicillin, amoxicillin+clavulanic acid for strains of *Escherichia coli* and *Salmonella sp*. However, *Staphylococcus aureus* strains were resistant to fusidic acid, to penicillin and to oxacillin; while *Enterococcus faecalis* was resistant to fusidic acid, penicillin, oxacillin and ticarcillin.

The antibacterial activity of the macerates towards tested microbial strains showed that the aqueous and methanolic macerates of the leaves were proportional to the tested concentration and active not only against Gram-positive and Gram-negative bacteria, but also on the fungal *species Candida albicans.*

The estimated MIC for *Escherichia coli, Enterococcus faecalis* and *Staphylococcus aureus* was in the order of 10 mg/mL, which seems more effective than towards *Salmonella sp, Klebseilla pneumoniae, Pseudomonas aeruginosa* and *C. albicans* which were in the order of 30 mg/mL.

These preliminary results confirm that the part of the studied plant had a very good antimicrobial activity that was proportional to the serial concentrations of the tested extracts.

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REFERENCES

[1]-ALLANE T., 2009 - Etude des pouvoirs antioxydant et antibactérien de quelques espèces végétales locales alimentaires et non alimentaires. Mémoire de Magister. Faculté des sciences de l'ingénieur, Département de technologie alimentaire, Université M'hamed BOUGAGA de Boumerdes (Algérie) ; 95p. Available on: http://dlibrary.univ-boumerdes.dz:8080/bitstream/123456789/806/1/Allane%20Taous.pdf;

[2]-OMS/ WHO. 1998 - Réglementation des médicaments à base de plantes: La situation dans le monde. WHO/TRM/98.1. Organisation mondiale de la santé, Genève, : 59p. ;

[3]-Google maps. Site géographique de Djeniene Bourezg (Wilaya de NAAMA-Algérie) ; 2017.Lien: https://www.google.com/maps/place/Wilaya+de+Na%C3%A2ma/@33.1995605,-0.8021968,8z/data=!4m2!3m1!1s0xd80933e66f1126d:0xe310586a7447bf74?force=lie;

[4]-GEORGIEVSKII V P, KOMISSARENKO NF, DMITROUK SE., 1990 - Substances bioactives des plantes médicinales, édition « Naouka » Novosibirsk; 336p. ;

[5]-MAJHENIC L, SKERGET M, KNEZ Z., 2007 - Antioxidant and antimicrobial activity of Guarana seed extracts. *Food Chem*; 104: 1258-1268. https://doi.org/10.1016/j.foodchem.2007.01.074 ;

[6]-BELYAGOUBI M. , 2006 - Effet de quelque essence végétale sur la croissance des moisissures de détérioration des céréales. Mémoire de Magister, Département de biologie, faculté de sciences, université Aboubekr Belkaid, Tlemcen-Algérie ;

[7]-NCCLS, 2002. Performance standards for antimicrobial disk susceptibility tests. Approved standard M02-A7. National Committee for Clinical Laboratory Standards, Villanova. (Etats-Unis) PA. USA ;

[8]-SOUSSY JC, Communiqué 2005. Comité de l'antibiogramme de la Société Française de Microbiologie ;

[9]-SACCHETTI G, MAIETTI S, MUZZOLI M, SCAGLIANTI M, MANSREDINI S, RADICE M, IRIMI R. 2005 - Comparative evaluation of 11 essentials oils of different origin as functional antioxydants, antiradicals and antimicrobial in food. *Food Chem*; 91: 621-632. https://doi.org/10.1016/j.foodchem.2004.06.031 ;

[10]-CELIKTAS OY, HAMES KOCABAS EE, BEDIR E, VERDAR SUCAN O, BASER KHC. 2007 - Antimicrobial activities of methanolic extract and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chem*; 100: 553-559. https://doi.org/10.1016/j.foodchem.2005.10.011 p;

[11]-VLIETINCK AJ, VANDEN BERGHE DA., 1991 - Can ethnopharmacology contribute to the development of antiviral drugs? *J Ethnopharmacol*; 32:141-153. https://doi.org/10.1016/0378-8741(91)90112-Q;

[12]-CLSI, 2012 - Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard, 9th ed., CLSI document M07-A9. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA;

[13]-BALOUIRI M, SADIKI M, KORAICHI IBNSOUDA S., 2016 - Methods for *In vitro* evaluating antimicrobial activity: A review. *J Pharm Anal*; 6(2): 71-79. http://dx.doi.org/10.1016/j.jpha.2015.11.005;

[14]-BOUHADJERA K. , 2004 - Contribution à l'étude chimique et biologique de deux plantes médicinales sahariennes *Oudneya africana* et *Aristida pungens*. Thèse de Doctorat. Université Aboubekr Belkaid,Tlemcen-Algérie ;

[15]-UDDIN G, RAUF A. 2012 - Phytochemical screening, antimicrobial and antioxidant activities of aerial parts of *Quercus robur* L. *Middle-East J Med Plants Res*; 1(1): 1-4. https://doi.org/10.5829/idosi.mejmpr.2011.1.1.1101;

[16]-DENNO RF, DINGLE H. 1981 - Insect life history patterns: habitats and geographic variation New York, Springer. [Papilio polyxenes Fabricius; Costa Rica]. Blau, William S., and Paul P. Feeny;

[17]-GÜLZ PG, MÜLLER M. 1992 - Seasonal variation in the composition of Epicuticular waxes of *Quercus robur* leaves. *Kristallogr C*; 47(11-12): 800-806. https://doi.org/10.1515/znc-1992-11-1204-10.5580/IJMB.42638;

[18]-LENFORT F, DOUGLAS CG. 1999 - An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species Acer, Fraxinus , Prunus and Quercus. *Ann For Sci*; 56(3): 259-263. https://doi.org/10.1051/forest:19990308;

[19]-ELBOUAMRI MC, ARSALANE L, KAMOUNI Y, BENNOUAR N, BERRAHA M, ZOUHAIR S. 2014 - Profil actuel de résistance aux antibiotiques des souches d'*Escherichia coli* uropathogénes et conséquences thérapeutiques. *Prog Urol*; 24 : 1058-1062. http://dx.doi.org/10.1016/j.purol.2014.09.035;

[20]-ELBAKKOURI J, BELABBES H, ZEROUALI K, BELAICHE A, MESSAOUIDI D, JD PERRIER GROS CLAUDE, ELMDAGHRI N. 2009 - Résistance aux antibiotiques d'*Escherichia coli* uropathogéne communautaire et consommation d'antibiotiques à Casablanca (Maroc). *Eur J Sci Res*; 36(1): 49-55;

[21]-NOURI M, ZIADI CHIBANE F. 2015 - Etude bactériologique et résistance aux antibiotiques de *Klebseilla pneumoniae*. Mémoire de Master. Faculté des sciences de la nature et de la vie, Departement de biologie animale, Université de Frères Mentouri Canstantine-Algérie, 50 p. Available on: http://fac.umc.edu.dz/snv/faculte/biblio/mmf/2015/62-2015.pdf ;

[22]-VIALE S, AVANCES C, BENNAOUM K, COSTA P. 2002 - Infections urinaires à *Salmonella* non typhique. *Prog Urol*; 12 : 1297-1298. Available on: https://www.urofrance.org/nc/science-et-recherche/base-bibliographique/article/html/infections-urinaires-a-salmonella-non-typhique.html;

[23]-WEILL FX, LAILLER R, BRISABOIS A. 2004 - Tendances récentes de la résistance aux antibiotiques des *Salmonella* d'origines animale et humaine. *Bull Epidemiol Hebd*; 32-33: 160-162. Available on: http://invs.santepubliquefrance.fr//beh/2004/32_33/beh_32_33_2004.pdf;

[24]-DUMITRESCU O, DAUWALDER O, BOISSET S, REVERDY ME, VANDENESCH F. 2010 - Résistance aux antibiotiques chez *Staphylococcus aureus :* les points-clés en 2010. *Med Sci* (Paris) ; 26(11) : 943-949. https://doi.org/10.1051/medsci/20102611943.

[25]-STUCKI K, NENDAZ M, HARBARTH S. 2014 - Infections à entérocoques : du plus simple au plus complexe. *Rev Med suisse*; 10 (446): 1918-1923. Available on: https://www.revmed.ch/RMS/2014/RMS-N-446/Infections-a-enterocoques-du-plus-simple-au-plus-complexe;

[26]-HERNANDEZ M, CASTILLO G, CINIGLIO C, RAMOS C, CHEN O, DE MAYORGA B, DURAN O, GONZALEZ E, GONZALEZ M,

AGUILAR C, CISTERNA O, DE CHIAL M. 2016 - Molecular characterization of *Pseudomonas aeruginosa* clinical isolates among patients of the Hospital Del Niño, Republic of Panama. *Internet J Microbiol*; 14(1): 1-9. https://doi.org/10.5580/IJMB.42638;

[27]-MLADENOVIC N, MLADENOVIC A, PAVLOVIC S, BASKIC D, ZDRAVKOVIC N. 2014 - The aqueous extract of *Quercus robur* L. (*Fagaceae*) shows promising antibacterial activity against *Klebsiella pneumonae*. *Global J Pathol Microbiol*; 2(2): 53-58. http://dx.doi.org/10.14205/2310-8703.2014.02.02.3;

[28]-SATISH S, MOHANA DC, RAGHAVENDRA MP, BABU S, RAVEESHA KA. 2009 - Antibacterial evaluation of some Iranian medicinal plants against som humain pathogenic bacteria. *Asian J Microbiol Biotech Env Sci*; 11(4): 735-738. Available on: http://www.envirobiotechjournals.com/article_abstract.php?aid=4368&iid=153&jid=1;

[29]-ANDRENSEK S, SIMONOVSKA B, VOVK I, FYHRQUIST P, VUORELA H, VUORELA P. 2004 -Antimicrobial and antioxidative enrichment of oak (*Quercus robur*) bark by rotation planar extraction using ExtraChrom®, *Int J Food Microbiol*; 92(2): 181-187. https://doi.org/10.1016/j.ijfoodmicro.2003.09.009;

[30]-CHUNG KT, WEI CI, JOHNSON MG. 1998 - Are tannins a double-edged sword in biology and health? *Trends in Food Sci Tech*; 9: 168-175. https://doi.org/10.1016/S0924-2244(98)00028-4 ;

[31]-BOUHADJERA K, BENDAHOU M, TABTI B. 2005 - Antimicrobial activity of extracts from Algerian *Aristida pungens* L. *Pak J Biol Sci*; 8(2): 206-210. https://doi.org/10.3923/pjbs.2005.206.210.

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