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Phenolic Extracts of *Zizyphus lotus* L. (Rhamnaceae) and *Ruta chalepensis* L. (Rutaceae) as Alternatives to Antibiotics and their Antimicrobial Effects on Clinical Multidrug-Resistant Pathogens

Nour El Houda Bekkar[1], Boumediene Meddah[1], Yavuz Selim Cakmak[2], Bahadir Keskin[3], Pascal Sonnet[4]

[1]Mustapha Stambouli University, Faculty of Life and Nature Sciences, Microbiological Engineering and Health Safety, Laboratory of Bioconversion, Mascara, Algeria
[2]Aksaray University, Faculty of Science and Letters, Department of Biotechnology and Molecular Biology, Aksaray, Turkiye
[3]Yildiz Technical University, Faculty of Arts & Science, Department of Chemistry, Istanbul, Turkiye
[4]Picardie Jules Verne University, AGIR Laboratory: Infectious Agents, Resistance and Chemotherapy, EA4294 UFR of Pharmacy, Amiens, France

ORCID IDs of the authors: NEH.B. 0000-0002-1194-3472; B.M. 0000-0001-7946-012X; Y.S.C. 0000-0001-8954-5485; B.K. 0000-0001-8502-8982; P.S. 0000-0003-0118-9151

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ABSTRACT

Objective: The phytochemical composition and the antibacterial and antifungal properties of *Zizyphus lotus* L. (ZL) leaves and *Ruta chalepensis* L. (RC) aerial parts harvested from Oran in northwest Algeria were assessed against multidrug-resistant (MDR) clinical pathogens.

Materials and Methods: The phenolic compounds identification in the hydromethanolic (MeOH.E) and the aqueous extracts (Aq. E) was done by HPLC-DAD analysis, while the phenolic, flavonoid and tannin contents were determined using quantitative methods. The antibacterial and antifungal activities were also determined. The synergistic effect between both plants was elucidated using the checkerboard dilution test.

Results: An important phenolic content was determined with higher concentrations in *Z. lotus* leaves extracts than *R. chalepensis*. The HPLC-DAD analysis allowed us to identify benzoic acid as the major phenolic compound in *Z. lotus* extracts, while catechin, quercetin and epicatechin were the major compounds identified in *R. chalepensis*. Important antimicrobial activity was observed against all the clinical pathogen strains. The most potent effect was estimated against MDR *Salmonella enterica* sp. *arizonae* with 20±0.1mm of growth inhibition zone diameter using $RC^{MeOH.E}$, while a diameter of 35.03±0.06 mm was measured using $ZL^{MeOH.E}$. Also, important anti-Candida activity was estimated. No synergistic interaction against the different microbial strains was determined by applying the combinations of both plants' extracts, with a fractional inhibitory concentration index superior to 4 (FIC_{index} >4).

Conclusion: *Z. lotus* and *R. chalepensis* can be exploited in the medical field as a potential source of antimicrobial components.

Keywords: Antibiotic alternatives, antimicrobial activity, multidrug-resistant pathogens, phenolic compounds, *R. chalepensis*, *Z. lotus*

Corresponding Author: Nour El Houda Bekkar E-mail: bekkarnour8@gmail.com; nourelhouda.bekkar@univ-mascara.dz

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INTRODUCTION

The increase of food-borne illnesses or collective food poisoning (CFP) and nosocomial infections transmitted in reanimation services and hospitals are considered a global health concern. The majority of the responsible microorganisms are multidrug-resistant (MDR) bacteria and microscopic fungi, germs with high pathogenicity expressed by the presence of genes for virulence and resistance to the various antibiotics commonly used in the medical field, which makes the therapeutic application of these drugs less efficient for the treatment of microbial infections, whatever food-borne disease or nosocomial infection. In addition, patients suffer from considerable side effects from antibiotic consumption, like vomiting, nausea, abdominal pain, loss of appetite and development of other microbial infections. Some antibiotic treatments induce the development of other infectious germs, such as candidiasis induced after therapeutic consumption of an antibiotics association for the treatment of gastric ulcers caused by *Helicobacter pylori* (1).

Furthermore, the most important side effect is the dysbiosis phenomenon. Antibiotics exert a noteworthy adverse effect on the gut microbial balance (*Bifidobacterium*, *Lactobacillus*, *Streptococcus*, and against *Enterobacteriaceae*). Thus, the immune system is stimulated by the attack of a broad spectrum of pathogenic microbes (barrier effect) and the penetration of foreign agents (chemicals or microbials) at the level of the gastrointestinal mucosa (2).

Therefore, there is increasing interest in the study of biomolecules that have a stronger antimicrobial effect than antibiotics, but do not pose any danger to the health of the organism. Phenolic extracts of medicinal plants have been reported to possess potent antioxidant and antimicrobial effects in the literature.

In Algeria, three species of the family Rhamnaceaeare are widely used as food and folk medicine: *Zizyphus spina-christi* (L.) Desf., *Zizyphus lotus* (L.) and *Zizyphus jujuba* Mill. (3). Studies have allowed the isolation of flavonoids (4,5), triterpenes (6), alkaloids (7), indole derivatives (8) and fatty acids in the genus *Zizyphus* (9). The sedative and hypnotic effects of saponins, flavonoids and fatty acids of *Ziziphus* species have also been demonstrated (10).

The health-promoting effects of the genus of *Ziziphus* in various diseases such as respiratory problems, scabies, pimples, mouth and gums inflammation or in memory enhancement have been indicated due to its bioactive compounds. This plant is also utilized in the cosmetic sector due to its efficient properties for bleaching the face and neck, and in hair growth (11,12).

Z. lotus is popularly called "*Sedra*" in Algeria and its delicious fruits known as "*Nbeg,*" are consumed fresh. *Z. lotus* is widely used in the field of nutrition, cosmetics and healthcare. It is consumed in Algeria as infusions and decoctions to treat a variety of diseases, including urinary tract infections and digestive disorders, and also acts as a hypoglycemic, hypotensive, antidiarrheal, and anti-ulcer agent (13-15).

Also, the fruit parts of this plant are used for the treatment of several illnesses: diarrhea, intestinal diseases and digestive problems, liver disorders, insomnia, skin infections and abscess (16-18). Various studies have reported that the plant has antibacterial, anti-inflammatory, anticancer, antifungal and antiulcerogenic activity, as well as analgesic and gastroprotective effects (19-24). *Z. lotus* fruits contain significant concentrations of health-promoting compounds: minerals, vitamins, amino acids, fatty acids and phenolic compounds (25).

Moreover, the bark, fruit, leaves, roots and seeds of *Z. lotus* have been reported to possess antimicrobial effects, antioxidant activity and antispasmodic and litholytic effects (26-30). In their recent study, Bencheikh et al. (31) demonstrated the nephroprotective effect of *Z. lotus* fruits in a gentamicin-induced acute kidney injury model in rats.

R. chalepensis (Rutaceae) is popularly called "*Fidjel,*" This plant species is of particular interest in traditional medicine due to its potential therapeutic effect against various human pathogens. *R. chalepensis* is known for its richness of secondary metabolites, such as essential oils, alkaloids (0.4-1.4%), flavonoids, coumarins (chalepensine), furocoumarines, phenols, tannins and saponins (32).

The biological properties of *R. chalepensis* extracts have been studied by several researchers. In their study, Loizzo et al. (33) showed that the leaf extracts of this plant exhibited important antioxidant and hypoglycemic activities. In another study, antimicrobial efficacy against *Streptococcus mutans*, a major etiological pathogen in dental caries was demonstrated for chalepensin extracted from *R. chalepensis* (34).

Szewczyk et al. (35) have demonstrated antioxidant and antimicrobial properties of phenolic extracts of *R. chalepensis* phenolic extracts. Khadhri et al. (36) and Adsersen et al. (37) determined the antiacetylcholinesterase (AChE) activities of ethanol extracts obtained from the leaf parts of this plant. *R. chalepensis* is also used in traditional medicine for the treatment of rheumatism, fever, mental disorders, dropsy, menstrual problems, anxiety and epilepsy disorders (38).

To the best of our knowledge, there have been no reports on the antibacterial and antifungal effects against multidrug-resistant clinical pathogens of phenolic components isolated from *Z. lotus* and *R. chalepensis* harvested from Oran-Tafraoui region in northwest Algeria. Thus, the objective of this work was to determine the quantitative contents of total phenolic compounds, flavonoids and tannins from the methanol and aqueous extracts of *Z. lotus* and *R. chalepensis*. The determination of qualitative and quantitative variation of polyphenolic compounds was performed using colorimetric methods and HPLC-DAD analysis. In addition, the antimicrobial activities against test bacteria and fungi were examined.

MATERIALS AND METHODS

Plant Sample Collection

Fresh samples of *Z. lotus* leaves were collected during the month of July 2017 and *R. chalepensis* aerial parts (leaves, flowers and small stems) during April 2017 from northwest Algeria's Oran-Tafraoui region. The collected plant parts were identified by a botanist from the Department of Biology of Mascara University, Algeria.

Microbial Strains: Isolation and Identification

The antimicrobial effect was assessed on pathogenic clinical isolates including Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Enterococcus faecalis*), Gram-negative bacteria (Enteropathogenic *Escherichia coli*, *Salmonella enterica* sp. *Arizonae*, *Proteus mirabilis*, *Hafnia alvei*) and pathogenic microscopic fungi (*Candida albicans*). All these microbial strains were isolated from different clinical samples (n=25): stool specimens of gastroenteritis patients of both sex (n=15; man and women), samples from the oral cavity and the periodontal pocket of periodontal disease patients (n=4) and urine of patients with urinary tract infections (n=6). The microbial identification was carried out using commercial kits (API STAPH, API 20E and API CANDIDA) that were administered according to the BioMerieux manual and adopting standard procedures. The coagulase and blood hemolysin tests were also performed (39,40).

Antibiotics Susceptibility Test

To complete the identification of the various isolated clinical strains, antibiotic susceptibility testing is necessary for the determination of antibiotic resistance profiles for each microbial strain. For that, susceptibility to different antibiotics was evaluated using the agar diffusion method and carried out according to the Clinical and Laboratory Standards Institute guidelines (41). The results were interpreted following the critical diameters mentioned by the FMS-AC (42) and the FMS-AC/EUCAST (43). After that, only the microbial strains presenting a multidrug-resistance were selected for the antimicrobial activity assays. The antibiotics tested were Spiramycin, Amoxycillin, Pristinamycin, Nitroxolin, Neomycin, Oxacillin, Colistin, Penicillin-G and Fluconazole.

Phenolic Compounds Extraction

Preparation of Hydromethanolic and Aqueous Extracts

For the preparation of hydromethanolic extracts (MeOH.E), powdered fresh leaves of *Z. lotus* and leaf, small stem and flower parts of *R. chalepensis* (50 g) were macerated in 500 mL of hydromethanolic solution of 80% concentration at room temperature (20°C) and in shaded glass vials that inhibit light penetration. The filtrates were then evaporated to dryness under vacuum using a rotary evaporator at 40°C. The aqueous extracts (Aq.E) of the tested materials were prepared by decoction procedure. 50 g of each plant material were boiled in 500 mL of distilled water at 100°C/30 min (44, 45). The prepared polyphenolic extracts (PPEs) were stored in small shaded vials at 4°C until use. The extraction yields (%) for each PPE were calculated as the ratio between the plant weights (m_1; g) and the dry extract weight (m_2).

Phytochemical Screening

The qualitative phytochemical analysis was done to identify the main chemical groups of bioactive substances contained in the leaves of *Z. lotus* and the aerial parts of *R. chalepensis*. Phenolic compounds, flavonoids, tannins, saponosides, anthocyanins, glycosides, terpenes and coumarins were analyzed in this study (46).

Phenolic Compounds

The detection of polyphenolic compounds was carried out by a test with ferric perchloride ($FeCl_3$) at 10%. To each 5 mL of the hydromethanolic extract and the aqueous extract, 1 to 2 drops of $FeCl_3$were added to observe the appearance of an intense black-green precipitate.

Flavonoids

The detection of flavonoids was carried out by a magnesium test. A few drops of concentrated HCl (2N) and a small amount of magnesium (Mg) were added to each 2 mL of the MeOH.E and Aq.E extracts with agitation for 3 minutes. The appearance of an orange or red color indicated the presence of flavonoids (cherry red color: flavonols; orange color: flavones; purplish red color: flavanones).

Tannins

1 mL of the MeOH.E and 10% of the Aq.E were mixed with 1 mL of distilled water and 1 to 2 drops of 10% diluted $FeCl_3$ solution. The test is considered positive by the appearance of a dark green color for catechic tannins (condensed tannins). The appearance of a dark blue color indicates the presence of gallic tannins.

Saponins

2 mL of the extract was added to 2 mL of a 1% lead acetate solution. The test is considered positive by the formation of a white precipitate. Thus, the presence of saponosides was determined qualitatively by the appearance, after agitation, of persistent foam for more than 15 min.

Anthocyanins

5 mL of each extract was added to 4 mL of 30% concentrated ammonia hydroxide (NH_4OH). The appearance of a red color indicates the presence of anthocyanins.

Glycosides

The demonstration of glycosides was carried out using concentrated sulfuric acid solution (96%). 150 mg of the MeOH.E and the Aq.E dissolved in 2 mL of methanol and distilled water respectively was mixed with a few drops of sulfuric acid solution (96%). The appearance of a blue-red color indicates the presence of glycosides.

Terpenes

The detection of terpenes in all extracts was performed by mixing 5 mL of phosphomolybdic acid and 5 mL of concentrated

sulfuric acid (96%) with each 5 mL of 10% MeOH.E and Aq.E solutions. The appearance of a blue color reveals the presence of terpenes.

Coumarins

The detection of coumarins was carried out using 2 g of the plant powder mixed with 20 mL of ethanol. The mixture was boiled for 15 min under reflux. After cooling and filtering, 10 drops of potassium hydroxide (KOH) and a few drops of 10% hydrogen chloride (HCl) were added to the extracts solutions. The formation of turbidity indicates the presence of coumarins.

Determination of Total Phenolic, Flavonoid and Tannin Contents

The total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) were determined according to the methods described by Boizot and Charpentier (47) using Folin Ciocalteu as reagent, Samatha et al. (48) using the 2% aluminum trichloride solution ($AlCl_3$) and Ba et al. (49) using vanillic acid, respectively. The concentrations of these phenolic contents were calculated after the absorbance measurements using a spectrophotometer (JENWAY model, 6400 spectrophotometer). All the determinations were performed in triplicate.

Chromatographic Analysis

Thin Layer Chromatography (TLC)

The qualitative determination and detection of the different bioactive components in the MeOH.E and Aq.E extracts were carried out by applying thin layer chromatography (TLC), as described by Sanogo et al. (50). In-brief, 5 µL of plant extracts solutions at a concentration of 20 mg/mL was applied on silica gel plates of the Silicagel 60 F_{254} type (Merck, Darmstadt, Germany). The plates were then deposited in a mobile phase, which consists of a solvents mixture (Butanol/acetic acid/water (60/15/35) (v/v/v)). After the phenolic components' migration and separation, the different spots observed in the silica gel plates were detected under UV light at 254 and 366 nm, and the relative migration rates (R_m) were estimated using the following formula: R_m= d/D, where d: Migration distance of the substance, D: Migration distance of the solvents mixture. Gallic acid, catechin, quercetin, rutin and vanillin were used as control.

High Performance Liquid Chromatography (HPLC-DAD)

The chemical composition of the MeOH.E and Aq.E extracts was determined according to the method described by Caponio et al. (51), with slight modifications. It was performed using an HP-Agilent 1290 Infinity HPLC equipped with a C_{18} column and diode array detector (DAD).

Antimicrobial Activity Assessment

Agar Diffusion Method

The agar-disc diffusion method was applied to determine the antimicrobial potency of the MeOH.E and Aq.E extracts of *Z. lotus* and *R. chalepensis* collected from the Tafraoui region in Oran against the MDR clinical strains previously isolated and identified. Briefly, sterile discs were impregnated in MeOH.E and Aq.E solutions at a concentration of 200 $mg.mL^{-1}$ then aseptically deposited on the previously inoculated Muller-Hinton plates with 0.5 McFarland of bacterial and fungal cultures in their exponential growth kinetics. The antimicrobial potency against all the clinical pathogens, determined as resistant strain, sensitive, very sensitive or extremely sensitive, was established according to the criteria of Poncé et al. (52).

Determination of the Antimicrobial Parameters

The microdilution titration method was done to determine the most important parameters in the antimicrobial effect evaluation: the minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC). The assays were done according to the method described by Chandrasekaran et al. (53), with slight modifications. After the plants' extracts concentrations and the adjusted microbial suspensions were blended out in equal volumes, the sterile 96-well microplates were aseptically incubated at 37°C and the microbial growth kinetics were measured after optical density lecture at 620 nm for bacteria and 450 nm for fungi at different time-kill kinetics: 0, 4, 18, 48 and 72 hours, using a Microplate Absorbance Reader (Tecan Spectra II Microplate Reader). The results were expressed as log germs/mL for each plant extract concentration. The minimum bactericidal and fungicidal concentrations (MBCs) were determined after inoculating a microbial suspension from each dilution that represented the MIC values on MHA agar. After incubation at 37°C /24 h, the viable bacteria and fungi cells were counted. The dilution for which no bacterial or fungal colony was counted represents the MBC and the MFC. Reports of MBC/MIC were calculated to determine the efficiency of PPEs as bactericidal or bacteriostatic.

Dilution Checkerboard Method

The microdilution checkerboard method was used to evaluate the interaction between *Z. lotus* and *R. chalepensis* phenolic extracts against MDR clinical pathogens. So this technique allowed us to have an idea whether a medicinal plant extract is more effective when used alone or in combination, in order to broaden its action spectrum on pathogenic microorganisms and to induce many more bactericidal and fungicidal effects.

During this study, we evaluated the synergistic, additive or antagonistic effect using the following combinations between the prepared extracts of both studied plants: $ZL^{MeOH.E}/RC^{MeOH.E}$ and $ZL^{Aq.E}/RC^{Aq.E}$.

The association interaction of both antimicrobial extracts was quantified after the determination of the MIC values for each PPE (previously determined) and by calculating the index of fractional inhibitory concentrations (FICI or ∑FIC) which are the lowest concentrations of the antimicrobial extracts in combination, completely inhibiting the microbial growth. A volume of 50 µL of Mueller Hinton broth was distributed in all the sterile cupules of the microplates. The first extract solution of *Z. lotus* was serially diluted along the abscissa, while the extracts of *R. chalepensis* were diluted along the ordinate. Subsequently, each solution was inoculated with 50 µL of the bacterial or fun-

gal cultures and the microplates were incubated at 37°C for 18 hours. The value of the combination is calculated using the FIC in the cupules where no microbial growth is observed, and considered effective MIC for the combination (54).

The FICIs were measured as follows: FICI=FIC_A + FIC_B, where FIC_A is the MIC of drug A (MeOH.E or Aq.E of *Z. lotus*) in the combination / MIC of drug A alone, and FIC_B = MIC of drug B (MeOH.E or Aq.E of *R. chalepensis*) in the combination / MIC of drug B alone. The combination is considered synergistic when the FICI is ≤ 0.5, additive: 0.5 < FICI ≤ 1, indifference: 1 <FICI ≤ 4 and antagonism: FICI >4 (55).

Statistical Analysis

The statistical analyses were performed using the SPSS software for comparing between the averages using the one-way and multivariate analysis of variance (ANOVA). Significant differences were also mentioned: p <0.05.

RESULTS

Clinical Strains Isolation and Identification

Results of the different bacterial and fungal strains isolation and identification are shown in Table 1.

Antibiotics Susceptibility Testing

The antibiogram profile for each microbial strain is shown in Table 2. Based on the critical diameters of antibiotic susceptibility stated by the French Society of Microbiology, the FSM-AC. (2013) and the FMS-AC/EUCAST. (2018), the results showed that only 8 clinical isolates among the 41 microbial strains were multidrug-resistant.

Extraction Yield, Qualitative and Quantitative Determination of Polyphenols

Results of the extraction yields, the phytochemical screening and quantification of the polyphenols, flavonoids and tannins in the polyphenolic extracts of *Z. lotus* and *R. chalepensis* are shown in Table 3.

The results showed that aqueous extracts represented the highest yield: 23.97±0% for *Z. lotus* and 30.83±0.0057% for *R. chalepensis*, followed by the crude methanolic extract where the proportions were about 17.68±0.015% for *Z. lotus* and 14.73±0.03% for *R. chalepensis*. No significant differences were determined between the yields of both plants PPE, whereas the highest yields among the different PPE were registered for $RC^{Aq.E}$ followed by $ZL^{Aq.E}$ (Table 3).

The phytochemical examination revealed the presence of seven biochemical groups: phenols, flavonoids, condensed tannins, glycosides, terpenes, coumarins and saponosides. We noted the richness in polyphenols, catechin tannins and terpenes in both tested plants with positive reactions of the phytochemical tests. In addition, abundant flavonoids were noted in the aqueous extract and hydromethanolic extract of *R. chalepensis* compared to *Z. lotus* extracts. However, a very abundant presence of saponosides was found in *Z. lotus* extracts compared to

Table 1. Microbial strains isolated from different biological samples.

Biological sample	N*		
Gastroenteritis	15	$S_1SP_{1\text{-}M}$	*S. aureus*
		$S_2SP_{1\text{-}M}$	*E. coli*
		$S_3SP_{1\text{-}M}$	*P. mirabilis*
		$S_4SP_{1\text{-}M}$	*C. albicans*
		$S_5SP_{2\text{-}W}$	*S. enterica* sp. *arizonae*
		$S_6SP_{2\text{-}W}$	*C. albicans*
		$S_{7\text{-}8}SP_{3\text{-}4\text{-}M}$	*E. coli*
		$S_9SP_{5\text{-}W}$	*E. coli*
		S_{10} $SP_{5\text{-}W}$	*S. enterica* sp. *arizonae*
		$S_{11}SP_{6\text{-}M}$	*E. coli*
		$S_{12}SP_{7\text{-}M}$	*S. enterica* sp. *arizonae*
		$S_{13\text{-}14}SP_{8\text{-}9\text{-}W}$	*E. coli*
		$S_{15}SP_{10\text{-}M}$	*E. coli*
		$S_{16}SP_{11\text{-}W}$	*S. enterica*
		$S_{17}SP_{11\text{-}W}$	*H. alvei*
		$S_{18}SP_{12\text{-}M}$	*P. mirabilis*
		$S_{19}SP_{12\text{-}M}$	*H. alvei*
		$S_{20}SP_{12\text{-}M}$	*E. faecalis*
		$S_{21}SP_{13\text{-}W}$	*E. coli*
		$S_{22}SP_{14\text{-}M}$	*E. coli*
		$S_{23}SP_{15\text{-}M}$	*E. coli*
Urinary tract infections	06	$S_{24}SP_{16\text{-}W}$	*S. aureus*
		$S_{25}SP_{17\text{-}W}$	*S. aureus*
		$S_{26}SP_{18\text{-}W}$	*E. coli*
		$S_{27}SP_{19\text{-}W}$	*S. aureus*
		$S_{28\text{-}29}SP_{20\text{-}21}$	*E. coli*
Periodontitis	04	$S_{30}SP_{22\text{-}W}$	*S. pyogenes*
		$S_{31}SP_{22\text{-}W}$	*Streptococcus* sp.
		$S_{32}SP_{22\text{-}W}$	*E. faecalis*
		$S_{33}SP_{23\text{-}M}$	*S. pyogenes*
		$S_{34}SP_{23\text{-}M}$	*Streptococcus* sp.
		$S_{35}SP_{23\text{-}M}$	*S. aureus*
		$S_{36}SP_{24\text{-}W}$	*Streptococcus* sp.
		$S_{37}SP_{24\text{-}M}$	*E. faecalis*
		$S_{38}SP_{25\text{-}W}$	*S. aureus*
		$S_{39}SP_{25\text{-}W}$	*Streptococcus* sp.
		$S_{40}SP_{25\text{-}W}$	*E. faecalis*
		$S_{41}SP_{25\text{-}W}$	*C. albicans*

*N: Number of samples, S: Strain, SP: Sample, M: Man, W: Women.

Table 2. Antibiotic resistance profiles of the pathogenic clinical isolates.

Microbial strains code	Clinical isolates	SP	AMX	PT	NI	N	OX	CT	P	P-G	FCA
S_1SP_1	S_1/ *S. aureus*	0^R	12^R	0^R	18^I	15^I	0^R	12^R	10^R	0^R	/
$S_{33}SP_{23}$	S_2/ *S. pyogenes*	0^R	15^R	0^R	22^I	15^I	0^R	12^R	0^R	0^R	/
$S_{20}SP_{12}$	S_3/ *E. faecalis*	23^S	19^I	20^I	12^I	0^R	0^R	0^R	21^I	0^S	/
S_8SP_4	S_4/ *E. coli* (EPEC)	0^R	0^R	0^R	20^I	15^R	0^R	11^R	0^R	0^R	/
S_3SP_1	S_5/ *P. mirabilis*	0^R	15^R	0^R	14^I	16^R	0^R	0^R	10^R	0^R	/
$S_{10}SP_5$	S_6/ *S. enterica* sp. *arizonae*	0^R	0^R	0^R	22^I	18^S	0^R	13^R	0^R	0^R	/
$S_{17}SP_{11}$	S_7/ *H. alvei*	0^R	0^R	0^R	20^I	20^S	0^R	13^R	0^R	0^R	/
S_4SP_1	S_8/ *C. albicans*	0^R	0^R	0^R	0^R	0^R	0^R	0^R	0^R	0^R	0^R

S: Strain, SP: Sample, SP: Spiramycin, AMX: Amoxycillin, PT: Pristinamycin, NI: Nitroxolin, N: Neomycin, OX: Oxacillin, CT: Colistin, P: Penicillin-G, FCA: Fluconazole, R: Resistant, S: Sensitive, I: Intermediate sensitivity.

Table 3. Polyphenolic compounds extraction yields, phytochemical screening and quantitative determination of polyphenols (mg/g DE) in *Z. lotus* and *R. chalepensis* harvested from Tafraoui region in Oran.

Plant Extracts	Yield (%)	TP	F	T	A_t	G	T_r	I	C	S/ FI	TPC	TFC	TTC
$ZL^{MeOH.E}$	17.68±0.015*	+++	++	+++	-	+	+++	-	-	+++/500	268.65±7*	109.45±2.87*	94.18±4.84*
$ZL^{Aq.E}$	23.97±0*	+++	++	+++	-	+	+++	-	-	+++/500	222.85±5.99*	71.51±2.34*	113.87±0.79*
$RC^{MeOH.E}$	14.73±0.03*	+++	+++	+++	-	+++	+++	-	++	+/125	214.06±4.71*	81.16±4.42*	18.97±1.79*
$RC^{Aq.E}$	30.83±0.0057*	+++	+++	+++	-	+++	+++	-	++	+/125	224.18±6.28*	59.83±1.96*	20.76±0*

TP: Total polyphenols, F: Flavonoids, T: Tannins, A_t: anthocyanins, G: Glycosides, T_r: terpenes, I: Iridoid, C: coumarins, S: saponins, FI: Foam index, TPC: Total phenol content, TFC: Total flavonoid content, TTC: Total tannin content. Measurements were performed in triplicate. Results are expressed as means± SD. $p<0.05$: Significant*.

R. chalepensis, which was illustrated by a marked foam index: IM=500. We also noted the presence of glycosides with a positive test reaction for *R. chalepensis* compared to *Z. lotus*.

The quantitative analysis of the methanolic and aqueous extracts was carried out by dosage of the main bioactive components: total polyphenols (PPT), flavonoids (TFC) and tannins (TTC). The contents of these phenolic compounds are shown in Table 3. We quantified variable important concentrations of the main chemical groups per gram of the dry extract. Both plants were detected to have higher doses of PPT ($ZL^{MeOH.E}$=268.65±7 mg GAE/g DE, $RC^{MeOH.E}$ =214.06±4.71 mg GAE/g DE, $RC^{Aq.E}$=224.18±6.28 mg GAE/g DE), of flavonoids ($RC^{MeOH.E}$=81.16±4.42 mg QE/g DE, $RC^{Aq.E}$ =59.83±1.96 mg QE/g DE) and tannins ($ZL^{Aq.E}$ =113.87±0.79 mg CE/g DE, $RC^{Aq.E}$ =20.76±0 mg CE/g DE) (Table 3).

The aqueous extract of *Z. lotus* showed a PPT content of 222.85±5.996 mg GAE/g DE, while the concentration in the MeOH.E extract was 268.65±7 mg GAE/g DE (Table 3).

The methanolic extract of *R. chalepensis* represented TPC, TFC and TTC of 214.06±0.053 mg GAE/g DE, 81.16±0.06 mg QE/g DE and 18.97±0.002 mg QE/g DE, respectively (Table 3).

Thin Layer Chromatography Analysis (TLC)

Results of the TLC analysis of the different PPE, as well as the standard phenols used: gallic acid, quercetin, catechin, rutin and vanillin are cited in Figure 1 and Table 4. The appearance of numerous spots after molecules' migration on the different TLC plates enabled us to conclude that both plant PPE are richer in various biological substances. Each spot is characterized by its frontal ratio and its color under UV light, at a wavelength of 254 and 366 nm (Figure 1).

Each spot gave a specific color or fluorescence under UV light, which indicates the separated chemical substance identity. Thirteen substances were migrated along the TLC plate of $ZL^{MeOH.E}$, while 8 substances were detected in $ZL^{Aq.E}$ (Table 4). However, for *R. chalepensis*, the large numbers of molecules were detected in the aqueous extract, of which 14 spots were observed in the TLC plate, while 10 molecule spots were detected for $RC^{MeOH.E}$ (Table 4; Figure 1).

High Performance Liquid Chromatography (HPLC-DAD)

The results of chromatogram profiles and phenolic compounds concentrations are shown in Figure 3 and Table 5. Various phe-

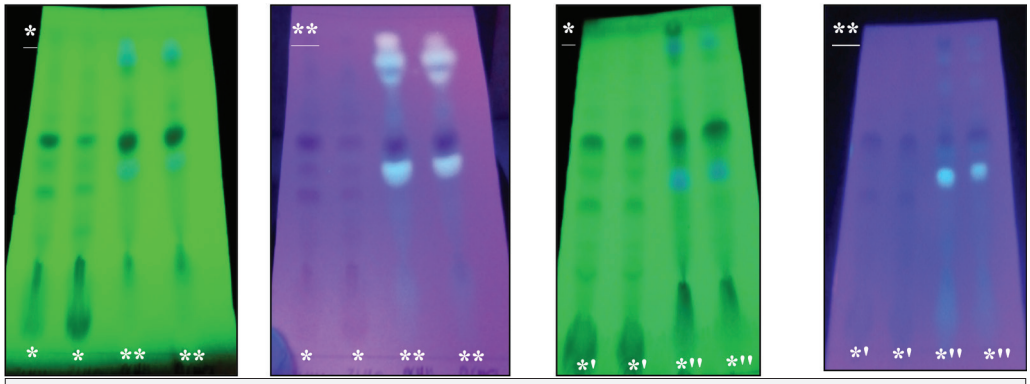

Figure 1. TLC profiles after UV revelation of the methanolic and aqueous extracts of *Z. lotus* and *R. chalepensis* harvested from Tafraoui region, in Oran (North-west Algeria). (*) 254 nm, (**) 366 nm, *: $ZL^{MeOH.E}$, **: $RC^{MeOH.E}$, *': $ZL^{Aq.E}$, *'': $RC^{Aq.E}$.

Table 4. Frontal ratios (F_R) of polyphenol spots obtained by TLC.

F_R of substances migrating along the TLC plates		
Solvent system used		**Eluent E1: butanol/acetic acid/water (60:15:35 ; v/v/v)**
Control substance	Gallic acid	0.84*
	Catechin	0.91
	Quercetin	0.94*
	Rutin	0.52*
	Vanillin	0.92
PPE	$ZL^{MeOH.E}$	0.07; 0.19; 0.21; 0.33; 0.39; 0.46; 0.52*; 0.57; 0.61; 0.68; 0.72; 0.77; 0.84*
	$ZL^{Aq.E}$	0.08; 0.23; 0.29; 0.39; 0.48; 0.57; 0.67; 0.84*
	$RC^{MeOH.E}$	0.21; 0.26; 0.42; 0.46; 0.52*; 0.59; 0.66; 0.71; 0.76; 0.84*
	$RC^{Aq.E}$	0.17; 0.26; 0.36; 0.45; 0.52*; 0.58; 0.62; 0.66; 0.69; 0.74; 0.78; 0.84*; 0.89; 0.94*

*F_R: Frontal Ratio.

Table 5. Phenolic compounds (µg/g DE) identified in the methanolic and aqueous extracts of *Z. lotus* leaves and *R. chalepensis* aerial parts collected from Oran-Tafraoui region, in western Algeria.

Plant Extracts	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**
$R_{t\ (min)}$	5.400	12.430	15.745	18.336	18.917	19.165	21.250	26.385	31.265	33.416	38.571	54.719	59.326	68.506	71.045
$ZL^{MeOH.E}$	3.54	3.90	2.01	NI	NI	NI	NI	NI	NI	NI	431.34	NI	NI	0.90	2.32
$ZL^{Aq.E}$	0.88	NI	NI	NI	NI	NI	NI	NI	NI	NI	50.56	NI	NI	NI	NI
$RC^{MeOH.E}$	1.39	78.38	35.38	6.18	4.29	55.47	1.06	3.76	NI	3.76	NI	NI	NI	NI	129.54
$RC^{Aq.E}$	7.15	24.42	0.98	0.61	NI	NI	1.57	NI	NI	NI	NI	NI	NI	NI	3.39

A: Gallic acid, B: Catechin, C: Chlorogenic acid, D: Caffeic acid, E: Hydroxybenzoic acid, F: Epicatechin, G: Syringic acid, H: Coumaric acid, I: Trans-ferrulic acid, J: Sinapic acid, K: Benzoic acid, L: Hesperidin, M: Rosmarinic acid, N: Cinnamic acid, O: Quercetin, R_t: Retention time in minutes (min), NI: Not identified.

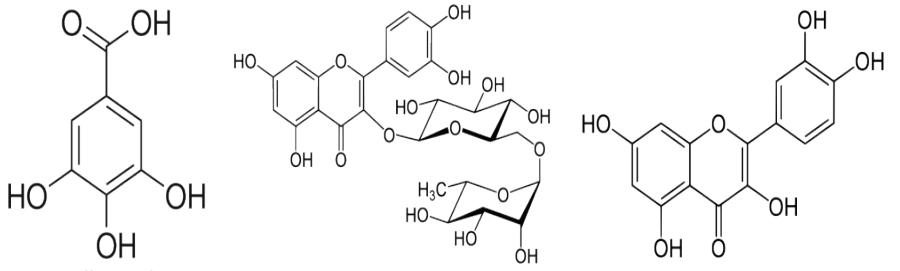

Figure 2. Chemical structure of bioactive compounds containing *Z. lotus* and *R. chalepensis* extracts.

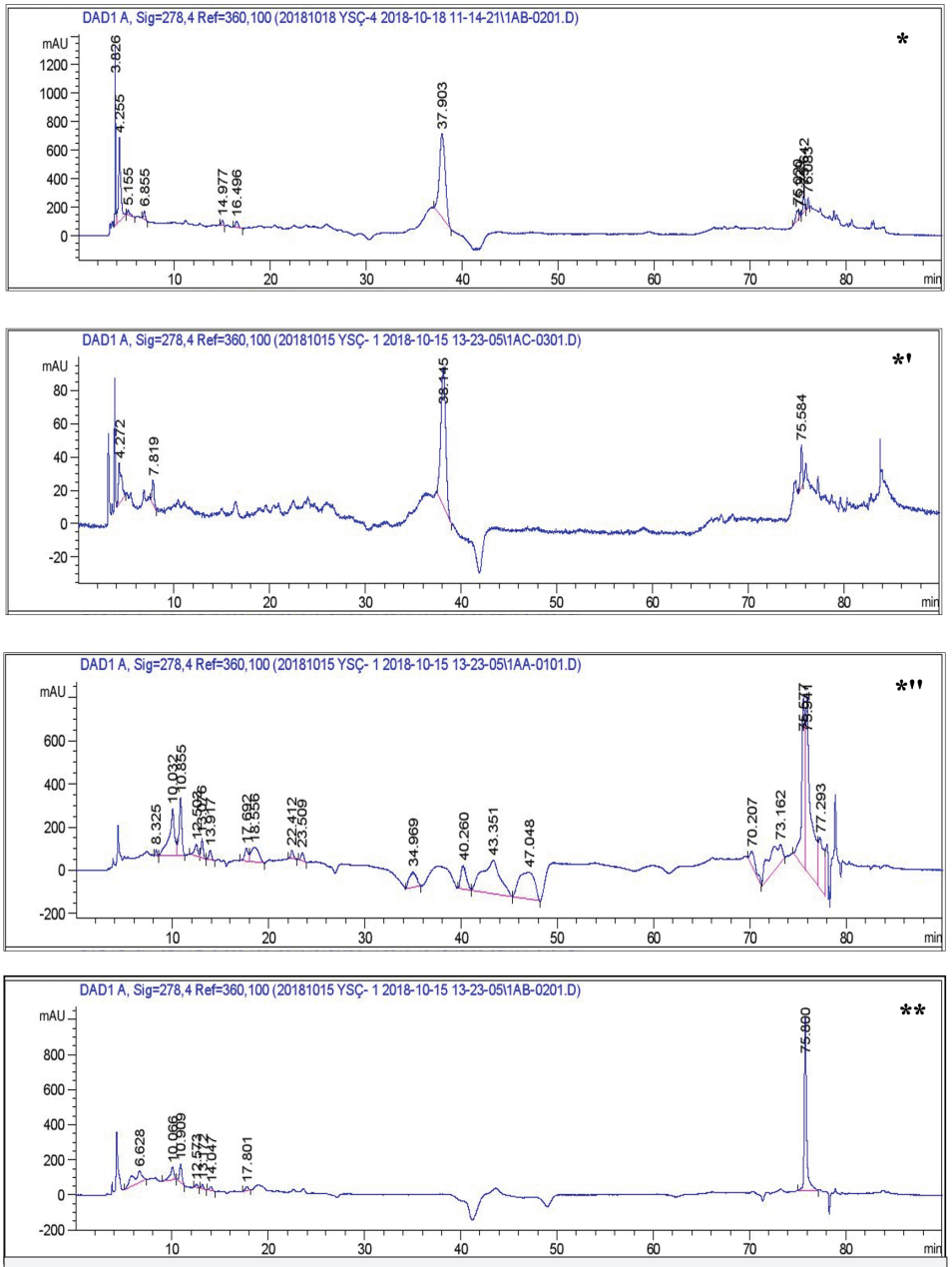

Figure 3. HPLC-DAD phenolic profiles of *Z. lotus* and *R. chalepensis* phenolic extracts detected at 278 nm. *: $ZL^{MeOH.E}$, *': $ZL^{Aq.E}$, *'': $RC^{MeOH.E}$, **: $RC^{Aq.E}$.

nolic components were identified and quantified in *Z. lotus* and *R. chalepensis* extracts using the HPLC-DAD analysis, including phenolic acids and flavonoids.

The HPLC results of *Z. lotus* polyphenolic extracts showed the presence of benzoic acid as a major phenolic component in methanolic and aqueous extracts. The highest concentration was quantified in the hydromethanolic extract of the plant (431.34 µg/g DE), while the lower content of this compound was determined in the aqueous extract (50.56 µg/g DE) (Table 5; Figure 3).

Other phenolic acids: Chlorogenic acid, gallic acid, cinnamic acid and flavonoids: Catechin and quercetin were also quantified in $ZL^{MeOH.E}$ and $ZL^{Aq.E}$ extracts with lower concentrations. Quercetin, catechin and chlorogenic acid were quantified only in the methanolic extract of *Z. lotus*.

Furthermore, ten phenolic compounds were identified in $RC^{MeOH.E}$. These bioactive components included seven phenolic acids (Chlorogenic acid, gallic acid, caffeic acid, hydroxybenzoic acid, syringic acid, coumaric acid and sinapic acid) and three flavonoids (Catechin, epicatechin and quercetin). Quercetin was qualified as the major phenolic compound in the hydromethanolic extract (129.54 µg/g), followed by catechin (78.38 µg/g DE) and chlorogenic acid (35.38 µg/g DE). For $RC^{Aq.E}$, catechin was identified as a major component, with a concentration of 24.42 µg/g DE, followed by gallic acid (7.15 µg/g DE) and quercetin (3.39 µg/g DE) (Table 5; Figure 3). The chemical structure of the main phenolic compounds identified and quantified in *Z. lotus* and *R. chalepensis* extracts is given in Figure 4.

Antimicrobial Activity

The results of the antimicrobial tests are shown in Tables 6 and 7 and in Figures 5-8. A potent antimicrobial effect was recorded on all bacterial strains using *R. chalepensis* and *Z. lotus* extracts, as well as significant antifungal activity on *C. albicans*, with diameters of the microbial growth inhibition zones exceeding 10 mm. The largest inhibition diameters were recorded using $ZL^{MeOH.E}$ (19.13±0.23 mm) on *C. albicans* (Table 6).

The MeOH.E and Aq.E of both plants were effective against different MDR Gram-positive and Gram-negative bacteria, as well as against *C. albicans*. In contrast, *S. pyogenes*, *E. faecalis*, *P. mirabilis*, *S. enterica* sp *arizonae* and *H. alvei* were the most sensitive isolates to the extracts of *Z. lotus* and *R. chalepensis*,

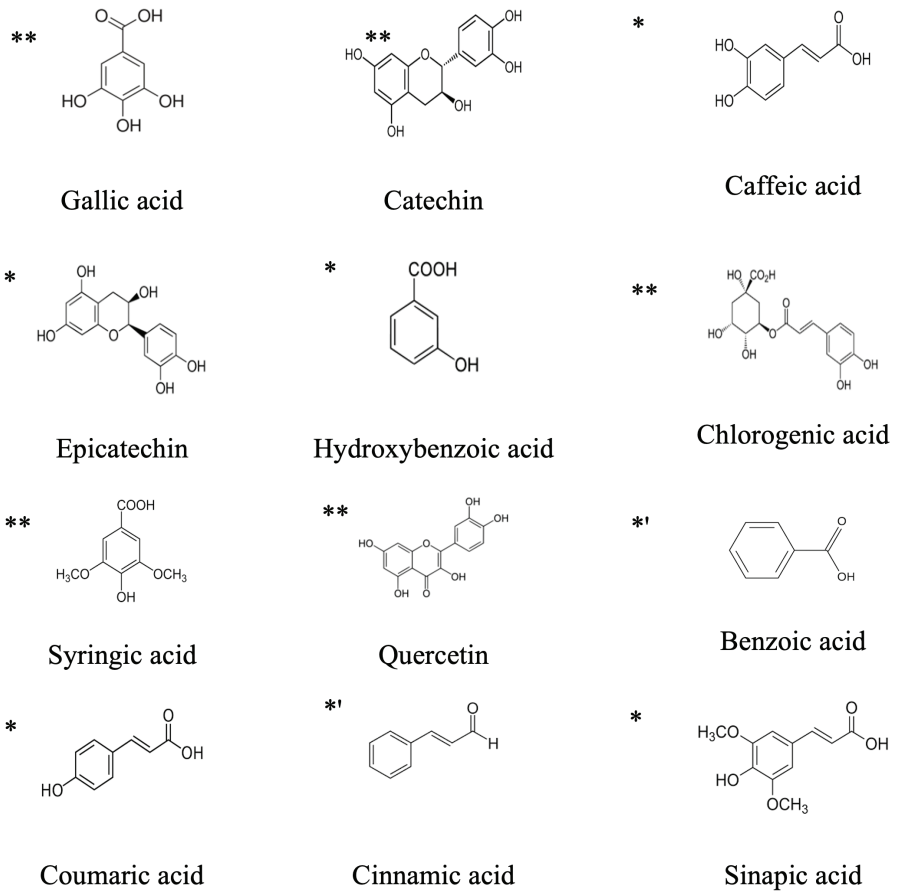

Figure 4. Main phenolic compounds containing *Z. lotus* and *R. chalepensis* extracts. **: Compounds quantified in both plant extracts, *: Compounds quantified only in *R. chalepensis* extracts, *': compounds quantified only in *Z. lotus* extracts.

Table 6. Antimicrobial activity of phenolic extracts of *Z. lotus* and *R. chalepensis* harvested in Tafraoui region-Oran (western Algeria), against pathogenic clinical germs.

Clinical isolates	Diameters of growth inhibition zones (ø mm)			
	$ZL^{MeOH.E}$	$ZL^{Aq.E}$	$RC^{MeOH.E}$	$RC^{Aq.E}$
S_1	11.13 ±0.23^{S*}	11.03 ±0.06^{S*}	10.13 ±0.15^{S*}	8.97 ±0.06^{S*}
S_2	24.03 ±0.06^{EHS*}	24.03 ±0.06^{EHS*}	NE	NE
S_3	24.06 ±0.12^{EHS*}	21.03 ±0.06^{EHS*}	15.13 ±0.23^{HS*}	NE
S_4	16.03 ±0.06^{HS*}	NE	9.03 ±0.06^{S*}	11.07 ±0.12^{S*}
S_5	22.06 ±0.12^{EHS*}	18.03 ±0.06^{HS*}	12.06 ±0.12^{S*}	10.1 ±0.17^{S*}
S_6	35.03 ±0.06^{EHS*}	25.06 ±0.12^{EHS*}	20 ±0.1^{EHS*}	NE
S_7	34.06 ±0.12^{EHS*}	33.03 ±0.06^{EHS*}	NE	NE
S_8	19.13 ±0.23^{HS*}	15 ±0^{HS*}	12.03 ±0.06^{S*}	9.16 ±0.2^{S*}

ø (mm): Diameters of growth inhibition zone in millimeter, S_1: *S. aureus*, S_2: *S. pyogenes*, S_3: *E. faecalis*, S_4: *E. coli* (EPEC), S_5: *P. mirabilis*, S_6: *S. enterica* sp *arizonae*, S_7: *H. alvei*, S_8: *C. albicans*, NE: No effect, R: Resistance (Ø < 8mm), S: Sensitivity (9 mm< Ø < 14 mm), HS: High susceptibility (15 mm< Ø <19 mm), EHS: Extremely high susceptibility (Ø >20 mm). The values are presented as the mean of three replicates±the standard deviation. p<0.05: Significant*.

Table 7. Quantitative analysis of the antimicrobial parameter: The minimum inhibitory and bactericidal concentrations (MICs, MBCs) against the clinical microbial isolates.

Clinical strains	MIC; MBC; MBC/MIC (mg/mL)			
	$ZL^{MeOH.E}$	$ZL^{Aq.E}$	$RC^{MeOH.E}$	$RC^{Aq.E}$
S_1	100; 200; 2	100; 100; 1	50; 100; 2	200; 200; 1
S_2	100; 200; 2	50; 200; 4	100; 200; 2	100; 200; 2
S_3	100; 200; 2	50; 100; 2	50; 100; 2	100; 200; 2
S_4	100; 200; 2	100; 200; 2	100; 200; 2	200; 200; 1
S_5	100; 100; 1	200; 200; 1	100; 200; 2	200; 200; 1
S_6	100; 100; 1	50; 200; 4	50; 100; 2	100; 100; 1
S_7	100; 100; 1	25; 100; 4	100; 200; 2	100; 200; 2
S_8	100; 100	50; 100; 2	50; 100; 2	50; 100; 2

with inhibition diameters of 24.03±0.06 mm against *S. pyogenes*, 24.06±0.12 mm and 21.03±0.06 mm against *E. faecalis*, 22.06±0.12 mm, 18.03±0.06 mm, and 24.06±0.12 mm against *P. mirabilis*, 34.06±0.12 mm and 33.03±0.06 mm against *H. alvei* using $ZL^{MeOH.E}$ and $ZL^{Aq.E}$ extracts, respectively.

The diameters of 35.03±0.06 mm, 25.06±0.12 mm and 20±0.1 mm against *S. enterica* sp. *arizonae* were determined using $ZL^{MeOH.E}$, $ZL^{Aq.E}$ and $RC^{MeOH.E}$, respectively (Table 6). However, the extracts $ZL^{Aq.E}$, $RC^{MeOH.E}$ and $RC^{Aq.E}$ did not exert antibacterial effect on *E. coli* and *S. pyogenes*, respectively (Table 6).

All these qualitative results were completed by the quantitative determination of the antimicrobial effect by determining the most important antimicrobial parameter, the minimum inhibitory concentration (MIC) for all the clinical microbial strains tested during this study. The results of the MIC values are shown in Table 7. Thus, an important decrease in microbial cells concentration was detected after the first 4 hours of direct contact between the plant extracts and the microbial strains, expressed by the decrease in log germs.mL^{-1} number as a function of microbial kill kinetics time (Figure 5-8).

The inhibitory properties of the MeOH.E and the Aq.E extracts of both plants on the different microbial strains were determined with the lowest MIC values of 25 mg/mL against *E. faecalis*, *H. alvei* and *C. albicans* using *Z. lotus* aqueous extracts (Table 7).

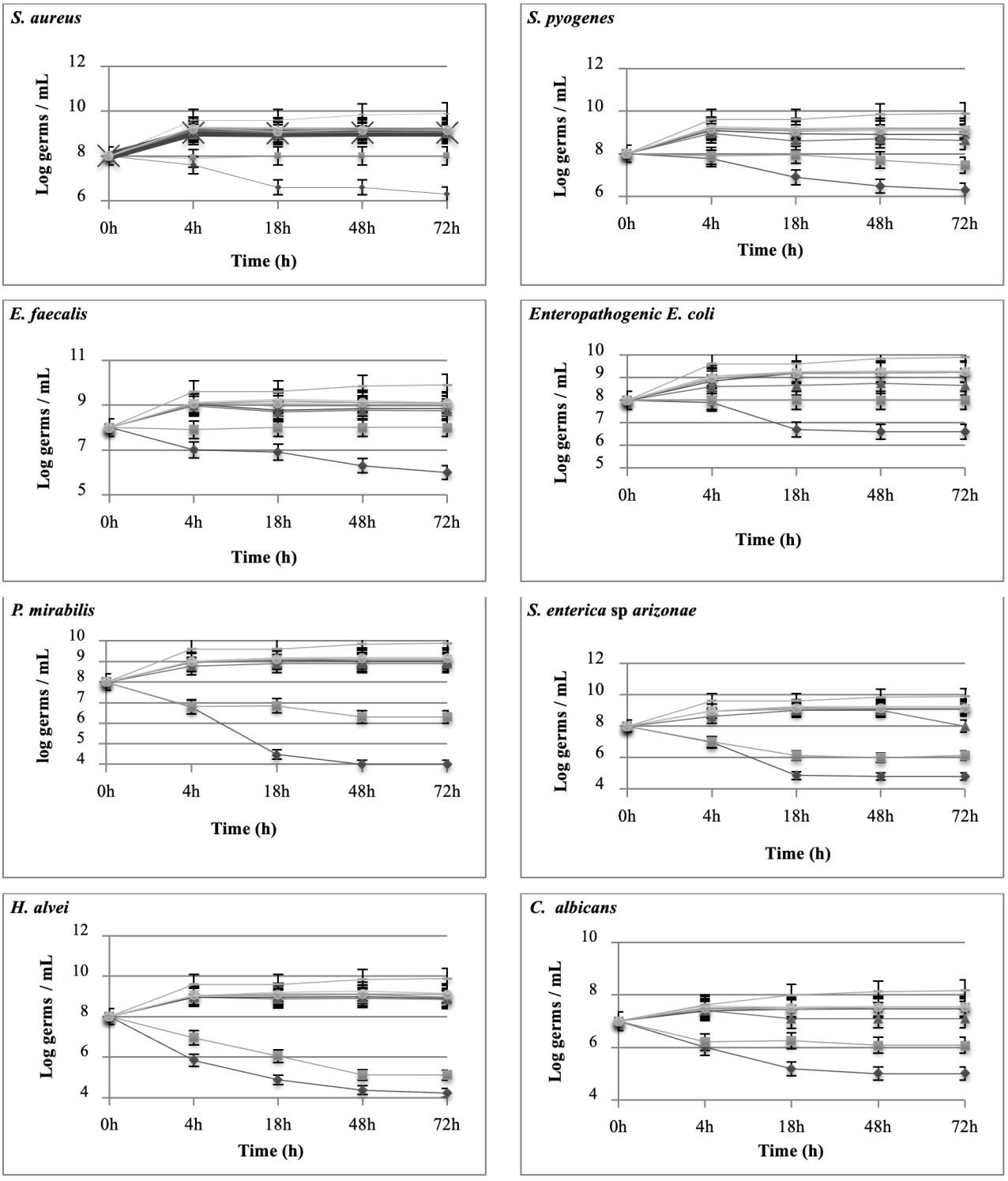

Figure 5. Microbial-kill kinetics of *Zizyphus lotus* hydromethanolic extract ($p < 0.05$). T: Control test. C1: 200 mg/mL, C2: 100mg/mL, C3: 50 mg/mL, C4: 25 mg/mL, C5: 12.5 mg/mL, C6: 6.25 mg/mL, C7: 3.13 mg/mL, C8: 1.56 mg/mL.

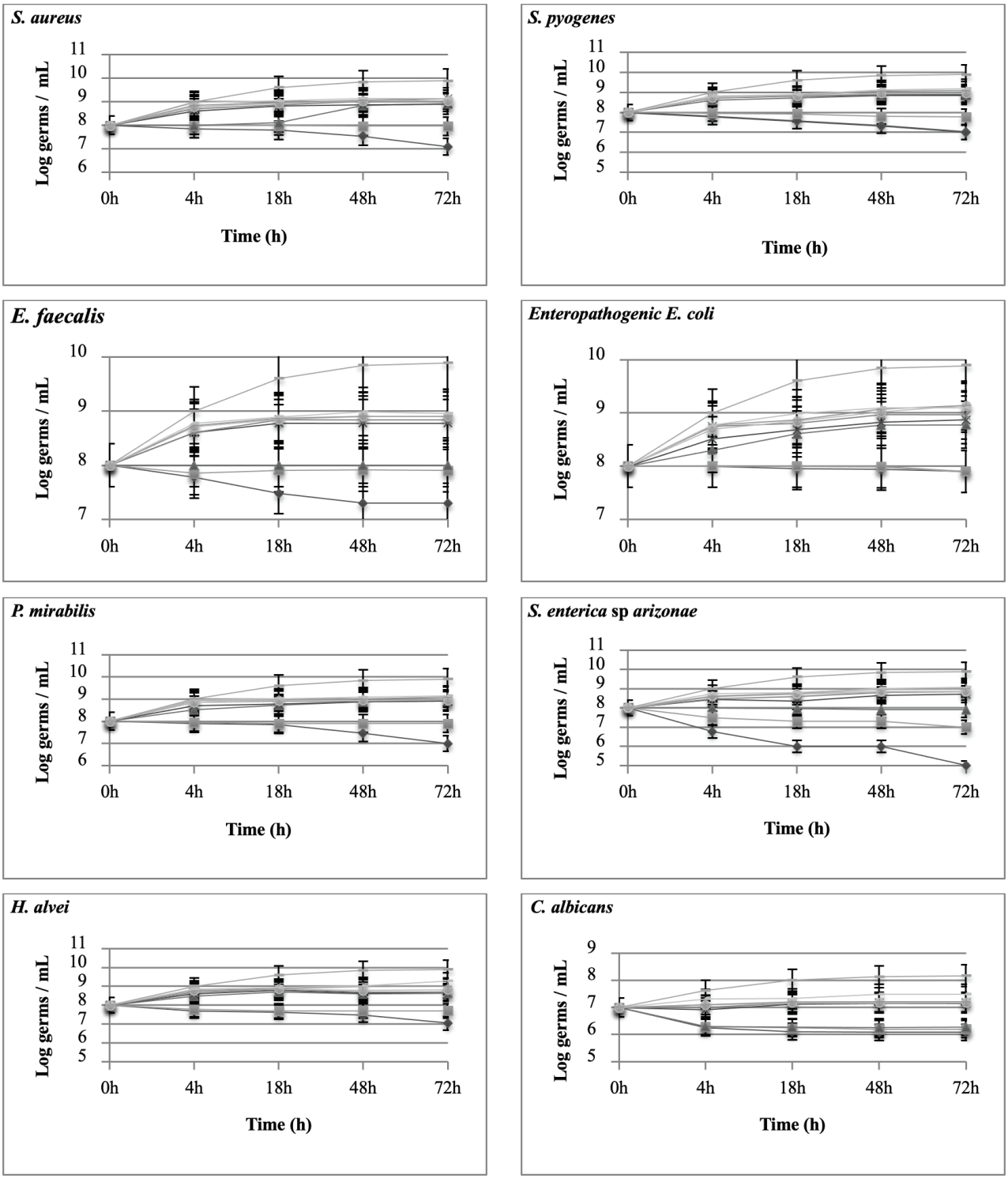

Figure 6. Microbial-kill kinetics of *Zizyphus lotus* aqueous extract ($p < 0.05$). T: Control test. C1: 200 mg/mL, C2: 100mg/mL, C3: 50 mg/mL, C4: 25 mg/mL, C5: 12.5 mg/mL, C6: 6.25 mg/mL, C7: 3.13 mg/mL, C8: 1.56 mg/mL.

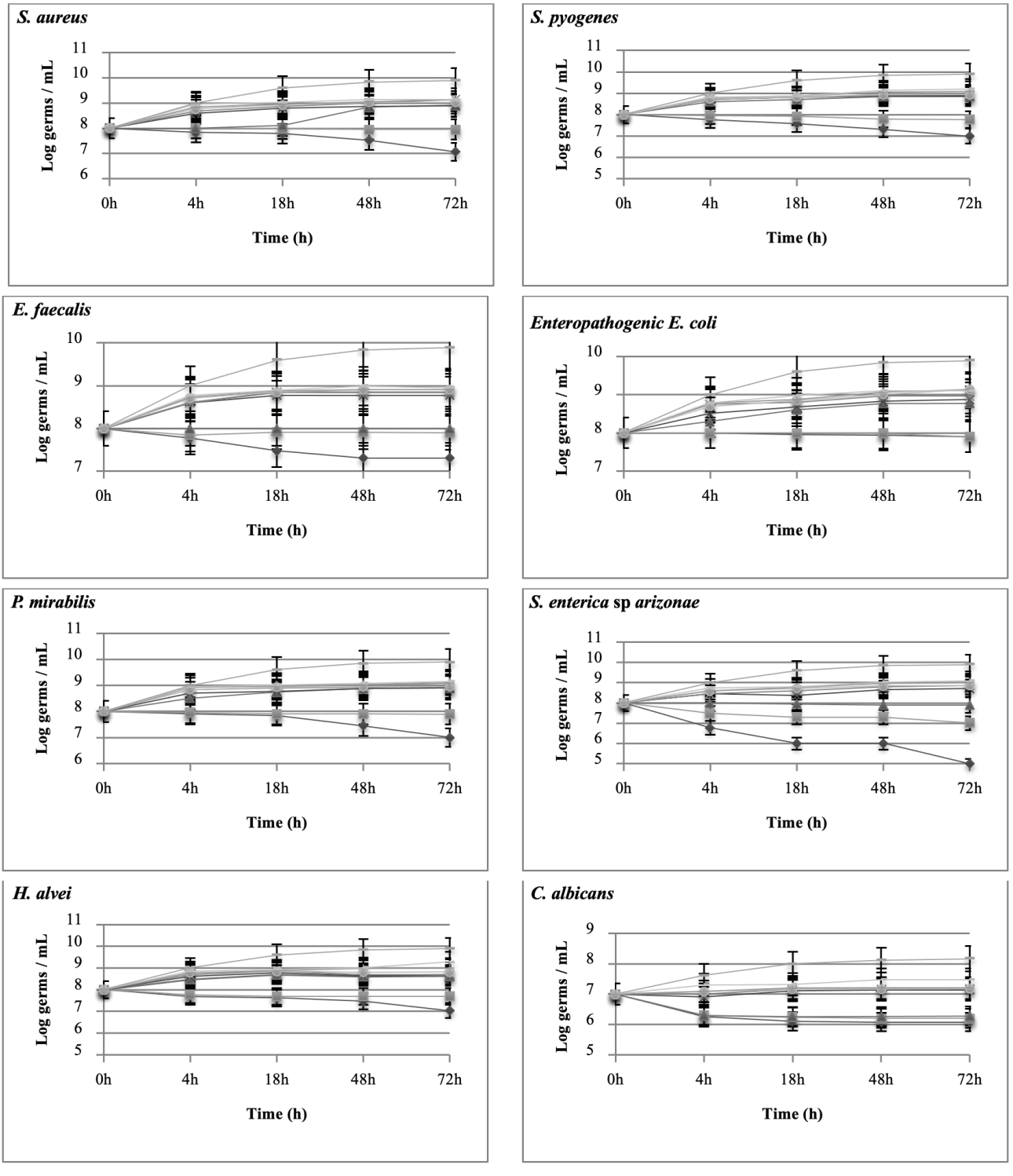

Figure 7. Microbial-kill kinetics of *R. chalepensis* hydromethanolic extract ($p < 0.05$). T: Control test. C1: 200 mg/mL, C2: 100mg/mL, C3: 50 mg/mL, C4: 25 mg/mL, C5: 12.5 mg/mL, C6: 6.25 mg/mL, C7: 3.13 mg/mL, C8: 1.56 mg/mL.

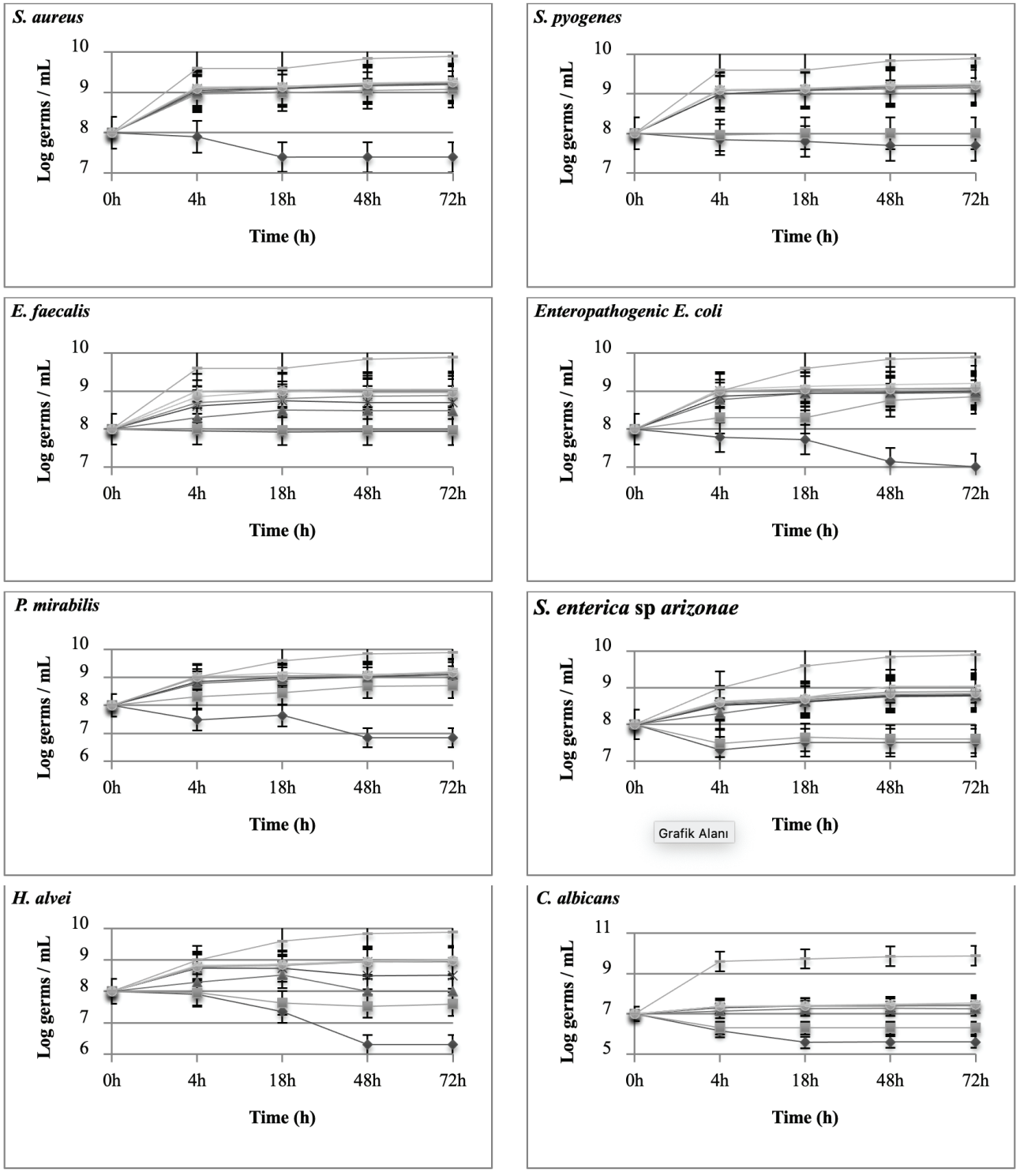

Figure 8. Microbial-kill kinetics of *R. chalepensis* aqueous extract (p <0.05). T: Control test. C1: 200 mg/mL, C2: 100mg/mL, C3: 50 mg/mL, C4: 25 mg/mL, C5: 12.5 mg/mL, C6: 6.25 mg/mL, C7: 3.13 mg/mL, C8: 1.56 mg/mL.

Table 8. FIC_{index} of the different combinations between *Z. lotus* and *R. chalepensis* phenolic extracts on the different microbial strains tested.

Clinical strains	$ZL^{MeOH.E}/RC^{MeOH.E}$			$ZL^{Aq.E}/RC^{Aq.E}$		
	MIC_{Cb}	FIC_{Id}	FIC_{index}	MIC_{Cb}	FIC_{Id}	FIC_{index}
S. aureus	200/200	2/4	6[a]	200/200	2/1	3[i]
S. pyogenes	200/200	2/2	4[i]	200/200	4/2	6[a]
E. faecalis	200/200	2/4	6[a]	200/200	4/2	6[a]
E. coli	200/200	2/2	4[i]	200/200	2/1	3[i]
P. mirabilis	200/200	2/2	4[i]	200/200	1/1	2[i]
S. enterica	200/200	2/4	6[a]	200/200	4/2	6[a]
H. alvei	100/100	1/1	2[i]	100/100	2/1	3[i]
C. albicans	200/200	2/4	6[a]	200/200	4/4	8[a]

a: antagonism, i: indifference, Cb: combination, Id: individual.

Also, the results of MBC/MIC reports allowed us to qualify both plants' extracts as bactericidal and fungicidal.

Checkerboard Test: Determination of the Fractional Inhibitory Concentrations (FIC test)

The checkerboard titration method was used to determine the FIC index of both plants' PPEs against MDR pathogenic bacteria and yeast. The results of the checkerboard assays are shown in Table 8. FICI values of the combination of *R. chalepensis* and *Z. lotus* extracts were greater than 0.5 (Table 8).

Therefore, the results demonstrated that *Z. lotus* extracts in combination with *R. chalepensis* showed no synergistic interaction. These combinations exerted antagonistic interactions on the microbial strains tested, with FIC index values greater than 4 (FIC_{index} >4). However, indifference interactions were recorded on *S. pyogenes*, *E. coli*, *P. mirabilis* and *H. alvei* by applying $ZL^{MeOH.E}$ and $RC^{MeOH.E}$ combination, and against *S. aureus*, *E. coli*, *P. mirabilis* and *H. alvei* using the combination of $ZL^{Aq.E}$ and $RC^{Aq.E}$, with an inhibitory fractional concentration index greater than 2 (FIC_{index} >2) (Table 8).

DISCUSSION

A total of 41 clinical strains were isolated during this study from the different biological samples (Table 1). The performance of antibiotics susceptibility testing is important to assure and confirm susceptibility to chosen empirical antimicrobial agents for particular infections, or to detect resistance in clinical microbial isolates. Results enabled us to entitle the eight selected microbial strains as multidrug-resistant pathogens, which carries in its genetic material several antimicrobial resistance genes.

According to Cheurfa et al. (56), the aqueous extract yield of *Z. lotus* roots is about 9.49% and 7.91% for the methanolic extract, which is incongruent with the results of our study, in which very interesting yields using the leaves of the plant were obtained. Thus, the study carried out by Zoughlache (57) showed that the extraction yields of the methanol extract and the aqueous extract of *Z. lotus* fruits were about 6.4% and 40.4%, respectively. This indicates that the polyphenolic extract content differs according to the plant part used. We found that the methanolic extract of *Z. lotus* leaves record higher yields compared to other parts of the plant, as shown by various studies.

The results obtained during this study are more interesting, because we obtained higher amounts of PPEs than those obtained by Mansour El-Said et al. (58), who obtained a lower yield of *Z. lotus* crude extracts (3.75%), and which may be explained by the use of high temperatures applying the Soxhlet extraction method. Thus, the content of polyphenolic compounds in *Z. lotus* was much higher compared to the yield obtained by Loizzo et al. (59), who found that the ultrasound extraction technique gives a content of 4.84% leaf methanolic extract. These results confirm that the technique used also influences the extraction yield of polyphenolic compounds.

The crude extract content differs from one specific plant part to another, which is confirmed by Attou (60), who recorded higher yields in *R. chalepensis* flowers harvested from Ain-Temouchent (32.15%), followed by leaves and stems, with a richness of the plant in methanol-soluble substances. So, during this study, in which we used the aerial part of the plant, we also obtained the highest yields of methanolic and aqueous extracts ($RC^{MeOH.E}$ =14.73%, $RC^{Aq.E}$ =30.83%) (Table 3).

These results allowed us to conclude that the harvest area has an immense influence on the extraction yields and chemical composition on bioactive compounds. Each harvest region is characterized by its climatic conditions (rain and outside temperature), which have an influence on the physical qualities of

medicinal plants (61). Other ecological factors can intervene in the development of the plant species: the altitude, the harvest period, the plant part harvested (leaves, flowers or stems, roots or aerial parts), the bioactive substances extraction technique, as well as the extraction time period, which influences not only the yield, but also the plant extract composition (62-64).

With regard to the obtained results of the phytochemical screening of the various PPEs, the presence of polyphenols, flavonoids, catechic tannins, coumarins, terpenes and saponins in the *R. chalepensis* aerial part was evident, which is in accordance with the study results demonstrated by Khadri et al. (65), while the results obtained by Alotaibi et al. (66) indicate that saponins were absent in the *R. chalepensis* extracts. Thus, the phytochemical tests performed by $ZL^{MeOH.E}$ and $ZL^{Aq.E}$ revealed the abundant presence of flavonoids: the orange color appearing in the extract solutions designates the presence of flavonoids of the flavone type, and the presence of catechin tannins.

All these results were in accordance with different recent studies by Borgi et al. (67), Chetibi and Diab (68) and Chelli et al. (69), who determined the presence of flavonoid and tannin components with high intensity in the methanolic and aqueous extracts prepared from different parts of *Z. lotus*.

In addition, the absence of gallic tannins, anthocyanins and iridoids (monoterpenes) was recorded in the leaves of *Z. lotus* and the aerial part of *R. chalepensis*. Thus, an absence of coumarins was observed in the *Z. lotus* leaves, which conforms to the results obtained by Saiah et al. (70). Moreover, our results are not in agreement with those of Belkadi and Hadj-Ali (71), who demonstrated an abundant richness of anthocyanins in *Z. lotus* leaves and fruits harvested from different regions of southern Algeria (Laghouat and Ghardaïa).

Phytochemical screening showed that *Z. lotus* contains tannins, terpenes and saponins as the main bioactive chemical compounds. These results are in agreement with those obtained by recent studies (72). Tannins are among the bioactive compounds of great interest in the medical field due to their potent anti-ulcer and gastroprotective properties (73). According to Ghazghazi et al. (74) and Borgi et al. (75), the *Z. lotus* leaves are rich in flavonoids, tannins and saponins of the dammarane type: jujuboside B, jujubogenin glycoside, as well as an important source of polyphenols, which is confirmed by the results of our study.

According to Gonzalez Trujano et al. (76), *R. chalepensis* is known for its greater richness in flavonoids, coumarins (chalepensin), phenols, tannins and saponins. This abundance of bioactive compounds allows these medicinal plants to have various pharmacological properties, which could justify their multiple indications or therapeutic uses in traditional medicine, including anti-inflammatory, analgesic, anti-ulcerogenic, antidiabetic, antioxidant and antimicrobial properties, and in treatment of intestinal disorders (77-79).

For both plants, it was noticed that the MeOH.E extracts contain higher concentrations of polyphenols, tannins and flavonoids compared to the aqueous extracts. The present study results revealed that *Z. lotus* is richer in polyphenolic compounds compared to *R. chalepensis*. This may be due to the plants' natures, each of which is characterized by its own composition and content of bioactive compounds. *R. chalepensis* harvested in Mascara is richer in polyphenol, with high levels compared with the plant harvested in Oran, which indicate that the harvest region has a great influence on the composition in secondary metabolite content of medicinal plants.

According to Neffati et al. (80), *Z. lotus* expressed significant levels on TPC, about 211 and 201.66 mg. Thus, Abdoul Azize et al. (81) reported significant amounts of polyphenols in *Z. lotus* extracts. According to Rsaissi et al. (82), *Z. lotus* fruits harvested from the El Brouj-Chaouia region in Morocco contain approximately 82.62 mg/kg, 46.21 mg/kg and 336.24 mg/kg, respectively of TPC, TFC and TTC. Comparing this with our results, we can conclude that *Z. lotus* leaves harvested in western Algeria are richer in polyphenols than other plant parts from other harvesting regions.

Saiah et al. (83) determined phenolic content ranging from 207.52±1.92 mg GAE/g DE and 21.91±0.31 mg QE/g DE on polyphenols and flavonoids, respectively, in *Z .lotus* harvested in Chlef. In our study, we determined higher content from the extracts (268.65±7 mg GAE/g DE and 109.45±2.87 mg EQ/g DE in $ZL^{MeOH.E}$). This difference in phenolic compound content may be related not only to the variety, but also to the influences of the extraction methods and conditions, maturity stage of the plant and the harvest region, biogenetic and environmental factors, the reagents used in the quantitative determination of polyphenol contents and spectrophotometer type used (84,85).

The hydromethanolic and aqueous extracts of *R. chalepensis* aerial part showed a TPC with values (Table 3) exceeding those demonstrated by Khadri et al. (86). They have determined a TPC in the ethanolic extract of 2.73±0.5 mg/g, and 3.90±0.3 mg/g in the Aq.E of *R. chalepensis* harvested from northern Tunisia. Therefore, as mentioned by Loizzo et al. (87), the hydromethanolic extract of *Ruta* harvested from Italy exhibited TPC, TFC and TTC contents with concentrations of 6.22 mg GAE/g DE, 6.59 mg QE /g DE and 0.72 mg epigallocatechin gallate equivalents/g DE, respectively. Comparing this with our results, we concluded that the Algerian medicinal plants we tested contain more bioactive substances than those collected from other harvest regions of other countries.

The literature analysis revealed a TPC in *R. chalepensis* ethanolic and Aq.E extracts of 54.13 and 51.28 mg/g, respectively (88). Kacem et al. (89,90) showed that *R. chalepensis* phenolic extracts are richer in total phenols, around 178 mg/g in the ethanol extracts and 152.09 mg/g in the Me.E extracts of the plant, with rutin as the main abundant flavonoid. Thus, Gali and Bedjou (91) determined the polyphenol and flavonoid contents of 12.2±0.84 µg AEG/mg DE and 3.43±0.13 µg QE/mg DE, respectively, in *R. chalepensis* aqueous extract. In addition, it has been

shown that the polyphenol content is much higher in both plants' hydromethanolic and aqueous extracts, which indicates that the polyphenolic extracts may be alternative plant-based drugs representing a rich and a potent reservoir of bioactive molecules. The highest content of polyphenols, flavonoids and tannins quantified in the different prepared extracts indicated that these plants were harvested during the optimal development stage and the harvest period when secondary metabolites production is much greater.

Comparing the TLC profiles of both plants' phenolic extracts, it can be observed that *Z. lotus* and *R. chalepensis* are richer in chemical substances based on the spot numbers on silica gel. The use of standard molecules in the phenolic compounds identification allowed us to determine the presence of gallic acid, which is a phenolic acid, in both plants' methanolic and aqueous extracts. For $ZL^{MeOH.E}$, the frontal ratios measurement for each of the spots revealed the presence of gallic acid with an F_R=0.84 and rutin: F_R=0.52 (Table 4; Figure 2). This is in agreement with the results obtained by Zoughlache (92) and Tlili et al. (93), who showed the presence of polyphenols and rutin in *Z. lotus* methanolic extract.

In addition, we detected the presence of coumarins and phenols in *R. chalepensis* extracts. According to Wagner and Bladt (94), any fluorescence detected at 366 nm on the TLC plates expresses the presence of hydroxycoumarines. Thus, the presence of quercetin was detected in $RC^{Aq.E}$: R_F=0.94, whereas it was absent in the methanolic extract (Table 4).

The different TLC plates visualized under UV at 254 nm presented colored spots including purple, brown, blue, red and green, which may correspond to several secondary metabolite classes. According to Markham (95), a black-violet color spot indicates the flavones' and flavonols' presence, a blue color spot indicates flavone or flavanone, a yellowish color indicates flavonol, an orange color indicates isoflavones, a yellow green color spot indicates aurones, a green color spot indicates chalcones and a blue green color spot reveals the presence of flavanone.

Based on the literature, we can conclude that *Z. lotus* extracts are rich in steroids, triterpenes, flavones, flavanones and chalcones. The absence of blue color spots in the chromatographic profile of *Z. lotus* extracts indicates the absence of coumarins, which is consistent with the qualitative analysis results of *Z. lotus* extracts obtained during this study. Thus, *R. chalepensis* extracts are rich in flavones, flavanones, steroids, chalcones and coumarins. These results are in agreement with those of Fasla (96), who showed the presence of flavonoids in *R. chalepensis* extracts.

The HPLC results revealed that *R. chalepensis* extracts exhibited higher flavonoid contents than *Z. lotus*. According to Bekkar et al. (97), the methanolic and aqueous extracts of *Z. lotus* harvested in Mascara-western Algeria had higher polyphenolic composition when compared with the results of the present research, using the same medicinal plant collected from other regions, which confirms the influence of the harvest region and geographical area in the plants' bioactive substance composition. High phenolic compound contents from *Z. lotus* were determined by Cacciola et al. (98), as well, as in our study.

Thus, our results were congruent with those described in the study by Marmouzi et al. (99), who detected the presence of gallic acid, catechin and chlorogenic acid in *Z. lotus* aqueous extract.

Z. lotus used in this study is characterized by a very abundant richness in benzoic acid, many studies have mentioned the antimicrobial properties of this chemical substance (100, 101). According to Mkadmini Hammi et al. (102), *Z. lotus* fruits "*Nbeg*" collected in Southern Tunisia are rich in flavonoid compounds. Our results suggest that *Z. lotus* leaves and *R. chalepensis* leaves, small stems and flowers can confer potent sources of natural antimicrobials

The results of the antimicrobial activity indicated that the phenolic extracts of *Z. lotus* and *R. chalepensis* are more effective in inhibiting the growth of *C. albicans*, which may justify its uses in the treatment of candidiasis.

With reference to antibiotic susceptibility testing, the PPEs of both plants were more active on the clinical strains, however, the inhibitory potency of *Z. lotus* was higher than *R. chalepensis*.

Some MDR strains have been found to be highly sensitive to antibiotics compared to other strains, as mentioned in Table 6. *P. mirabilis*, *S. enterica* and *H. alvei* were determined to be the strains most sensitive to *Z. lotus* extracts, with potent growth inhibition expressed by very large diameters compared to the antibacterial potency of *R. chalepensis*.

The potent bactericidal and fungicidal effects of *Z. lotus* could be elucidated by the abundance of major phenolic components, particularly the benzoic acid known as a potent antimicrobial. In addition, the phenolic compounds identified in the phenolic extracts of *R. chalepensis* are appreciated as vigorous antibacterial and antifungal.

Our findings were more interesting than those obtained by Tukue and Karismala (103), who showed that the aqueous extract of *R. chalepensis* exerts an antibacterial effect with an inhibition growth diameter of 5 mm against *S. aureus*, while as a result of the antibacterial effect, the extracts used in this study gave diameters exceeding 10 mm with bactericidal and fungicidal effects (Table 7).

In comparison with the findings of Hamza and Meziani (104), the *Z. lotus* leaves used in our study exerted highly effective antimicrobial activity. Furthermore, the present results are found to be consistent with those of Chelli Chentouf et al. (105), who indicated that the Me.E extract of the plant harvested from the Mascara region in western Algeria exerts potent antibacterial effects on *S. aureus* and *E. coli* at a concentration of 200 mg/mL.

Our results are in agreement with those of Elaloui et al. (106) and Lahmer and Messai (107), who showed that the leaf ex-

tracts of *Z. lotus* exert an important antibacterial effect on *S. aureus* and *E. coli*.

In the present study, the most potent microbial growth inhibitory effect was obtained by *Z. lotus* when compared with *R. chalepensis*. This could be described by the abundance of secondary metabolites in *Z. lotus* leaf extracts, in particular phenols known for their antimicrobial activities (108). This activity was related to the high level not only of monoterpene hydrocarbons, but also of tannins that bind to bacterial cell walls (109,110).

Naili et al. (111) demonstrated that the Me.E extract of *Z. lotus* harvested from Libya was less effective against Gram-negative bacterial strains, while the results of our study indicated that the PPEs of this plant, harvested from Oran in western Algeria, were effective on both Gram-negative and Gram-positive strains. All these results justified the therapeutic applications of these medicinal plants as antimicrobials (112-115).

Previous studies have shown the effectiveness of plant extract combinations in the expression of a synergistic effect against pathogenic bacteria. Amirouche and Belkolai (116) demonstrated that a combination of sage and tea tree essential oils had a synergistic effect against *S. aureus*.

In addition, medicinal plants show their most important synergistic effect not only for antimicrobial activity but also on other biological activities. Ghali and Rafed (117) reported the synergistic effects of the aqueous extracts of *Allium ursinum* and *A. porrum* with an important anti-hemolytic activity. On the other hand, it was determined during this study that the effect of the combinations of both plants' phenolic extracts is lower compared to the effect exerted by each plant extract alone.

Therefore, *Z. lotus* and *R. chalepensis* extracts cannot be used in combination, due to the antagonism effect exerted on the different microbial strains tested, and therefore, the combination of these extracts can limit and reduce the bactericidal and fungicidal effect on MDR pathogens.

CONCLUSION

Research on natural bioactive substances extracted from medicinal plants is of particular interest, as it contributes to the national effort to conserve medicinal plants and to promote local traditional medicine. Our work was carried out to determine the phytochemical profiles and *in vitro* antimicrobial activity of *Z. lotus* and *R. chalepensis* collected from Oran in northwest Algeria. This study demonstrated that phenolic compounds extracted from the leaves of *Zizyphus lotus* and the leaves, small stems and flowers of *R. chalepensis* exhibited antibacterial activities on the MDR clinical isolates, as well an important antifungal effect against the MDR *C. albicans* strain. According to our study results, both plants' PPEs could be used in the medical field to tackle the emergence of antimicrobial resistance due to their potent antimicrobial activities on the MDR bacteria and fungi.

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