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Oral Acute Toxicity, Influence on the Gastrointestinal Microbiota and *In Vivo* Anti-salmonellosis Effect of *Zizyphus lotus* (L.) and *Ruta chalepensis* (L.) Essential Oils

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Abstract

Introduction: The aim of this study was to evaluate the chemical composition of *Zizyphus lotus* and *Ruta chalepensis* essential oils (EOs), the oral acute toxicity, influence on the gastrointestinal microbiota and the *in vivo* anti-salmonellosis effect.

Materials and Methods: The EOs were isolated using the steam distillation process, and bioactive components were identified by gas chromatography–mass spectrometry (GC-MS) analysis. Oral acute toxicity, influence on the gastrointestinal flora composition and the anti-salmonellosis effect were elucidated using *in vivo* methods on experimental animals.

Results: The GC-MS allowed us to identify 33 and 58 components in *Z. lotus* and *R. chalepensis*, respectively. Di-isooctyl phthalate (89.857%) was found to be the major compound identified in *Z. lotus*. The main compounds in *R. chalepensis* were 2-undecanone (26.528%) followed by 2-nonanone (13.404%). The LD_{50} of EOs was found to be greater than 5000 mg/kg. Also, no negative influence to intestinal microbiota was detected. An important decrease in *S. enterica* ssp *arizonae* cells achieving a bactericidal effect was recorded in rats treated with the EOs of both plants at a dose of 400 mg/kg. In parallel, an important significant ($P<0.05$) increase in lymphocytes number was observed for all tested animals. A decrease in alkaline phosphatase (ALP), amino alanine transferase (ALT) and aspartate aminotransferase (AST) levels was observed. Furthermore, a reduced blood erythrocyte sedimentation rate (ESR) was recorded in treated animals.

Conclusions: The *Z. lotus* and *R. chalepensis* act effectively as anti-salmonellosis agents, which support the use of these plants to cure gastrointestinal infections.

Keywords: *Zizyphus Lotus* (L.), *Ruta Chalepensis* (L.), Essential Oils, Acute Toxicity, Gastrointestinal Microbiota, Anti-salmonellosis

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Introduction

The emergence of multidrug resistant (MDR) bacteria has become a worldwide menace to public health safety. It has been determined since 1940 and up to 2016, when the first cases of *Enterobacteriaceae* carrying transferable resistance to colistin were reported. Wherefore, a great interest has been dedicated to the use of natural compounds as antibiotic alternatives.[1,2] *Salmonella enterica* ssp. *arizonae*, which is classified into non-typhoidal *Salmonella* (NTS) is an uncommon human pathogen with serious human infections. It represents a major health concern that affects antimicrobial treatment, because of its high resistance to various antibiotics.[3] This pathogen was the second leading cause of the foodborne disease. In Algeria, NTS represents one of the primary causes of salmonellosis. Some studies showed the detection of multiple *Salmonella* serovars contaminating a wide variety of raw meat and processed meat products.[4,5] A study by the Pasteur Institute of Algeria revealed that 11% of food poisoning cases were caused by *Salmonella* spp. in 2011.[6] Another study in the Algerian east, in Skikda, persistence of gastroenteritis infection within the poultry industry has prompted an investigation for NTS in this sector in 2013.[7] In cases of severe enteric disease to the multi-resistant *Salmonella* strains, the search for alternative and effective antimicrobials such as EOs is critical because of treatment failure with antibiotics.

Essential oils (EOs) have been widely investigated in recent years due to their antimicrobial properties against a wide spectrum of MDR bacteria. They have also been qualified as highly popular therapeutic treatments and have been used much more in traditional medicine and are of great scientific interest. These alternative products are known to contain an immense variety of bioactive components that may contribute

to various medical properties.[8]

The present study investigates the chemical composition on bioactive compounds of EOs extracted from two medicinal plants: *Zizyphus lotus* (*Rhamnaceae*) and *Ruta chalepensis* (*Rutaceae*) collected from Mascara, western Algeria and their antimicrobial effect *in vivo*.

Zizyphus lotus is abundant in Algeria and popularly famous as "*Sedra*" and the edible fruit is called "*Nbeg*". This plant is a very thorny fruit shrub, abundantly present in Mediterranean countries and widely found in Mascara (Western Algeria). The *Z. lotus* is used in nutrition, health and cosmetic sectors. Besides, this plant presents a delicious red fruit (jujube) that is consumed by the local population. Recently, several scientific reports have been published on the health benefits and nutritional potential of the bioactive compounds in *Z. lotus*. The nutritional virtues of *Z. lotus* are mainly based on its composition rich in vitamin E, vitamin C, fibers, fatty acids, amino acids, calcium, magnesium and considerable amounts of sugars.[9]

This plant has been found to be rich on polyphenols (flavonoids and tannins), tri-terpenes, anthraquinones, alkaloids (cyclopeptides and isoquinolides) and the saponosides which are abundant in the extracts of *Z. lotus* seeds and root bark.[10,11] Several parts, especially, leaves, fruits and roots are used in traditional medicine for the treatment of various diseases, such as insomnia and anxiety,[12] treatment of digestive disorders, obesity, urinary troubles and skin infections.[13,14] In Algeria, *Z. lotus* is also used for the treatment of liver diseases[15] and the root barks are known for their antidiabetic properties.[16]

Therefore, this plant is used for treatment of bronchitis and urolithiasis,[17] as anti-inflammatory and analgesic,[18] antibacterial and antifungal,[19,20] antioxidant,[21] litholytic and antiulcerogenic.[22] The fruit and leaves are used traditionally as emollient, in the treatment of intestinal diseases, and the crushed roots are employed in the treatment of leukemia eye disease.[23] In recent years, there has been a great interest in studying this plant, as it has been shown to improve hyperglycemia in rodents.[24] Moreover, the root extracts from this plant were reported to exert an immunosuppressive effect by inhibiting T-cell proliferation and IL-2 mRNA expression.[24,25] More recently, in a study on the protective effect of *Z. lotus*, Bencheikh et al[26] demonstrated that aqueous extracts of *Z. lotus* fruits exhibited a hepatoprotective effect against hepatic lesions induced by Carbon tetrachloride (CCL.sub.4) in rats.

Ruta chalepensis, commonly known as "*Fidjel*" is strongly scented sub-shrubs native to the Mediterranean region.[27] This plant represents a potential source of natural products with biological activities. *R. chalepensis* extracts, EOs and isolated compounds have shown a diverse potential for the treatment of different diseases. The aerial parts (leaves, flowers and small stems) are used for medicinal purposes as antimicrobials,[28] antioxidants,[29] and possessing anti-cholinesterase effects.[30] In addition to its described emmenagogue, abortifacient, anthelmintic and spasmolytic effects,[31] moreover, it is also characterized by its anti-inflammatory properties,[32,33] and possessing a depressant activity on the central nervous system.[34] Recent studies have revealed the potent effect of *R. chalepensis* as hypoglycemic,[35] and possessing an anti-mutagenic effects against potassium bromate by decreasing the sperm cell abnormalities.[36]

In their study, Abdelrahim et al[37] determined that *R. chalepensis* exhibits an increase in good cholesterol (high-density-lipoprotein, HDL), and decrease the triglyceride levels in serum when added to the high-cholesterol diet applied in Wistar rats. Besides, they demonstrated that the plant might beneficially affect the lipid profile of rats treated with paracetamol, since it lowered the low-density-lipoprotein (LDL), triglycerides and total cholesterol serum levels when added to their high cholesterol diet.

To the best of our knowledge, this is the first study which has demonstrated the *in vivo* antimicrobial activity of *Z. lotus* and *R. chalepensis* EOs against *S. enterica* ssp *arizonae*. Thus, no reports have been found about the EOs of both plants growing in Mascara, western Algeria. Therefore, the aim of this study was to evaluate the chemical composition profiles of EOs extracted from *Z. lotus* leaf and *R. chalepensis* aerial parts (leaves, flowers and small stems) using gas chromatography–mass spectrometry (GC-MS) analysis.

The oral acute toxicity and effect of these EOs on the composition and diversity of gastrointestinal microbiota were also investigated using *in vivo* and *in vitro* methods. We aimed to examine the efficiency of both plants EOs for its antimicrobial effect and its potential role on the treatment of gastroenteritis infection to *Salmonella enterica* ssp *arizonae* induced in Wistar rats.

Materials and Methods

Plant Material

The *Z. lotus* leaves were collected in July 2017 and the aerial parts of *R. chalepensis* (leaves, flowers and small stems) were collected in April 2017 during the flowering stage from the El-Mamounia region in Mascara, western Algeria and were identified by a botanist from the Department of Biology, University of Mascara, Algeria.

Bacterial Strain

The MDR *Salmonella enterica* ssp *arizonae* used in this study was isolated from stool specimens of patients with gastroenteritis and identified in Meslem-Taib hospital Laboratory, of Mascara-Algeria, as well at the Laboratory of Bioconversion, Microbiological Engineering and Health Safety of the Department of Biology, University of Mascara.

The bacterial identification was carried out for the purpose of the bacterial identity confirmation using macroscopic examination on Salmonella-Shigella agar, microscopic examination (gram stain) and using biochemical tests of commercials kits, miniaturized multi-test systems API 20E, which was applied according to the BioMerieux manual. Bacterial strain was maintained during its exponential phase of growth on agar nutrient medium, Brain Heart Infusion Broth (BHIB) and selenite F broth (SFB) medium at 4°C.[38,39]

Experimental Animals

Wistar rats of male sexes, with an average weight of ±5 g (150-

200 g body weight b.w., n=5) were used during this study. The animals were supplied by the Animal Care Facility of the Faculty of Life and Nature Sciences, University of Mascara, Algeria. They were randomly assigned to different groups. Three groups of five animals each were used for the oral acute toxicity study: control group n°1, group n°2 of animals treated with ZLEO, and group n°3 of animals treated with RCEO. The same group organization was used to study the influence of EOs on the gastrointestinal flora. While, for the anti-salmonellosis effect elucidation, five groups of five animals each were used: Negative Control Group (NCG) n°1, Positive Control Group (PCG) n°2, group n°3 of animals infected and treated with ZLEO (ITG_{ZLEO}), group n°4 of animals infected and treated with RCEO (ITG_{RCEO}), group n°5 of animals infected and treated with the antibiotic: neomycin (ITG_{ATB}). All these groups were kept under standard environmental conditions (at 25±2°C, 12/12 h light/dark cycle). They were provided with standard rodent pellets diet and had free access to water *ad libitum*. Before testing, the animals were fasted for 13h with access to water and all experimental procedures were performed in accordance with the ethical guidelines of the Organization for Economic Cooperation and Development (OECD).[40]

Essential Oil Isolation

The extraction of EOs from *Z. lotus* leaves and the aerial parts (leaves, flowers and small stems) of *R. chalepensis* was carried out using the steam distillation process. Briefly, each 45 g of the plant were subjected to steam distillation in distilled water for 3 hours according to the current European Pharmacopoeia.[41] The obtained EOs were collected, dried over anhydrous sodium sulfate and stored at +4°C in brown sealed glass vials until used. The extraction of these EOs was done in three replications for each plant and the extraction yield (w/w) was expressed as the weight of EO volume on the weight of the plant used.

Essential Oil Analysis

The separation, identification and quantification of the various bioactive volatile compounds containing in the EO samples of both plants was carried out on a Shimadzu gas chromatograph (GC), Agilent GC Model 7890B equipped with HP 5977A Mass spectrometer. Analytical conditions: Agilent 122-7062 DB-WAXN capillary column (60m_250 micrometer, 0.25-micrometer film thickness), helium as a carrier gas with a linear velocity in column of 19 cm/s and 37.862 psi of pressure. The carrier gas split flow was about 250 mL/min, split ratio: 100:1 and septum purge flow: 3 mL/min. The oven temperature program was between 70 and 260°C with an equilibration time of 1 minute and was then maintained at 250°C. The injection in the split mode of 2 µL of the substance to be analyzed was carried out using a micro-syringe with a size of 10 µL. The components were identified by comparing their relative retention times and mass spectra with the data from the library of EO constituents, Wiley, Mass-Finder and Adams GC/MS libraries. The percentage composition determination was based on peak area normalization without using correction factors.

Oral Acute Toxicity Study

The oral acute toxicity of *Z. lotus* and *R. chalepensis* EOs was performed according to the OCDE guidelines.[40] The test was performed on 15 male rats: *Rattus norveagicus* divided into three groups (control and test groups) of five animals each: control group n°1, group n°2 of animals treated with ZLEO, group n°3 of animals treated with RCEO. These animals were fasted 13 hours before the experiment, then weighed and treated with EO samples by esophageal gavage: oral administration. Animals in the control group received 10 mL/kg b.w. of sterile physiological water 9% (NaCl), and those of the test groups were given a single unique-dose (5000 mg/kg b.w.) of each *Z. lotus* and *R. chalepensis* EOs on the first day of the experiment.

Upon treatment, these animals were fasted for additional 3 hours. During the experiment, animals were weighted and symptoms of toxicity including mobility, aggression, sensitivity to pain and noise, tail state, stool and urine condition and color, brittleness, anxiety state, weight loss, sweating, painful palpation, vomiting, fever and diarrhea were noted. Furthermore, death was monitored 14 days following treatment. Animals were then anesthetized nasally with chloroform, sacrificed and organs (liver, kidneys, heart, lung and spleen) were removed, and their relative weights were determined.

Essential Oil Influence on Gastrointestinal Tract Microbiota

Isolation and Enumeration on Selective Agar Medium Method

In this experiment, 15 rats were used and they were fasted 13 hours before the study. The animals were divided into three groups (control and 2 test groups) as mentioned previously. The control group received by oral gavage, 10 mL/kg b.w. of 9% NaCl and the test groups received a single unique-dose (5000 mg/kg) of *Z. lotus* and *R. chalepensis* EOs, respectively. After a single oral administration, the treated and untreated rats were fasted and after 18 hours, they were anesthetized for further dissecting under aseptic conditions to remove the digestive tracts.

Determination of CFU (colony forming unit) counts: Isolation and enumeration on selective agar medium was applied for quantitative CFU counts determination of respective groups of intestinal bacteria in 1 g of substrate. Nutrient and selective agar medium in Petri dishes were inoculated with 100 µL of the ileum and colon solution samples, previously prepared (1 g of the organ in 9 mL of 9% NaCl) for each animal group (control and test groups). The homogenized samples of the ileum and colon were prepared in advance by sequential diluting based on decimal dilution system application (from 10^{-1} to 10^{-5}). Total aerobic mesophilic flora (TAMF) was counted on Plate Count Agar (PCA), total anaerobic flora (TANF) on Columbia Agar, *Enterobacteriaceae* on Hektoen Agar and Methylene Blue Eosin Agar (EMB), *Staphylococcaceae* on Chapman Agar and *Streptococcaceae* on Bil Eosin Agar (BEA). The bacterial colonies counts were performed after incubation at 37°C for 18-24 hours with the presence of CO_2 for strict anaerobic germs. *Lactobacillus* sp were counted on Man Rogosa Sharpe Agar (MRS) and incubated at 37°C for 48-72 hours. The

enumeration of viable bacteria in each sample was made on the Petri dishes, presenting between 30 and 300 colonies, and expressed in Log CFU/g of sample according to the following formula[42]:

Log CFU/g = Number of colonies / (dilution x inoculated volume)

The identification of the different bacterial colonies which had appeared in each selective medium was confirmed by macroscopic and microscopic (Gram stain) examinations.[43] This analysis was carried out with the aim of evaluating the influence of ZLEO and RCEO on the composition and diversity of the intestinal flora, as well as on the implantation of probiotic bacteria at the level of intestinal mucosa, in comparison with the control group of animals which had not received any treatment.

In Vivo Anti-salmonellosis Effect

For the evaluation of the EOs ability of both plants to treat gastroenteritis induced by *S. enterica* ssp *arizonae* in Wistar rats, 25 male animals weighted 200 kg ±5 g b.w. were used. The animals were divided into five groups of five rats each. The control group, which consisted of the NCG, received 10 mL/kg of normal saline water, while the PCG was given 2 mL of *S. enterica* ssp *arizonae* suspension prepared previously in BHIB broth, and of 4×10^6 CFU/mL concentrations in exponential phase.

Groups 3, 4 and 5 were firstly administered 10 mL/kg of *S. enterica* ssp *arizonae* (4×10^6 log CFU/mL) induced diarrhea. Then, after three days (optimal period for developing symptoms of the disease), animals in groups 3 and 4 were daily treated with oral administration of a single dose (400 mg/kg) of ZLEO and RCEO respectively for 7 days, while, animals in group 5 were administered 200 mg/kg of neomycin as standard antibiotic treatment.

Clinical signs observed in animals of all test groups were noticed and the body weight was recorded. At the end of experiments, all rats were deprived of food for 13 hours and they were anesthetized and sacrificed to complete the study. Blood samples taken from the abdominal aorta of each animal were collected in tubes containing an EDTA-type anticoagulant and were used for further analysis.

Detection of Salmonella enterica ssp arizonae in Faecal Flora

The objective of the experiment was to detect *S. enterica* ssp *arizonae* strain in the faecal flora of the treated and untreated animals. The stool samples taken in aseptic conditions from animals of each group (control and test groups) were immediately transported to the Laboratory of Bioconversion, Microbiological Engineering and Health Safety of Mascara University for bacteriological analysis.

Determination of CFU counts: Stool specimens (1 g wet weight samples in 9 mL of NaCl 9%) and decimal dilutions were prepared to achieve the 10^{-4} dilution. *S. enterica* ssp *arizonae* was counted on Salmonella-Shigella agar medium after inoculation of 100 µL stool solution in each Petri dish and was incubated at 37°C for 24 hours. Enumeration of the infectious germ expressed in Log CFU/g was made on colorless colonies and identification was carried out using biochemical tests of API systems: API 20E.

Hematological Analysis

Hematological parameters comprising white blood cells (WC), red blood cells (RC), Granulocytes (GRA), hematocrit (Ht), platelets (PLT), hemoglobin (Hb), Mid-range percent (MID) including basophils, eosinophils and monocytes, lymphocytes (LYM), mean corpuscular hemoglobin (TMCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and mean corpuscular volume (MCV) were analyzed using DIATRON automaton hematology (Abacus 380).

Biochemical Parameters

Biochemical parameters including alanine aminotransferse (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and erythrocyte sedimentation rate (ESR) were analyzed in the serum as inflammation and infection markers. These analyses were performed using commercial kits (Hospitex Diagnostics std, Germany).

Statistical Analysis

Replicates were prepared for all experiments. The results were given as means and their standard deviations (means ± SD). The means were compared by using the one-way and multivariate analysis of variance (ANOVA). The differences between individual means were deemed to be significant at $P < 0.05$.

Results and Discussion

Yield and Essential Oils Composition (GC-MS)

The relative amount (w/w%) of EOs from the two selected medicinal plants is presented in Table 1.

Zizyphus lotus EO was pale yellow, characterized by a liquid aspect and an aromatic odor, close to that of the plant, while RCEO was yellowish with a strong odor and an oily liquid aspect. Statistical analysis showed no significant differences between the yields of the EOs of both plants, while the highest amount was obtained for RCEO (4.99±0.86%) compared to ZLEO (4.16±0.036%).

The EO yields obtained in the present study were comparable to what has been reported by Benammara et al[44] who found that *R. chalepensis* EO has lower yields. They showed that *R. chalepensis* collected from Remchi (Tlemcen) and Naama in western Algeria gave a yield of 1.17 and 0.19%, respectively, while, a highest yield (7.23%) was obtained by Boumediene[45] using *R. chalepensis* collected from Sidi-Bel Abbes in western Algeria.

According to Dob et al[46] the EO yield of the aerial parts of *R. chalepensis* is 0.27%, which is not in agreement with the

Table 1. Yield Extraction of *Zizyphus lotus* and *Ruta chalepensis* EOs (w/w%)

Botanical Name	Samples Code	Part Used	Yield (%)
Zizyphus lotus	ZLEO	Leaves	4.16±0.036
Ruta chalepensis	RCEO	Aerial parts: leaves, flowers and small stems	4.99±0.86

Values are given as means ± SD (n=3).

results of the present study.

The yield of RCEO obtained during this study was more interesting and higher than those reported by various studies. Thus, high yields of ZLEO were recorded during this study for the first time. This indicates that plants harvested from Mascara-western Algeria contain higher levels of EOs. In addition, the harvest region has a greater influence on the yield of the EOs of plants.

The chemical composition of *Z. lotus* and *R. chalepensis* EOs was determined by GC-MS analysis. Results are shown in Table 2 and Table 3. A total of 33 components were identified for *Z. lotus* and 58 components for *R. chalepensis* that comprised 94.65% and 81.82% respectively of the total EO. The chemical profile of ZLEO allowed us to quantify 89.857% of Di-isooctyl phthalate as major compound, followed by linalol at 2.149%. Other minor components were also identified in this plant but

Table 2. Chemical Composition of *Zizyphus lotus* Leaf EO (ZLEO) Collected From Mascara in Western Algeria

Number	Compounds	Retention Time	Percent
01	α-Pinene	4.833	0.007
02	N-butyl acetate	5.556	0.015
03	Sabinene	8.16	0.029
04	α-Terpinene	8.857	0.012
05	D-Limonen	9.567	0.03
06	Eucalyptol-1.8 cineole	10.047	0.03
07	ɤ-Terpinene	11.292	0.166
08	P-cymene	12.148	0.351
09	Methyl heptenone	14.493	0.099
10	2-Nonanone	16.294	0.077
11	1,1'-Bicyclohexyl CAS no: 92-51-3	17.639	0.038
12	1 Octen 3 ol	18.178	0.035
13	Trans sabinen hydrate	18.708	0.017
14	Aldehyde C 10-decanal	19.12	0.015
15	2-Decanone CAS no: 693-54-9	19.509	0.009
16	Camphor crystal syn	20.172	0.036
17	2-Nonanol CASno: 628-99-9	20.252	0.053
18	Linalol	20.98	2.149
19	Linalyl acetate	21.267	0.147
20	2-Undecanone CAS#: 112-12-9	22.532	0.204
21	Caryophylene	22.61	0.268
22	Safranal	23.851	0.039
23	α-Terpineol	25.395	0.038
24	D-germacrene	25.892	0.011
25	2-Undecanol	26.092	0.061
26	Carvone- spearmint oil	26.501	0.028
27	Profarnesal	30.154	0.098
28	β-Ionone	32.812	0.035
29	Methyl Eugenol+97 ION	34.878	0.136
30	Thymol	40.812	0.559
31	Diisooctyl phthalate	56.894	89.857
	Total components		94.65

Table 3. Chemical Composition of *Ruta chalepensis* EO (RCEO) Collected From Mascara in Western Algeria

Number	Compounds	Retention Time	Percent
01	α-Pinene	4.837	0.025
02	Isopropyl-2-methyl butyrate	5.215	0.046
03	N-Butyl acetate	5.568	0.028
04	β-Pinene	6.611	0.023
05	Sabinene	6.915	0.02
06	D-limonen	9.581	0.44
07	Eucalyptol-1.8 cineole	10.066	0.255
08	Trans-2-Hexenal	10.174	0.072
09	ɤ-Terpinene	11.301	0.042
10	Methyl hexyl ketone	12.711	0.046
11	Methyl heptenone	14.51	0.021
12	2-Propanol, 1-butoxy- CAS no : 5131-66-8	14.728	0.175
13	2-Octanol, acetate CAS no: 2051-50-5	15.471	0.283
14	2-Nonanone CAS no: 821-55-6	16.403	13.404
15	Aldehyde C9- Nonanal	16.472	0.041
16	1,1'-Bicyclohexyl CAS no: 92-51-3	17.659	0.348
17	Methyl octyl ketone	18.475	0.056
18	2-Nonanol, acetate CAS no: 14936-66-4	18.669	10.094
19	Octyl acetate	19.009	0.034
20	2-Decanone CAS no: 693-54-9	19.534	1.178
21	Benzaldehyde	20.135	0.049
22	Camphor crystal syn	20.196	0.116
23	2-Nonanol CAS no: 628-99-9	20.291	3.265
24	Linalol	20.985	0.034
25	2 Undecanone Methyl nonyl ketone	21.16	0.127
26	Octanol--Alcohol C 8	21.325	0.066
27	Nonyl acetate	21.945	0.063
28	Isobornyl acetate	22.086	0.043
29	2-Undecanone CAS no: 112-12-9	22.696	26.528
30	Safranal	23.876	0.036
31	Pinocarveol	24.193	0.031
32	Alcohol C9	24.313	0.067
33	2-Undecanol, acetate CAS no: 14936-67-5	24.445	4.851
34	2-Dodecanone CAS no: 6175-49-1	24.634	0.778
35	Neral-Citral	25.087	0.044
36	α-Terpineol	25.411	0.567
37	2-Dodecanone	25.664	0.548
38	2-Undecanol CAS no: 1653-30-1	26.127	2.966
39	Carvone- spearmint oil	26.524	0.025
40	Methyl salicylate	27.608	0.041
41	2-Tridecanone CAS no: 593-08-8	28.86	1.077
42	P-cymene 8 ol	29.84	0.046
43	Anethole	30.431	0.028
44	β-Ionone	32.821	0.14
45	Methyl eugenol	34.877	0.072
46	Dimethyl antranilate	36.708	0.069
47	Elemol	37.158	0.498

Table 3. Continus.

48	ɤ-Decalactone	38.932	0.196
49	ɤ-Eudesmol	39.855	0.117
50	P-vinylguaiacol	40.236	0.332
51	Elemicine +alpha eudesmol	41.254	0.156
52	Methyl antranilate	41.374	0.017
53	β-Eudesmol	41.495	0.168
54	Indole	45.382	0.019
55	Dulcinyl	46.762	1.756
56	Ethyl piperonylacetate CAS no: 7116-48-5	46.847	0.349
57	Dodecanoic acid	47.178	0.18
58	Phytol	48.01	0.496
59	Chalepensin	61.193	9.364
	Total components		81.82

at low percentages, taken as example thymol (0.559%), methyl eugenol+97 ION (0.136%), p-cymene (0.351%), ɤ-terpinene (0.166%), linalyl acetate (0.147%), 2-undecanone (0.204%) and caryophyllene (0.268%). In addition, results indicated the most richness of *Z. lotus* EO on terpenes and phthalate esters (Table 2). The dominance in Di-isooctyl phthalate enabled us to classify this EO as Di-isooctyl phthalate chemotype. To the best of our knowledge, this is the first report on the EO composition of *Zizyphus lotus* leaves collected from Mascara, Western Algeria.

Other studies on the same plant genus *Zizyphus* collected in Iran reported the presence of α-pinene, D-limonen, P-cymene, caryophylene and α-terpineol in the Eos of leaves, in addition to geranyl acetone (14%), hexadecanoate (10%), ethyl octadecanoate (9.9%), hexadecanol (9.7%) and ethyl octadecanoate (8%) as major components.[47] A study by Ourzeddine et al[48] on the chemical composition and antioxidant activity of EO extracted from *Z. lotus* fruit, harvested from Ouled Fadhel (region of Batna eastern Algeria), showed that ethyl hexadecanoate (12%), decanoic acid (11%), ethyl dodecanoate (9.4%), ethyl hexadec-9-enoate (7.9%), dodecanoic acid (6.5%), ethyl tetradecanoate (6.1%), tetradecanoic acid (5%), ethyl decanoate (4.8%), octanoic acid (3.1%), ethyl undecanoate (2.8%), nonanoic acid (2.4%) and undecanoic acid (2.1%) are the predominant components in the EO sample.

The GC-MS analysis of *R. chalepensis* EO allowed us to determine another chemical profile, different to the one obtained with *Z. lotus* leaf EO. The EO of *R. chalepensis* harvested at Mascara-El-Mamounia region in western Algeria has a very interesting chemical polymorphism (Table 3).

The 2-Undecanone was the major compound identified in this plant at 26.528% with abundance of 2-nonanone (13.404%), followed by 2-nonanol acetate (10.094%), chalepensin (9.364%), 2-undecanol acetate (4.851%), 2-nonanol (3.265%), 2-undecanol (2.966%), 2-tridecanon (1.077%), 2-decanone (1.178%) and dulcinyl (1.756%) (Table 3).

Other minor components were also detected and quantified in *R. chalepensis* EO such as 2-dodecanone CAS no: 6175-49-1 (0.778%), α-terpineol (0.567%), elemol (0.489%), p-vinylguaiacol (0.332%), phytol (0.496%), bicyclohexyl (0.348%), 2-octanol acetate (0.283%), eucalyptol-1.8 cineole (0.255%), d-limonen (0.44%), ɤ-decalactone (0.196%), β-eudesmol (0.168%), dodecanoic acid (0.18%), ɤ-eudesmol (0.117%) and crystal camphor (0.116%). Camphor was identified for the first time in RCEO during the present study. It is an oxygenated mono-terpene known for its antifungal and antibacterial properties.[49,50]

According to these results, this plant can be classified as 2-undecanone chemotype, which is in agreement with previous studies.[51,52] Thus, Rustaiyan et al[53] showed that the EO of *R. chalepensis* collected from Iran is dominated by 2-undecanone (52.5%). While, Abdellaoui et al[54] reported that the main components of *R. chalepensis* EO were 2-octanol acetate (30.98%), 2-undecanone (25.94%), 2-nonanone (16.28%) and 5-dodecanone acetate (9.35%), followed by others components with lower percentages, which were 2-nonanol (2.54%) and 2-decanone (2.42%).

Mejri et al[55] reported the identification of four compounds representing 89.52% of the oil, which were 2-undecanone as the major compound, 2-decanone, 2-dodecanone and 2-tridecanone. Other studies indicated the richness of *R. chalepensis* in 2-undecanone, 2-nonanol and 2-dodecanone that were qualified as the major constituents of the EO.[56] The content and nature of the predominant compounds in EOs vary considerably depending on the origin of the medicinal plant.

Acute Toxicity

The current investigation supports the safety profile of *Z. lotus* and *R. chalepensis* EOs, in order to use these natural drugs for treatment of acute diseases. Results of body gain evaluation and organs weight are mentioned in Figure 1 and Table 4. From daily observations, all the animals were in good health. The animal groups treated with EOs of *Z. lotus* and *R. chalepensis* (GTZLEO and GTRCEO respectively) showed no negative clinical signs and behavior changes. No toxicity

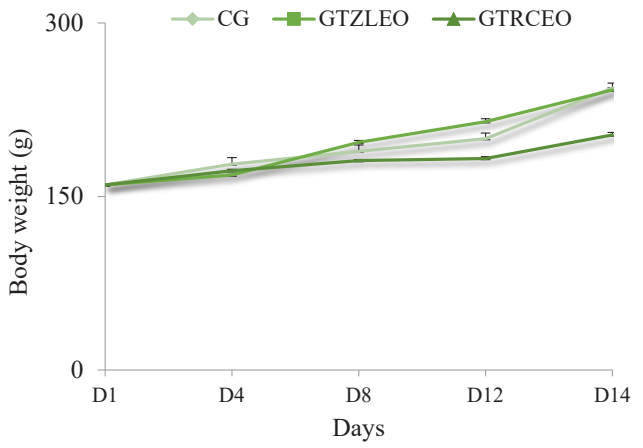

Figure 1. Body Weight Evolution (g) During the 14 Days After the Administration of Single Unique-Dose (5000 mg/kg) of *Zizyphus lotus* and *Ruta chalepensis* EOs ($P<0.05$). Each value represents the average between five animals per group. D: day, CG: Control Group, GTZLEO: Group Treated with *Z. lotus* EO, GTRCEO: Group Treated with *R. chalepensis* EO.

Table 4. Organs Weight Changes (g) (n= 5, $P<0.05$)

Animal Groups	Liver	Kidneys	Heart	Lung	Spleen
CG	10.011 ±0.001	1.6852 ±0.002	0.887 ±0.02	1.3678 ±0.001	0.6396 ±0.001*
GTZLEO	11.75 ±0.03	2.128 ±0.022	0.876 ±0.026	1.202 ±0.05	0.62 ±0.014*
GTRCEO	9.138 ±0.058	1.942 ±0.063	0.796 ±0.009	1.216 ±0.019	0.63 ±0.014*

CG: Control Group, GTZLEO: Group of animals Treated with *Z. lotus* EO, GTRCEO: Group of animals Treated with *R. chalepensis* EO.
* Results are considered as not significant.

signs or deaths in all groups were recorded during the 14 consecutive days of the experiment. There were no changes in their general behavior or other physiological conditions such as locomotion and access to food. However, some side effects were determined during the first 3 hours following the oral administration of 5000 mg/kg of RCEO, including reduced mobility of animals, anxiety and somnolence of animals treated with *R. chalepensis* EO, while no effect was determined in animals treated with *Z. lotus* EO. These effects disappear after 18 hours of treatment. The somnolence and decrease in locomotor activity could reflect the sedative or tranquilizing effect of *R. chalepensis* EO.

Therefore, the EOs of *Z. lotus* leaves and *R. chalepensis* aerial parts were considered non-toxic and the oral LD_{50} of both plants were greater than 5000 mg/kg. Moreover, the animals did not present considerable variations in the body weights among control (untreated rats) and treated animal groups. As summarized in Figure 1, the body weight of the rats increased relatively during the study. When compared with the control animals group, ZLEO and RCEO treatments induced significant changes ($P<0.05$) in the body weight of Wistar rats. A significant increase ($P<0.05$) in body weight of animals treated with *Z. lotus* EO was observed after the fourth day of the experiment when compared with control animals group. In addition, the body weight of animals, which had taken the EO of *R. chalepensis* was less than those of the control animals group and the treated animals group with *Z. lotus* EO (Figure 1).

Similarly, the oral ingestion of *Z. lotus* and *R. chalepensis* EOs caused significant changes ($P<0.05$) in the organs weight (liver, kidneys, heart, lung, and spleen) of the animals (Table 4). While, no significant change was recorded in the spleen weight of treated animal groups when compared with the control group (untreated animals).

The results of the present study are in accordance with those of El Hachimi et al[57] who have determined that *Z. lotus* is non-toxic orally in a single dose of 5000 mg/kg. In their studies on the toxicity of *Z. lotus*, Bakhtaoui et al[58], Abdelhafidh et al [59] have documented that the extracts of this plant could be administered at a dose range of 2500 to 5000 mg/kg without any side effects in Wistar rats. Furthermore, Bencheikh et al[26] and Touiti et al[60] determined that the oral administration of *Z. lotus* at a dose limit of 2000 mg/kg body weight do not represent any mortality or behavioral changes of the animals. More recently, Gadiri et al[61] and Kandimalla et al[62] demonstrated that the oral administration of 2000 mg/kg of *Zizyphus jujuba* extracts do not induce any toxic symptoms or death during the 14 days of the observation period and therefore, this plant was considered to be safe up to the dose of 2000 mg/kg. In addition, Gelayee et al[63] determined that the oral LD_{50} of *R. chalepensis* is higher than 2000 mg/kg. A recent study on the same plant genus demonstrated that the oral LD_{50} of *Ruta graveolens* L. (*Rutaceae*) is greater than 4000 mg/kg.[64]

Influence of Essential Oils on the Gastrointestinal Tract Microbiota

This study examined the influence *in vivo* of EOs on gut microbiota composition. The results of gastrointestinal microbiota analysis in the ileum and colon parts of the intestine, of the treated animals with the EOs of both plants (test groups) and the untreated animals (control group), by conventional culture techniques are shown in Figure 2 and Figure 3. According to the obtained results, it can be clearly observed that the different groups of bacteria involved in the intestinal microbiota composition of the control group (untreated rats) were also examined in the intestine of treated animals with the EOs of both plants.

The results reflected the richness of the ileum and colon parts in bacterial communities in the intestine of all animals treated with the EOs of both plants, which had significantly ($P<0.05$) increased by ZLEO and RCEO treatments. Significantly higher counts ($P<0.05$) of strict aerobic (TAMF) and anaerobic (TANMF) bacteria were detected in both parts of the intestine (ileum and colon) of all treated animal groups than in those of the control group (untreated rats) (Figure 2 and Figure 3). A concentrations of 8.166±0.05 log CFU/g in the ileum and 8.179±0.128 log CFU/g in the colon, on total aerobic mesophilic bacteria, expressed as Log N (Log number of bacterial colonies enumerated in the ileum and colon specimens (log CFU/g) were determined in the intestine of the animals in the control group. While concentrations of

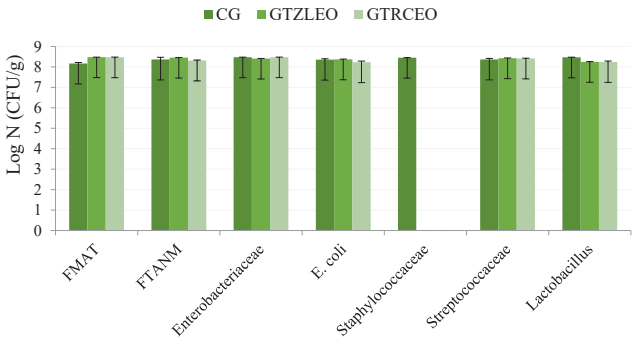

Figure 2. *In Vitro* Evaluation of the Intestinal Flora Composition in the Ileum Part of the Intestine ($P<0.05$). CG: Control Group, GTZLEO: Group Treated with *Z. lotus* EO, GTRCEO: Group Treated with *R. chalepensis* EO.

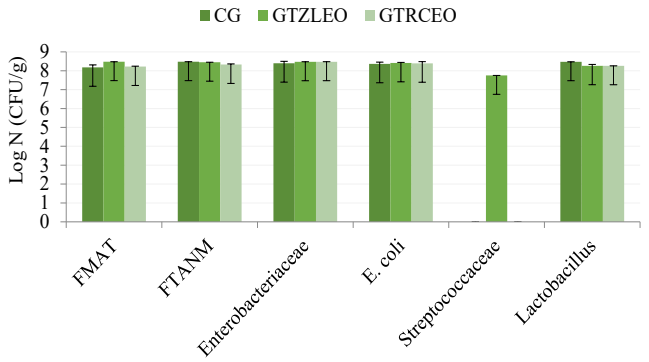

Figure 3. *In Vitro* Evaluation of the Intestinal Flora Composition in the Colon Part of the Intestine ($P<0.05$). CG: Control Group, GTZLEO: Group Treated with *Z. lotus* EO, GTRCEO: Group Treated with *R. chalepensis* EO.

8.472±0.007 log CFU/g in the ileum and 8.221±0.023 log CFU/g in the colon were calculated in the intestine of animals treated with RCEO, 8.476 ±0.001 log CFU/g in the ileum and 8.477 log CFU/g in the colon, on total aerobic bacteria were determined in the intestine of animals treated with ZLEO. These results indicate that *Z. lotus* and *R. chalepensis* EOs are able to selectively stimulate the growth of bacteria in the intestine which can improve the organism physiology. Whereas, no significant difference was observed between groups of animals treated with ZLEO and RCEO, on total aerobic mesophilic flora counts in ileum part (8.476±0.001 log CFU/g and 8.472 ±0.007 log CFU/g, respectively), as well as between control animals group and GTRCEO on total anaerobic bacteria counts (8.362±0.107 log CFU/g and 8.312±0.024 log CFU/g, respectively).

The genus *Staphylococcus* was detected in the ileum part of the control animals group, which was present in significantly ($P<0.05$) higher levels (8.449±0.003 log CFU/g). Thus, it was not detected in the intestine of treated animals with the EOs of both plants, which explain the great potency of *Z. lotus* and *R. chalepensis* in the inhibition and destruction of the pathogenic *Staphylococcus* cells among the intestinal flora. An abundant presence of *Streptococcus* bacteria at the ileum level of treated animals with the EOs of both plants was recorded with higher amounts (8.425±0.013 and 8.412±0.012 log CFU/g in the intestine of animals treated with ZLEO and RCEO, respectively) than in control animals group (8.365±0.05 log CFU/g) (Figure 2).

The presence of *Enterobacteriaceae* was extremely abundant in the colon intestinal microbiota of treated rats with EOs compared with control animals group (Figure 3). Higher concentrations of 8.413±0.03 and 8.393±0.49 log CFU/g in *E. coli* counts were determined in the colon flora of treated animals by ZLEO and RCEO respectively, while a concentration of 8.363±0.09 log CFU/g was calculated in the colon microbiota of animals in the control group. A significant difference ($P<0.05$) in the composition on total aerobic mesophilic bacteria, total anaerobic flora, *Streptococcus* and *Lactobacillus* in the colon part were registered, while no significant difference was determined between the treated and untreated animal groups as regards to the composition on *Enterobacteriaceae* and *E. coli* species.

The appearance of characteristic colonies of *Lactobacillus* in MRS agar for the different parts of the intestine of the treated animals with *Z. lotus* and *R. chalepensis* EOs indicated that this bacterial genus is tolerant and has a great ability for growth in the presence of antimicrobial agents. However, a significant decrease on the number of these bacterial cells was observed in both the intestine parts of treated animals compared to the control group (8.466±0.008 and 8.473±0.007 log CFU/g concentrations in the ileum and colon parts respectively of untreated animals group). Although, no significant difference was noticed when comparing both test groups (Figure 3). Concentrations of 8.247±0.013 and 8.240±0.05 log CFU/g on *Lactobacillus* cells were enumerated in the ileum part of the intestine of animals treated with ZLEO and RCEO respectively (Figure 2). Whereas higher concentrations were calculated in the colon microbiota of all animals test group: 8.261 ±0.003 and 8.258 ±0.14 log CFU/g of *Lactobacillus* cells in the intestine of animals treated with ZLEO and RCEO respectively (Figure 3).

According to our results, these EOs can be considered as prebiotic products for probiotic bacteria development, such as *Enterobacteriaceae* (*Escherichia coli*), *Streptococcus* sp and *Lactobacillus* that exhibit an important role in the inhibition of pathogenic microorganisms and the immune system developments. It can well be determined that the EOs of these plants can be used as stimulators of probiotic bacterial growth and in the treatment of gastric pathologies without inducing any negative influence on the composition and diversity of gastrointestinal tract microbiota, thus consumption of commercial probiotic coupled with these natural drugs can be performed. While, our goal was to find and search for natural and effective alternative drugs that can be used in the treatment of microbial infections, without having any influence on the intestinal microbiota composition, and especially on probiotic bacteria.

Limited studies on the influence of plant bioactive compounds on the composition of intestinal flora have been reported. In a study by Yamakoshi et al[65] they demonstrated that when administering the grapes seeds extracts, the number of *Bifidobacterium* increased when at the same time, the colony of *Enterobacteriaceae* decreased.

In addition, Tzounis et al[66] have evaluated the influence of flavonols derived from cocoa on the composition of the intestinal microbiota and they have determined a statistically significant growth of *Bifidobacterium* and *Lactobacillus*.

Wiciński et al[67] have approved that the administration of bioactive compounds extracted from medicinal plants exerted an influence on gastrointestinal microbiota, by increasing the level of probiotic bacteria of the genus *Bifidobacterium* and *Lactobacillus*, and decreasing the level of pathogenic microorganisms such as *Clostridium sp*. Therefore, the results of the present study confirmed the possibility of applying the

EOs of plants as potential prebiotic compounds.

In Vivo Anti-salmonellosis Effect against Salmonella enterica ssp arizonae

Disease Clinical Signs Determination and Body Weight Evolution

The present study aimed to valorize the medicinal value of *Zizyphus lotus and Ruta chalepensis* in the treatment of intestinal infection induced by pathogenic MDR bacteria. This infection is a real public health problem in developing countries. The chemical drugs used in the treatment of this disease are less efficient on these bacteria. For the assessment of the antimicrobial effect against *S. enterica* ssp *arizonae*, clinical signs and body weight evolution were determined.

After the induction of the gastroenteritis infection to *S. enterica* ssp *arizonae* and an incubation period of three days, the untreated animals of the PCG developed mild to severe diarrhea, characterized by the presence of liquid and bloody stools with mucus. The color of the stool samples in the infected untreated animals group was blackish tarry, and for other animals was dark green with a disagreeable odor.

Reduced mobility was observed in most animals before treatment with loss of appetite and weight. A painful sensation during defecation and colon inflammation were also observed. No mortality was recorded for both animal groups treated with *Z. lotus* and *R. chalepensis* EOs, however, a mortality of 2/5 and 1/5 rats was noted in the infected untreated animals (PCG) and those infected treated with the antibiotic (ITG_{ATB}), respectively.

Clinical signs began to decrease at the second day of treatment with both *Z. lotus* and *R. chalepensis* EOs (D5), at a dose of 400 mg/kg b.w. An increase in body weight was recorded in most of the treated animals according to the treatment period (Table 5 and Figure 4).

Thus, a change in the appearance and color of faecal matter of the treated rats with the EO of each plant was determined compared with animals in the PCG. The appearance became almost the same as of the stool specimens of the negative control group animals, with the disappearance of the displeasing odor and a color change from tarry black or dark green to light brown or dark brown.

However, there was a slight significant ($P<0.05$) increase in the body weight of animals treated with *R. chalepensis* EO when compared with other animals groups, which reflects the fact that this treatment has an influence on body weight gain. In addition, a reduction in colon inflammation was observed in animals treated with the EOs of both plants with normal access to food, water and a normal locomotor activity. For the animals in PCG, a permanent decrease in body weight was recorded with the mortality of two animals in this group, which indicate that the enteric infection induced in Wistar rats had a significant influence on the organism functions and physiology.

Table 5. Examination of Clinical Signs

Clinical Signs	NCG	PCG	ITG_{ATB}	ITG_{ZLEO}	ITG_{RCEO}
Number of Animals	5	5	5	5	5
Mobility	N	R	R	N	N
Stool Condition	N	A	A	N	N
Vomiting	A_b	A_b	A_b	A_b	A_b
Fever	A_b	P_r	P_r	A_b	A_b
Loss of Appetite	A_b	P_r	P_r	P_r	P_r
Diarrhea	A_b	P_r	P_r	A_b	A_b
Mortality	A_b	2/5	1/5	A_b	A_b

NCG: Negative control group, PCG: Positive control group, ITG_{ATB}: Infected treated group with the antibiotic, ITG_{ZLEO}: Infected treated group with *Z. lotus* EO, ITG_{RCEO}: Infected treated group with *R. chalepensis* EO.
N: Normal, A: Abnormal, R: Reduced, A_b: Absence, P_r: Presence.

Detection of Salmonella enterica ssp arizonae in Faecal Flora

The results of *S. enterica* ssp *arizonae* enumeration in faecal flora are shown in Figure 5. A significantly higher decrease ($P<0.05$) was observed on *S. enterica* ssp *arizonae* cells in the

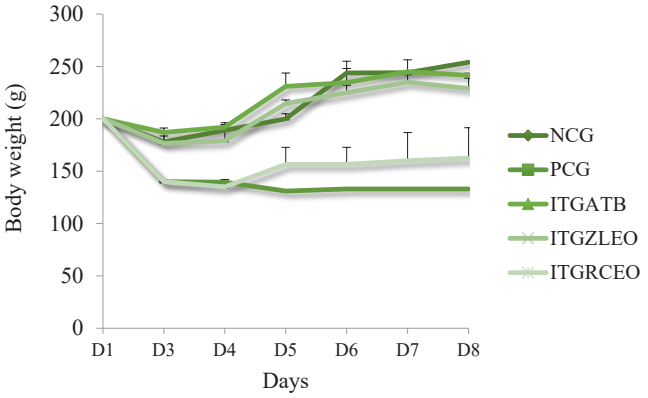

Figure 4. Body Weight (g) Changes as Function of the Experiment Period (8 days) ($P\leq0.05$). NCG: Negative control group, PCG: Positive control group, ITG_{ATB}: Infected treated group with the antibiotic, ITG_{ZLEO}: Infected treated group with *Z. lotus* EO, ITG_{RCEO}: Infected treated group with *R. chalepensis* EO.

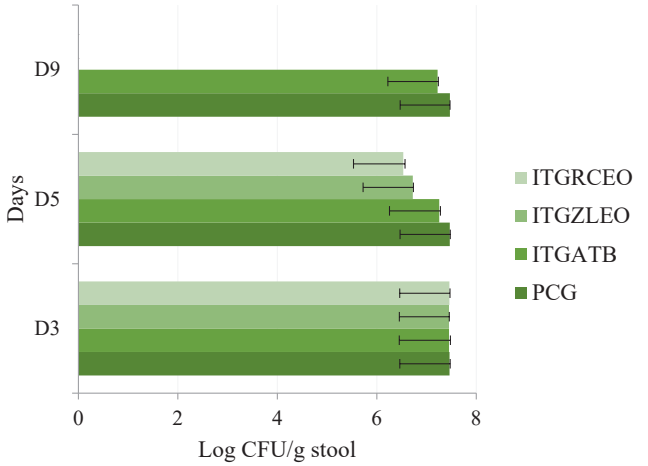

Figure 5. Effect of Antibiotic and EOs Therapy With *In Vitro* Inhibitory Activity Against Colonizing Multidrug Resistant *Salmonella enterica* ssp *arizonae* strain ($P<0.001$). PCG: Positive control group, ITG_{ATB}: Infected treated group with antibiotic, ITG_{ZLEO}: Infected treated group with *Z. lotus* EO, ITG_{RCEO}: Infected treated group with *R. chalepensis* EO.

faecal flora of animals treated with *Z. lotus* and *R. chalepensis* EOs when compared with stool bacterial content of animals in the PCG (Figure 5).

The therapeutic application of *Z. lotus* and *R. chalepensis* affected faecal content on *Salmonella enterica* ssp *arizonae* cells (Figure 5). Statistically significant differences ($P<0.05$) were observed between positive control and test groups after therapeutic application of neomycin, *Z. lotus* and *R. chalepensis* EOs. The number of *S. enterica* count in faecal flora of animals in the PCG was about 7.47 log CFU/g of stool samples. A significant decrease ($P<0.05$) in S. *enterica* cells count was determined after the therapeutic application of the EOs of both plants. Concentrations of the infectious germ ranged from 7.47 log CFU/g to 6.72 log CFU/g the second day of treatment with ZLEO, while it ranged from 7.47 log CFU/g to 7.25 log CFU/g the second day of treatment with neomycin, and to 7.23 log CFU/g the sixth day of treatment with the same antibiotic used (Figure 5). However, no bacterial cells of *S. enterica* ssp *arizonae* were counted in the faecal flora of animals after the sixth day of treatment with *Z. lotus* and *R. chalepensis* EOs.

Our results revealed that treatment with the EOs of both plants has led to a significant reduction of the MDR *Salmonella* concentration in faecal flora. These results indicated that both plants exhibited an important toxicity against *S. enterica* ssp *arizonae* after six days of treatment, in which no *Salmonella* was detected in the faecal microbiota (D9) (Figure 5).

Results of this experiment showed an important decrease of *S. enterica* ssp *arizonae* strain in the faecal flora of the treated animals with *R. chalepensis* EO, after the second day of oral administration of 400 mg/kg RCEO in which no *S. enterica* ssp *arizonae* cells were counted when comparing with those of treated rats through *Z. lotus* EO. Moreover, a slight decrease of the germ was observed in the faecal flora of treated rats with the antibiotic. These results indicated that alternative treatment against *S. enterica* ssp *arizonae* induced diarrhea, by oral administration of 400 mg/kg of *Z. lotus* and *R. chalepensis* EOs for seven days on a daily basis were more effective than the standard chemical drug used (neomycin). Also, results indicated the most efficiency of both plants as antimicrobials, as shown by various studies.[68,69] Yahia et al[70] demonstrated the greatest potency of bioactive compounds extracted from *Z. lotus* as antimicrobials by exhibiting an important antibacterial effect against *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli*. Besides, Degu et al[71] demonstrated that *R. chalepensis* at a dose of 400 mg/kg body weight possessed a significant anti-diarrheal activity. Therefore, this research was the first study to demonstrate the *in vivo* antimicrobial effect of *Z. lotus* leaf and *R. chalepensis* EOs growing in Mascara, Algeria against *S. enterica* ssp *arizonae* induced diarrhea in Wistar rats.

Hematological Analysis

The quantification of the hematological parameters using DIATRON automaton hematology (Abacus 380) is shown in Table 6. According to the results of the present study, the EOs of *Z. lotus* and *R. chalepensis* in the acute treatment (400 mg/kg b.w.) of gastroenteritis to *S. enterica* ssp *arizonae* induced a significant difference ($P<0.05$) in the leukocyte formula between the different groups. A significant increase ($P<0.05$) in white blood cells and lymphocytes was determined in the blood samples of the different animal groups (PCG and test groups).

Furthermore, a significant and important increase was observed in the lymphocytes of animals in the PCG after inducing the enteric infection (72.1±0%) compared to the NCG (46±0%) (Table 6). These increases in immune cells are specifically intended against all types of bacterial infections, which allowed us to prove the development of the *in vivo* gastroenteritis infection to *S. enterica* ssp *arizonae* in Wistar rats.

An increase in these parameters indicated the activation of the immune system because of the enteric infection development. A significantly slight decrease ($P<0.05$) in these immune cells was detected in the infected animals treated with the EOs of both plants comparing with the PCG

Table 6. Hematological Parameters. Values are given as mean±SD (n=2), P<0.05

Hematological Parameters	NCG	PCG	ITG_{ATB}	ITG_{ZLEO}	ITG_{RCEO}
WBC ($\times10^9$/L)	5.665±0.021	9.035±0.021	9.915±0.007	7.915±0.26	5.895±0.02
LYM (%)	46±0	72.1±0	60.4±0	56.05±0.07	68.5±0.14
MID (%)	10.2±0.14	9.7±0.141	11.65±0.212	14.7±0.14	8.95±0.21
GRA (%)	43.55±0.212	18±0	28.05±0.07	28.1±0	23.1±0
RBC ($\times10^9$/L)	8.85±0.07	8.505±0.021	8.3±0.014	8.755±0.21	7.7±0.14
Hb (g/dL)	16.25±0.212	13.35±0.212	14.05±0.07	14.5±0.14	12.2±0.14
Ht (%)	46.9±0.141	40.03±0.049	39.95±0.014	41.52±0.14	36.78±0.01
MCV (fL)	53±0	47±0	48±0	46.5±0.7	48±0
TMCH (pg)	17.3±0.282	15.5±0.565	17.05±0.07	16.15±0.07	16.05±0.07
MCHC (g/dL)	32.9±0	33.85±0.07	35.35±0.07	34.5±0.14	33±0
PLT ($\times10^9$/L)	574.5±5	706.5±2.12	646±1.41	651±1.41	480.5±0.71
THT	0.34±0	0.455±0.007	0.405±0.007	0.4±0	0.31±0.01

WBC: White blood cells, RBC: Red blood cells, GRA: Granulocytes, Ht: Hematocrit, PLT: Platelets, Hb: Hemoglobin, MID: Mid-range percent including basophils, eosinophils and monocytes, LYM: Lymphocytes, TMCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume.

(Table 6). In addition, a significant decrease in granulocytes for all tested groups compared to the NCG was recorded with a slight increase in the mid-range percent (basophiles, eosinophils and monocytes) for the treated animal groups with neomycin and ZLEO. These cells are involved in innate immunity mechanisms.

An increase in the thrombocytes or platelets (PLT) number was observed, except the ITG_{RCEO} in which these platelets were less than measured in the NCG. A slight decrease in hemoglobin and hematocrit levels were observed in animals of all tested groups, which indicated that the administration of *Z. lotus* and *R. chalepensis* EOs for enteric infection treatment could not present a risk of anemia.

Biochemical Parameters

Results of biochemical parameters dosage (ALT, AST, ALP and ESR) are mentioned in Figure 6 and Figure 7. Daily oral acute treatment of the gastroenteritis with *Z. lotus* and *R. chalepensis* EOs induced a significant decrease in ALT and AST activity when compared to the PCG of the untreated animals (Figure 6).

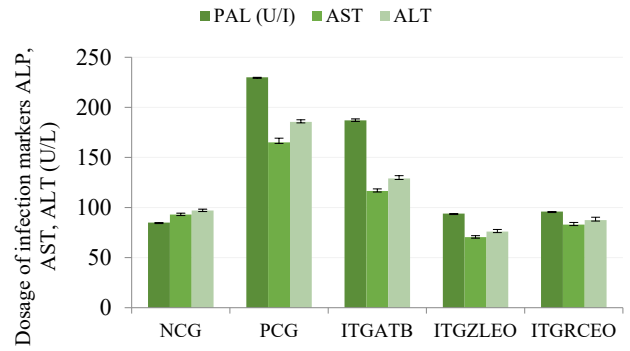

Figure 6. Biochemical Markers of Hepatic (Transaminases) Toxicity After Administration of EOs of *Zizyphus lotus* leaves and *Ruta chalepensis* Aerial Parts at a Dose of 400 mg/kg and Neomycin at a Dose of 200 mg/kg for 7 Consecutive Days ($P<0.05$).

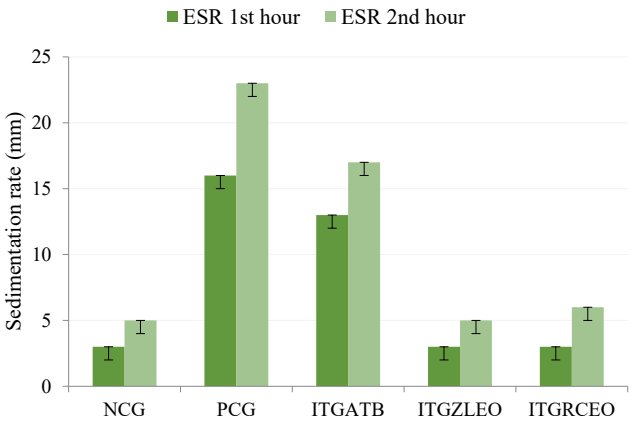

Figure 7. Biochemical Markers of Inflammation and Bacterial Infection (ESR) of Rats After the Administration of *Zizyphus lotus* and *Ruta chalepensis* EOs at a Dose of 400 mg/kg and the Antibiotic at a Dose of 200 mg/kg for 7 Consecutive Days ($P<0.05$).

The ALT and AST are more specific to liver damages.[72] The decreased activity of these enzymes could express the hepatoprotective property of both plants EOs. In addition, there was a decrease in ALP levels in treated animals with *Z. lotus* and *R. chalepensis* EOs compared to the infected untreated rats (PCG) and those treated with the antibiotic (ITG_{ATB}), in which we observed high levels in the ALP parameter (Figure 6).

The biochemical parameters changes were associated with increased sedimentation rates (ESR) for all tested groups comparing with animals of the negative control group (Figure 7). The measured ESR in the PCG was more important and higher (10 mm) than that obtained in the NCG, which indicated and proved the development of *S. enterica* ssp *arizonae* infection. The ESR levels began to decrease after the treatment of the gastroenteritis with the neomycin, *Z. lotus* and *R. chalepensis* EOs (Figure 7).

These results indicate that EOs of *Z. lotus* and *R. chalepensis* has the potential to protect from liver injury causing the abnormal variation in the plasma biochemical parameters caused by *S. enterica* ssp *arizonae* infection. It seems that the hepatoprotective effect of ZLEO and RCEO on liver damage, caused by pathogenic germs infections, may be due to the phytochemical content of *Z. lotus* and *R. chalepensis* EOs on bioactive compounds possessing hepatoprotective properties.

In addition, in a recent study on another species of the genus *Zizyphus*, researchers demonstrated that *Z. jujuba* is rich on bioactive substances known to have hepatoprotective activities against liver damages.[73] Moreover, Geth et al[74] determined the hepatoprotective effect of *R. chalepensis* when administered as a protective and therapeutic treatment. In the present study, the antimicrobial effect of *Z. lotus* and *R. chalepensis* EOs against *S. enterica* ssp *arizonae* can be attributed to the presence of high amounts of various bioactive components possessing a great potential against pathogenic microbial cells, and having hepatoprotective effect against liver damages when administered as therapeutic treatment against gastroenteritis induced by pathogenic germs.

Conclusions

The chemical composition on bioactive compounds and *in vivo* antimicrobial effect of *Zizyphus lotus* and *Ruta chalepensis* EOs collected from Mascara in western Algeria was determined in this study for the first time. Few studies on the EO of *Zizyphus lotus* have been reported, while, various studies have elucidated the biological properties of *Ruta chalepensis* EOs. The GC-MS analysis allowed us to identify and quantify various bioactive components in the Eos of both plants. Di-isooctyl phthalate was the major compound detected in *Z. lotus* with higher percentages, while, 2-undecanone was the major component in *R. chalepensis*.

The *in vivo* study of the oral acute toxicity allowed us to determine that the LD_{50} of *Z. lotus* and *R. chalepensis* EOs were greater than 5000 mg/kg b.w. and thus to confirm the non-toxicity of these EOs. Oral administration of the EOs of both plants had no negative influence on the diversity and composition of the gastrointestinal microbiota, while an increase in cell counts of aerobic and anaerobic bacteria were

registered. We have determined the presence of probiotic bacteria (lactobacilli) in the intestine of treated animals, which indicated no influence of these EOs on the implementation of probiotic bacteria.

Besides, the present study demonstrated that EOs of *Z. lotus* leaf (400 mg/kg) and *R. chalepensis* aerial part (400 mg/kg) possessed anti-salmonellosis effects on *S. enterica* ssp *arizonae* induced diarrhea in Wistar rats. It can be also concluded that the efficiency of the EOs of both plants in gastroenteritis treatment is mainly due to its effective antibacterial effect. In addition, the anti-salmonellosis activities of these EOs are attributed to the presence of various bioactive components in both medicinal plants. These findings can support the use of EOs extracted from *Z. lotus* leaves and *R. chalepensis* aerial parts in folk medicine for the treatment of various microbial infections and diseases.

Authors' Contributions

NB, BM and SP described the work plan for carrying out the study. NB prepared the samples and animal groups including in the study, carried out all the experiments and wrote the manuscript with the agreement of all the authors. NB also analyzed the results. BK performed the GC-MS analysis. All authors contributed to the manuscript revision, read and approved the submitted final document.

Conflict of Interest Disclosures

The authors declare that there is no conflict of interest.

Ethical Approval

For animal experimentations, adequate measures were taken to minimize pain and discomfort of the animals, and all experimental procedures were in accordance with the ethical guidelines of the organization for economic cooperation and development (OECD).

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References

1. Nair DVT, Venkitanarayanan K, Kollanoor Johny A. Antibiotic-resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. Foods. 2018;7(10):167. doi:10.3390/foods7100167.
2. Brun-Buisson C. Consommation d'antibiotiques et résistance aux antibiotiques en France: Nécessité d'une mobilisation déterminée et durable. Ed., ANSM. Agence Nationale de Sécurité des Médicaments et des produits de santé. Santé publique France ; 2016. https://www.ansm.sante.fr/content/download/98417/1249747/version/3/file/Resistance+Antibiotiques-nov2016.pdf.
3. Di Bella S, Capone A, Bordi E, et al. *Salmonella enterica* ssp. arizonae infection in a 43-year-old Italian man with hypoglobulinemia: a case report and review of the literature. J Med Case Rep. 2011;5:323. doi:10.1186/1752-1947-5-323.
4. Bouzidi N, Aoun L, Zeghdoudi M, et al. *Salmonella* contamination of laying-hen flocks in two regions of Algeria. Food Res Int. 2012;45(2):897-904. doi:10.1016/j.foodres.2011.05.027.
5. Mezali L, Hamdi TM. Prevalence and antimicrobial resistance of *Salmonella* isolated from meat and meat products in Algiers (Algeria). Foodborne Pathog Dis. 2012;9(6):522-529. doi:10.1089/fpd.2011.1032.
6. Mouffok F. Situation en matière TIA en Algérie de 2010 à 2011. in Deuxième congrés magrébin sur les TIA, Tunis. Tunis; 2011. http://mail.pasteur.dz/pasteur-old-2048/presentation.php?page=72. Accessed June 26, 2015.
7. Djeffal S, Bakour S, Mamache B, et al. Prevalence and clonal relationship of ESBL-producing *Salmonella* strains from humans and poultry in northeastern Algeria. BMC Vet Res. 2017;13(1):132. doi:10.1186/s12917-017-1050-3.
8. Listorti V, Battistini R, Ercolini C, et al. In vitro susceptibility of multidrug resistant strains of *Salmonella* to essential oils. Nat Prod Commun. 2020;15(1):1-8. doi:10.1177/1934578x19878904.
9. Chouaibi M, Mahfoudhi N, Rezig L, Donsì F, Ferrari G, Hamdi S. Nutritional composition of *Zizyphus lotus* L. seeds. J Sci Food Agric. 2012;92(6):1171-1177. doi:10.1002/jsfa.4659.
10. Borgi W, Chouchane N. Activité anti-inflammatoire des saponosides des écorces de racines de *Zizyphus lotus* (L.). Revue des Régions Arides; 2006: 283-286.
11. Abdoul-Azize S. Potential benefits of jujube (*Zizyphus lotus* L.) bioactive compounds for nutrition and health. J Nutr Metab. 2016;2016:2867470. doi:10.1155/2016/2867470.
12. Yoon JI, Al-Reza SM, Kang SC. Hair growth promoting effect of *Zizyphus jujuba* essential oil. Food Chem Toxicol. 2010;48(5):1350-1354. doi:10.1016/j.fct.2010.02.036.
13. Adzu B, Amos S, Amizan MB, Gamaniel K. Evaluation of the antidiarrhoeal effects of *Zizyphus spina-christi* stem bark in rats. Acta Trop. 2003;87(2):245-250. doi:10.1016/s0001-706x(03)00114-1.
14. Lahlou M, El Mahi M, Hamamouchi J. [Evaluation of antifungal and mollusuicidial activities of Moroccan *Zizyphus lotus* (L.) Desf]. Ann Pharm Fr. 2002;60(6):410-414.
15. Kumar SP, Asdaq SB, Kumar NP, Asad M, Khajuria DK. Protective effect of *Zizyphus jujuba* fruit extract against paracetamol and thioacetamide induced hepatic damage in rats. Internet J Pharmacol. 2009;7(1):13667.
16. Glombitza KW, Mahran GH, Mirhom YW, Michel KG, Motawi TK. Hypoglycemic and antihyperglycemic effects of *Zizyphus spina-christi* in rats. Planta Med. 1994;60(3):244-247. doi:10.1055/s-2006-959468.
17. Khouchlaa A, Tijane M, Chebat A, Hseini S, Kahouadji A. Enquête ethnopharmacologique des plantes utilisées dans le traitement de la lithiase urinaire au Maroc. Phytothérapie. 2017;15(5):274-287. doi:10.1007/s10298-016-1073-4.
18. Borgi W, Ghedira K, Chouchane N. Antiinflammatory and analgesic activities of *Zizyphus lotus* root barks. Fitoterapia. 2007;78(1):16-19. doi:10.1016/j.fitote.2006.09.010.
19. Ghazghazi H, Aouadhi C, Riahi L, Maaroufi A, Hasnaoui B. Fatty acids composition of Tunisian *Ziziphus lotus* L. (Desf.) fruits and variation in biological activities between leaf and fruit extracts. Nat Prod Res. 2014;28(14):1106-1110. doi:10.1080/14786419.2014.913244.
20. Adeli M, Samavati V. Studies on the steady shear flow behavior and chemical properties of water-soluble polysaccharide from *Ziziphus lotus* fruit. Int J Biol Macromol. 2015;72:580-587. doi:10.1016/j.ijbiomac.2014.08.047.
21. Khouchlaa A, Bouyahya A, Ait Lahcen S, Youssef B, Nadia D, Tijane M. Phytochemical screening, evaluation of antioxidant activity and litholytic effect of *Zizyphus lotus* L. extracts. World J Pharm Res. 2017;6(3):1354-1367. doi:10.20959/wjpr20173-7966.
22. Borgi W, Bouraoui A, Chouchane N. Antiulcerogenic activity of *Zizyphus lotus* (L.) extracts. J Ethnopharmacol. 2007;112(2):228-231. doi:10.1016/j.jep.2007.02.024.
23. Ghedira K. *Zizyphus lotus* (L.) Desf. (Rhamnaceae): jujubier sauvage. Phytothérapie. 2013;11(3):149-153. doi:10.1007/s10298-013-0776-8.
24. Bennamar C, Baghdad C, Belarbi M, Subramaniam S, Hichami A, Akhtar Khan N. Antidiabetic and antioxidant activities of *Zizyphus lotus* L aqueous extracts in Wistar rats. J Nutr Food Sci. 2014;S8:004. doi:10.4172/2155-9600.S8-004.
25. Abdoul-Azize S, Bendahmane M, Hichami A, et al. Effects of *Zizyphus lotus* L. (Desf.) polyphenols on Jurkat cell signaling and proliferation. Int Immunopharmacol. 2013;15(2):364-371. doi:10.1016/j.intimp.2012.11.013.
26. Bencheikh N, Bouhrim M, Kharchoufa L, Choukri M, Bnouham M, Elachouri M. Protective Effect of *Zizyphus lotus* L. (Desf.) fruit against

CCl(4)-induced acute liver injury in rat. Evid Based Complement Alternat Med. 2019;2019:6161593. doi:10.1155/2019/6161593 .
27. Hammami I, Smaoui S, Hsouna AB, Hamdi N, Triki MA. *Ruta montana* L. leaf essential oil and extracts: characterization of bioactive compounds and suppression of crown gall disease. EXCLI J. 2015;14:83-94. doi:10.17179/excli2014-655.
28. Arámbula CI, Diaz CE, Garcia MI. Performance, chemical composition and antibacterial activity of the essential oil of *Ruta chalepensis* and *Origanum vulgare*. J Phys Conf Ser. 2019;1386:012059. doi:10.1088/1742-6596/1386/1/012059.
29. Ouerghemmi I, Bettaieb Rebey I, Rahali FZ, et al. Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian *Ruta chalepensis*. J Food Drug Anal. 2017;25(2):350-359. doi:10.1016/j.jfda.2016.04.001.
30. Gali L, Bedjou F. Antioxidant and anticholinesterase effects of the ethanol extract, ethanol extract fractions and total alkaloids from the cultivated *Ruta chalepensis*. S Afr J Bot. 2019;120:163-169. doi:10.1016/j.sajb.2018.04.011.
31. Di Stasi LC, Oliveira GP, Carvalhaes MA, et al. Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest. Fitoterapia. 2002;73(1):69-91. doi:10.1016/s0367-326x(01)00362-8.
32. Iauk L, Mangano K, Rapisarda A, et al. Protection against murine endotoxemia by treatment with *Ruta chalepensis* L., a plant with anti-inflammatory properties. J Ethnopharmacol. 2004;90(2-3):267-272. doi:10.1016/j.jep.2003.10.004.
33. Atta AH, Alkofahi A. Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. J Ethnopharmacol. 1998;60(2):117-124. doi:10.1016/s0378-8741(97)00137-2.
34. Gonzalez-Trujano ME, Carrera D, Ventura-Martinez R, Cedillo-Portugal E, Navarrete A. Neuropharmacological profile of an ethanol extract of *Ruta chalepensis* L. in mice. J Ethnopharmacol. 2006;106(1):129-135. doi:10.1016/j.jep.2005.12.014.
35. Loizzo MR, Falco T, Bonesi M, Sicari V, Tundis R, Bruno M. *Ruta chalepensis* L. (Rutaceae) leaf extract: chemical composition, antioxidant and hypoglicaemic activities. Nat Prod Res. 2018;32(5):521-528. doi:10.1080/14786419.2017.1326491.
36. Elhaddad NS, Efkerine EM, Khatab HA, Eldurssi IS, Belkacem EM. Anti-mutagenic action of *Ruta chalepensis* against rat sperm cell abnormalities-induced by potassium bromate. Annu Res Rev Biol. 2020;35(2)41-51. doi:10.9734/arrb/2020/v35i230187.
37. Abdelrahim DN, Takruri HR, Al-Ismail KM. Effect of introducing the Jordanian common rue (*Ruta chalepensis* L.) on blood lipid profile in adult male Sprague Dawley rats toxified with Paracetamol. J Saudi Soc Food Nutr. 2020;13(1):17-23.
38. Evans DG, Evans DJ Jr, Tjoa W. Hemagglutination of human group A erythrocytes by enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. Infect Immun. 1977;18(2):330-337. doi:10.1128/iai.18.2.330-337.1977.
39. Fricker CR, Girdwood RW, Munro D. A comparison of enrichment media for the isolation of salmonellae from seagull cloacal swabs. J Hyg (Lond). 1983;91(1):53-58. doi:10.1017/s0022172400060022.
40. Organization for Economic Cooperation and Development (OECD). Repeated dose oral toxicity test method? OCDE guidelines for testing of chemicals. Paris, France: OECD; 2008.
41. European Pharmacopoeia. Guide for the elaboration of monographs on herbal drugs and herbal drug preparations. European Directorate for the Quality of Medicines & HealthCare; 2007. https://www.edqm.eu/medias/fichiers/Technical_Guide_for_the_Elaboration_of_Monographs_on_herbal_drugs_and_herbal_drug_preparations_2007.pdf.
42. Béraud M. The technician biological analyzes theoretical and practical guide. TeDc Lavoisier; 2001:988-990.
43. Finney M, Smullen J, Foster HA, Brokx S, Storey DM. Evaluation of Chromocult coliform agar for the detection and enumeration of *Enterobacteriaceae* from faecal samples from healthy subjects. J Microbiol Methods. 2003;54(3):353-358. doi:10.1016/s0167-7012(03)00068-x.
44. Benammara CH, Benkada D, Benammar F. Analyse par CPG/SM de l'huile essentielle de Ruta chalepensis (Fidjel) de Tlemcen. In: IIème Symposium sur les plantes aromatiques et médicinales, Marrakech; 2006.
45. Boumediene N. Contribution à l'étude de l'activité biologique d'une espèce du genre Rata de Djebel Tessala (*Algérie occidentale*) et à la faisabilité d'un Plan de conservation. In: Département d'Agro-Foresterie, Université Abou Bekr Belkaid: Tlemcen; 2014. http://dspace.univ-tlemcen.dz/bitstream/112/7350/1/BOUMEDIENE.pdf.
46. Dob T, Dahmane D, Gauriat-Desrdy B, Daligault V. Volatile constituents of the essential oil of *Ruta chalepensis* L. subsp. *Angustifolia* (Pers.) P. Cout. J Essent Oil Res. 2008;20(4):306-309. doi:10.1080/10412905.2008.9700019.
47. Ghannadi A, Tavakoli N, Mehri-Ardestani M. Volatile Constituents of the Leaves of *Ziziphus spina-christi* (L.) Willd. from Bushehr, Iran. J Essent Oil Res. 2003;15(3):191-192. doi:10.1080/10412905.2003.9712109.
48. Ourzeddine W, Fadel H, Mechehoud Y, et al. Chemical composition and antioxidant activity of the fruit essential oil of *Zizyphus lotus* (L.) Desf. (Rhamnaceae). Int J Pharmacogn Phytochem Res. 2017;9(2):228-232. doi:10.25258/phyto.v9i2.8067.
49. Alvarez-Castellanos PP, Bishop CD, Pascual-Villalobos MJ. Antifungal activity of the essential oil of flowerheads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens. Phytochemistry. 2001;57(1):99-102. doi:10.1016/s0031-9422(00)00461-1.
50. Demetzos C, Angelopoulou D, Perdetzoglou D. A comparative study of the essential oils of *Cistus salviifolius* in several populations of Crete (Greece). Biochem Syst Ecol. 2002;30(7):651-665. doi:10.1016/s0305-1978(01)00145-4.
51. Haddouchi F, Chaouche TM, Zaouali Y, Ksouri R, Attou A, Benmansour A. Chemical composition and antimicrobial activity of the essential oils from four *Ruta* species growing in Algeria. Food Chem. 2013;141(1):253-258. doi:10.1016/j.foodchem.2013.03.007.
52. Akkari H, Ezzine O, Dhahri S, et al. Chemical composition, insecticidal and in vitro anthelmintic activities of *Ruta chalepensis* (Rutaceae) essential oil. Ind Crops Prod. 2015;74:745-751. doi:10.1016/j.indcrop.2015.06.008.
53. Rustaiyan A, Khossravi M, Sultani-Lotfabadi F, Yari M, Masoudi S, Monfared A. Constituents of the essential oil of *Ruta chalepensis* L. from Iran. J Essent Oil Res. 2002;14(5):378-379. doi:10.1080/10412905.2002.9699892.
54. Abdellaoui K, Acheuk F, Miladi M, Boughattas I, Omri G. Phytochemistry, biochemical and insecticidal activities of *Ruta chalepensis* essential oils on *Tribolium confusum*. Agric For. 2018;64(3):31-45. doi:10.17707/AgricultForest.64.3.03.
55. Mejri J, Chakroun I, Abderrabba M, Mejri M. Study of hydro-distillation process of *Ruta chalepensis* L. essential oil. Res J Agric Environ Manage. 2014;3(10):511-518.
56. Bouabidi W, Hanana M, Gargouri S, et al. Chemical composition, phytotoxic and antifungal properties of *Ruta chalepensis* L. essential oils. Nat Prod Res. 2015;29(9):864-868. doi:10.1080/14786419.2014.980246.
57. El Hachimi F, Alfaiz C, Bendriss A, Cherrah Y, Alaoui K. Activité anti-inflammatoire de l'huile des graines de *Zizyphus lotus* (L.) Desf. Phytothérapie. 2017;15(3):147-154. doi:10.1007/s10298-016-1056-1.
58. Bakhtaoui FZ, Lakmichi H, Megraud F, Chait A, Gadhi CEA. Gastro-protective, anti-*Helicobacter pylori* and, antioxidant properties of Moroccan *Zizyphus lotus* L. J Appl Pharm Sci. 2014;4(10):81-87. doi:10.7324/japs.2014.40115.
59. Abdelhafidh K, Mhadhbi L, Mezni A, Badreddine S, Beyrem H, Mahmoudi E. Protective effect of Zizyphus lotus jujube fruits against cypermethrin-induced oxidative stress and neurotoxicity in mice. Biomarkers. 2018;23(2):167-173. doi:10.1080/1354750X.2017.1390609.
60. Touiti N, Bousta D, Boukhira S, Chebaibi M, Achour S. Phytochemical screening, acute and sub-acute toxicity of aqueous extract from a mixture of some recipe of *Herniaria glabra* L., *Opuntia ficus-indica*, *Zea mays* L. and *Zizyphus lotus* L. used traditionally against renal lithiases. Phcog Res. 2020;12:60-4. doi:10.4103/pr.pr_89_19.
61. Gadiri N, Tigrine C, Lakache Z, Kameli A. Anti-inflammatory, antinociceptive, and antioxidant potentials of Algerian *Zizyphus jujuba* Mill. fruits and leaves in vivo and in vitro in different

experimental models. Phytothérapie. 2020;18(6):362-368. doi:10.3166/phyto-2019-0164.
62. Kandimalla R, Dash S, Kalita S, et al. Protective effect of bioactivity guided fractions of *Ziziphus jujuba* Mill. root bark against hepatic injury and chronic inflammation via inhibiting inflammatory markers and oxidative stress. Front Pharmacol. 2016;7:298. doi:10.3389/fphar.2016.00298.
63. Gelayee DA, Abdelwuhab M, Asrie AB. In vivo antiplasmodial activity evaluation of the leaves of *Ruta chalepensis* L. (Rutaceae) against *Plasmodium berghei*. Discovery Phytomedicine. 2017;4(1):1-7. doi:10.15562/phytomedicine.2017.37.
64. Akbar S. *Ruta graveolens* L. (Rutaceae). In: Handbook of 200 Medicinal Plants. Cham: Springer; 2020. p. 1567-1576. doi:10/1007/978-3-030-16807-0_162.
65. Yamakoshi J, Tokutake S, Kikuchi M, Kubota Y, Konishi H, Mitsuoka T. Effect of proanthocyanidin-rich extract from grape seeds on human fecal flora and fecal odor. Microb Ecol Health Dis. 2001;13(1):25-31. doi:10.1080/089106001750071672.
66. Tzounis X, Rodriguez-Mateos A, Vulevic J, Gibson GR, Kwik-Uribe C, Spencer JP. Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. Am J Clin Nutr. 2011;93(1):62-72. doi:10.3945/ajcn.110.000075.
67. Wiciński M, Gębalski J, Mazurek E, et al. The influence of polyphenol compounds on human gastrointestinal tract microbiota. Nutrients. 2020;12(2):350. doi:10.3390/nu12020350.
68. Ait Abderrahim L, Taibi K, Ait Abderrahim C. Assessment of the antimicrobial and antioxidant activities of *Ziziphus lotus* and *Peganum harmala*. Iranian Journal of Science and Technology. 2019;43(1):409-414. doi:10.1007/s40995-017-0411-x.
69. Boughendjiou A. Yield, chemical composition and antibacterial activity of *Ruta chalepensis* L. essential oil growing spontaneously in Algeria. Pharm Pharmacol Int J. 2019;7(1):33-36. doi:10.15406/ppij.2019.07.00230.
70. Yahia Y, Benabderrahim MA, Tlili N, Bagues M, Nagaz K. Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two *Ziziphus* Mill. species. PLoS One. 2020;15(5):e0232599. doi:10.1371/journal.pone.0232599.
71. Degu A, Kefale B, Alemayehu D, Tegegne GT. Evaluation of the antidiarrheal activity of hydromethanol crude extracts of *Ruta chalepensis* and *Vernonia amygdalina* in mice. Evid Based Complement Alternat Med. 2020;2020:8318713. doi:10.1155/2020/8318713.
72. Peirs C. Contribution à l'étude phytochimique de *Galega officinalis* L. (Fabaceae) [dissertation]. in. Toulouse, France: Faculté des Sciences Pharmaceutiques; 2005.
73. Bai L, Cui X, Cheng N, et al. Hepatoprotective standardized EtOH-water extract of the leaves of *Ziziphus jujuba*. Food Funct. 2017;8(2):816-822. doi:10.1039/c6fo01690a.
74. Geth EM, Eldurssi IS, Algassi AA, Abdalla GM, Hamad MA. Histopathological effects of potassium bromate on liver male rat's and possible protective role of *Ruta chalepensis* L. (Rutacae) oil extract. Asian J Pharm Res Dev. 2019;7(2):93-97. doi:10.22270/ajprd.v7i2.473.