Acta Biologica Szegediensis

Volume 67(1):123-144, 2023
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs

DOI:10.14232/abs.2023.1.123-144

ARTICLE

Acute toxicity and therapeutic application of *Zizyphus lotus* and *Ruta chalepensis* phenolic extracts in treatment of gastroenteritis induced by *Salmonella enterica* subsp. *arizonae*

Nour El Houda Bekkar[1]*, Foufa Bouabsa[1], Boumediene Meddah[1], Bahadir Keskin[2], Yavuz Selim Cakmak[3], Abdenacer Tou[4]

[1]Laboratory of Bioconversion, Microbiological Engineering and Health Safety, Faculty of Life and Nature Sciences, University Mustapha Stambouli of Mascara, 29000, Algeria.
[2]Department of Chemistry, Faculty of Arts & Science, Yildiz Technical University, TR34210 Istanbul, Turkey.
[3]Department of Biotechnology and Molecular Biology, Faculty of Science and Letters, Aksaray University, Aksaray, Turkey.
[4]Anatomopathology Department, University Hospital of Sidi-Bel-Abbes, Sidi-Bel-Abbes, Algeria.

ABSTRACT This study aimed to evaluate the antigastroenteritis effect against *Salmonella enterica* subsp. *arizonae* after therapeutic application of hydromethanolic extracts (MeOH.E) and aqueous extracts (Aq.E) of *Zizyphus lotus* (ZL) and *Ruta chalepensis* (RC). Acute oral toxicity was elucidated using *in vivo* methods and antigastroenteritis effect were evaluated using *S. enterica* subsp. *arizonae*-induced diarrheal model. Furthermore, test groups were treated with 400 mg/kg of the MeOH.E and Aq.E of each plant, while the control group was given neomycin (200 mg/kg) as standard antibiotic treatment, positive and negative controls were given the infectious germ (4×10^6 cells/mL) and 0.9% saline solution NaCl (10 ml/kg), respectively. Both plants extracts showed no toxicity for all the animals, so the LD_{50} was found to be greater than 5000 mg/kg. Moreover, an important bactericidal effect, using both plants extracts was determined against *S. enterica* subsp. *arizonae* cells in the intestine. In parallel, a decrease in alkaline phosphatase, amino alanine transferase and aspartate aminotransferase levels was observed with reduction in blood erythrocyte sedimentation rate in all treated animals. Thus, these results could be exploited in the medical field for the formulation of potent antibacterial drugs that cure severe gastrointestinal infections. **Acta Biol Szeged 67(1):123-144 (2023)**

KEY WORDS
gastroenteritis
phenolic extracts
Ruta chalepensis
Salmonella enterica subsp. *arizonae*
therapeutic application
Zizyphus lotus

ARTICLE INFORMATION
Submitted
15 May 2023
Accepted
9 September 2023
***Corresponding author**
E-mail: bekkarnour8@gmail.com
nourelhouda.bekkar@univ-mascara.dz

INTRODUCTION

Despite the advancement of medicine, diarrheal diseases and gastroenteritis are still the second leading cause of death because of the development of bacterial resistance to various antibiotics, and the emergence of multidrug-resistance (MDR) bacteria in many industrial sectors: collective restaurants, hospitals, and reanimation services (WHO 2017). Even if sufficient antibiotics are available for treating diarrhea of the gastrointestinal diseases, most of patients suffer from adverse effects like nausea, fever, vomiting, gastric and intestinal pains and constipation.

As an example, more than 50% of people were infected with *H. pylori* worldwide (Crowe 2019). The triple or quadruple therapy with different antibiotics in clinical has gradually failed to eradicate the *H. pylori* infection mainly due to the increased drug resistance (Can et al. 2005). Moreover, these antibiotics disrupted the balance of the gastrointestinal flora, metabolism, and the immunity and even increased the risk of other diseases (Belkaid et al. 2014; Kim et al. 2017).

Other adverse effects are headaches with or without vomiting. Intolerance to the drugs due to their adverse effects leads to noncompliance with the treatment, culminating in treatment failure and resistance to these drugs (Shakya Shrestha et al. 2016). Because of this, there is a necessity for researching into medicinal plants to investigate alternative drugs from natural products. Internationally, traditional medicines report for 80% of the healthcare needs of the majority of developing countries (Mohammed et al. 2009). Plant extracts have various natural advantages, such as safety, immunomodulation, and anti-pathogen abilities. They are usually used alone or in combination with antibiotics or probiotics to treat gastrointestinal diseases (Archana et Geetha 2022; Coimbra et al. 2022).

Accordingly, the application of natural drugs may be

served as a potential treatment to inhibit the synergistic infections caused by *pathogenic MDR bacteria.*

However, the usages of medicinal plants in folk medicine for gastroenteritis and diarrhea treatments were handed down from generation to generation, empirically, without having any ideas of the plausible mechanisms, safety, and efficacy of herbal treatments (Mujumdar et al. 2001). For this, the present study is conducted to evaluate the safety profile of *Zizyphus lotus* and *Ruta chalepensis* before its therapeutic applications in treatment of various diseases, and this by determining the toxicity effect of polyphenolic extracts (PPEs) of *Z. lotus* leaves and *R. chalepensis* aerial parts. Also, by determining the influence of these PPEs in the composition and diversity of the intestinal microbiota, especially in probiotic bacteria, that leads an important protective effect of the gastrointestinal mucosa. Also, we determined the therapeutic efficient dose of PPE of both plants in treatment of the gastroenteritis induced by *Salmonella enterica* subsp. *arizonae* and diarrhea model in Wistar rats.

Briefly, we will mention the great interest in using these medicinal plants and some biological activities which are studied and determined by many recent studies. Both plants are popularly known in Algeria and the most used in the treatment of various pathologies. Previous studies have demonstrated the antidiarrheal effects of *Z. lotus* and *R. chalepensis* (Hani et al. 2020; Degu et al. 2020). Zazouli et al. (2022) and Letaief et al. (2021) have determined an important antiproliferative activity of *Z. lotus* extract against cancer cell lines, in addition of an interesting antimicrobial activity. Thus, Dhibi et al. (2022) have demonstrated the antioxidant activities of phenolic compounds extracted from *Z. lotus* leaves. Abdoul-Azize (2016) have determined the richness in polyphenols, cyclopeptide alkaloids, dammarane saponins, vitamins, minerals, amino acids, and polyunsaturated fatty acids that were supposed to be responsible for the different biological activities of *Z. lotus*, including antimicrobial, anti-inflammatory, hypoglycemic, antioxidant, and immunomodulatory effects.

In a recent study carried out by Bekkar et al. (2021a), they have determined important antimicrobial effects of *Z. lotus* and *R. chalepensis* phenolic extracts on clinical pathogenic microorganisms, with a great antibacterial effect against *Salmonella enterica* subsp. *arizonae*. Borgi et al. (2007) have mentioned the antiulcerogenic properties of *Z. lotus* extracts. In addition to the biological effects, this plant represents a potential role in the field of nutrition, because of its richness in nutritional components essential for the organism health.

Z. lotus fruits are consumed by local population in North Africa and especially in Algeria. The nutritional value of this plant is essentially based on its rich composition in vitamin C, vitamin E, fibers, fatty acids, amino acids, calcium, magnesium, and sugars. The vegetable oils contribute to foods flavor, taste, and texture (Chouaibi et al. 2012). Besides, Chouaibi et al. (2012) have declared that the seeds of this plant are rich in fat and protein and the seed oil contained various bioactive components that could be of a great economic interest with several applications in food, cosmetics, and medicinal industries. In a recent study by Szewczyk et al. (2022), they have determined that *R. chalepensis* extract exhibited an important radical scavenging effect with an antibacterial activity against *Staphylococcus aureus*. Althaher et al. (2020) have demonstrated an interesting apoptotic activity of the essential oil of this plant against experimental breast carcinoma.

R. chalepensis methanolic extracts have been mentioned as potent antidiabetic agents (Al-Ismail et al. 2022). Besides, Velàzquez et al. (2006) have showed that the methanolic aerial parts extract of this plant exert a significant antisecretory activity on cholera toxin-induced intestinal secretion in a rat jejunal loops model. Other biological activities of *R. chalepensis* extracts have been investigated. This plant is known for its anti-inflammatory properties, as potent antibacterial and antifungal, having a cytotoxic effect on cancer cell-lines, for its antiparasitic activity, antilarvicidal and insecticidal effects (Nahar et al. 2021).

In addition to all these biological effects of both plants which are studied and determined by numerous studies, *Z. lotus* and *R. chalepensis* are much more used in traditional Algerian medicine for the treatment of many pathologies, especially the intestinal disorders, by preparing herbal teas: infusion or decoction based on these medicinal plants. Despite this traditional claim and previous studies, it was not completely investigated scientifically to validate its therapeutic effects. Therefore, the present study aimed to scientifically validate its importance to corroborate

Figure 1. *Zizyphus lotus* (A) leaves and *Ruta chalepensis* (B) aerial part (leaves, flowers and small stems).

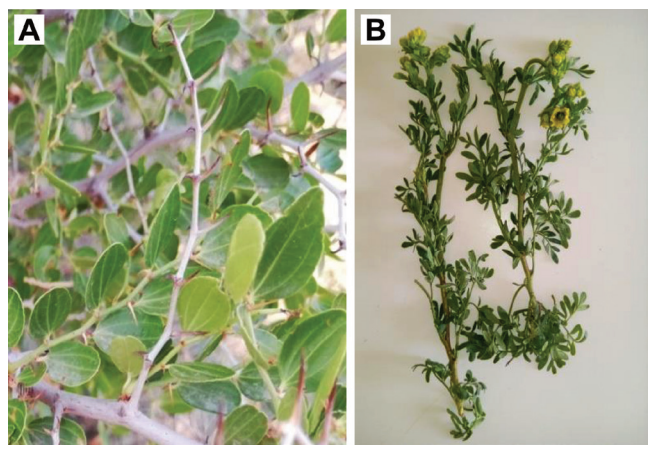


Table 1. Antibiotic resistance profile of *Salmonella enterica* subsp. *arizonae* strain.

	Growth inhibition zone diameters (mm)								
Antibiotics	SP	AMX	PT	NI	N	OX	CT	P	P-G
S. enterica subsp. *arizonae*	0^R	0^R	0^R	22^I	18^S	0^R	13^R	0^R	0^R

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.

the traditional claim in Algeria.

Our research team previously published the phenolic composition of the methanolic and the aqueous extracts of *Z. lotus* and *R. chalepensis*. The phenolic extracts of *Z. lotus* were shown to be rich in phenolic acid compounds, especially the benzoic acid (1333.59 µg/g DE). The major identified compounds in *R. chalepensis* extracts were the hydroxybenzoic acid (56.60 µg/g DE), chlorogenic acid (44.60 µg/g DE), epicatechin (38.80 µg/g DE), catechin (26.30 µg/g DE) and gallic acid (13.22 µg/g DE) (Bekkar et al. 2021a). Furthermore, the efficiency of essential oils of these plants in the treatment of enteric infection induced in Wistar rat model was elucidated in a recent study (Bekkar et al. 2021b).

MATERIALS AND METHODS

Plant material

Plant material used during this study were leaves of *Z. lotus*, known under the vernacular name "*Sedra*" in Algeria and the aerial parts (leaves, flowers, and small stems) of *R. chalepensis* "*Fidjel*", collected during the flowering stage, from the region of El-Mamounia, in Mascara-Algeria. Both plants specimens were identified by a botanist from Mascara University's Department of Biology. *Z. lotus* and *R. chalepensis* were rinsed in clean water, air-dried at room temperature, and then transformed into a fine powder that was used to prepare the various polyphenolic extracts (PPE).

Bacterial isolate

The MDR strain of *Salmonella enterica* subsp. *arizonae* ($S_{10}SP^5$) used for induction of enteric infection was isolated from fecal matter samples of gastroenteritis patients and identified in Meslem-Tayeb Hospital, Laboratory of Microbiological Analysis of Mascara (Algeria), and the Laboratory of Bioconversion, Microbiological Engineering and Health Safety of the Department of Biology, Mascara University. Table 1 represents the antibioresistance profile of this bacterial strain determined according to the critical bacterial growth inhibition diameters mentioned by FMS-AC/EUCAST: French Society of Microbiology-Antibiogram Committee/European Committee on Antimicrobial Susceptibility Testing (2020). Bacteria were cultured in Brain Infusion Heart (BHIB) Broth at 37 °C overnight. Subsequently, diluted in sterile physiological water 0.9% (NaCl) to achieve bacterial suspensions of 4×10^6 cells/mL concentration in exponential phase of microbial growth.

Experimental animals

The *in vivo* experiments were realised on male Wistar rats with an average weight of 150-200 ± 5 g body weight (b.w.). Animals were supplied by the Animal Care Facility of the Faculty of Life and Nature Sciences, University of Mascara, Algeria. Animals were divided into different groups which are mentioned in the experimental design section, 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*. Furthermore, 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).

Polyphenolic extracts preparation

Extraction of phenolic compounds from *Z. lotus* leaves and *R. chalepensis* leaves, flowers, and small stems was carried out using two techniques: maceration at room temperature with magnetic stirring for 24 h to prepare hydromethanolic extracts (MeOH.E), as described by Romani et al. (2006) with minor modifications, and decoction in distilled water for the aqueous extracts (Aq.E), following the method of Chavan et al. (2001). Our team has previously conducted and reported a phytochemical investigation on these plant phenolic extracts (PPE), which included qualitative screening for bioactive compounds, quantitative assessments of total phenolics, flavonoids, and tannins, and the characterization of various phenolic substances via High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD). The study also encompassed evaluating the in vitro antioxidant and antimicrobial properties, as detailed in Bekkar et al. (2021a).

Experimental design

For the oral acute toxicity evaluation, the Wistar rats were randomly divided into five groups of 5 rats each (n = 5): negative control group (NCG) n°1 contains healthy Wistar rats, not treated with the PPE, group n°2 of animals treated with $ZL^{MeOH.E}$, group n°3 of animals treated with $ZL^{Aq.E}$, group n°4 of animals treated with $RC^{MeOH.E}$ and group n°5 of animals treated with $RC^{Aq.E}$. The same group distributions were used to study the influence of PPE on the gastrointestinal microbiota composition.

Whereas, for the therapeutic application of PPE in treatment of gastroenteritis induced by *S. enterica* subsp. *arizonae*, seven groups (n = 5) were used: NCG group contains healthy Wistar rats, not infected with the clinical MDR isolate of *S. enterica* subsp. *arizonae*, and not treated with the extracts of *Z. lotus* and *R. chalepensis*, positive control (PCG) group B contains Wistar rats infected with MDR *S. enterica* subsp. *arizonae* isolate without treatment with both plants extracts, group C contains Wistar rats infected with the pathogenic bacteria and treated with antibiotic: neomycin (ITG^{N}), group D and E contains Wistar rats infected and treated with $ZL^{Me.E}$ and $ZL^{Aq.E}$ respectively, while group F and G contains Wistar rats infected with *Salmonella* isolate and treated with $RC^{Me.E}$ and $RC^{Aq.E}$, respectively. Before experimental analysis, the animals were fasted for 13h with access to water only. All the experimental procedures were performed in accordance with the ethical guidelines of the Organization for Economic Cooperation and Development (OECD).

Acute oral toxicity assessment

Acute oral toxicity of *Z. lotus* and *R. chalepensis* PPE was carried out according to the OECD guidelines (2008). Experiments were performed on 15 Wistar rats of 160 ± 10 g b.w. divided into five groups as mentioned previously in the experimental design. These animals were fasted 13h before the experiment, weighed and then treated with both plants extracts by esophageal gavage.

Wistar rats of the NCG received 10 mL/kg b.w. of sterile physiological water 0.9% (NaCl), and those of the test groups were given a single unique dose: 5000 mg/kg b.w. of each plant extract: $ZL^{MeOH.E}$, $ZL^{Aq.E}$, $RC^{MeOH.E}$ and $RC^{Aq.E}$. Subsequently, Wistar rats were fasted for additional 3h after treatment. The body weight gain, undesirable side effects and toxicity symptoms including mobility, aggressiveness, somnolence, stool and urine aspect and color, anxiety state, weight loss, vomiting, fever, and diarrhea were examined during this experiment. Moreover, death was monitored 14 days following the treatment. After the 14^{th} days of the experiment, Wistar rats were anesthetized and then sacrificed. The organs: liver, kidneys, heart, lung, and spleen were removed, and their relative weights were determined.

The assessment of the toxicity signs in Wistar rats treated with the single higher concentration of PPE was made at the holistic level of each animal, and at the level of organs and biological functions.

Effects of Z. lotus and R. chalepensis polyphenolic extracts on gastrointestinal microbiota

Investigation composition and diversity of the gastrointestinal microbiota

Fifteen (15) Wistar rats were utilized in the experiment, following a 13-hour fasting period. The rats were divided into five groups, including a control group and four test groups, as previously described. The NCG received 10 mL/kg body weight of 0.9% NaCl solution via oral gavage, while the test groups were administered a single unique dose of 5000 mg/kg body weight of *Z. lotus* and *R. chalepensis* PPE, respectively.

Following the treatment, the Wistar rats underwent a 3-hour fasting period and were subsequently anesthetized under aseptic conditions after 18 h. The digestive tracts were surgically removed, and samples from the ileum and colon (1 g each) were homogenized in 9 mL of sterile 0.9% physiological saline (NaCl). A sequential dilution based on a decimal dilution system was applied to achieve a 10^{-5} dilution.

Nutrient and selective agar mediums in Petri dishes received inoculation with 100 µL of the diluted solutions from the ileum and colon samples. Incubation was carried out at 37 °C for 24 to 72 h, depending on the bacterial strains under investigation.

The bacterial groups examined during this study were the total mesophilic aerobic flora (TMAF) in Plate Count Agar (PCA), the total anaerobic flora (TANF) in Colombia Agar, Enterobacteriaceae in Hektoen Agar, *Escherichia coli* in Methylene Blue Eosin Agar (EMB), Staphylococcaceae on Chapman Agar, Streptococcaceae on Bil Eosin Agar (BEA) and the probiotic bacteria of the genus *Lactobacillus* isolated in Man Rogosa Sharp Agar (MRS).

The enumeration of viable bacteria in each sample was made on Petri dishes presenting between 30 and 300 colonies, and results were expressed as Log Colony Forming Unit (CFU) per gram of sample according to the following formula (Béraud 2001):

$$\text{Log CFU/g} = \text{Number of colonies} / (\text{Dilution} \times \text{Inoculated volume}^{100\ \mu L}).$$

Biochemical parameters determination

Blood samples were collected from the abdominal aorta of the Wistar rats following their sacrifice and placed into heparin tubes for subsequent analysis. Commercial kits provided by Hospitex Diagnostics (Germany) were

utilized for the accurate quantification of these biochemical parameters.

These parameters included lipid profiles, such as Total Cholesterol (TC), Triglycerides (Tg), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL). Additionally, hepatic parameters were assessed, including Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Direct Bilirubin (DB), Total Bilirubin (TB), and Total Serum Protein (TSP). Renal parameters, namely Blood Urea (BU) and Blood Creatinine (BC), were also measured. Furthermore, Glycemia (Gs) levels were determined.

Histological analysis

The histological analysis was performed on liver samples, from which thin fragments were prepared and previously fixed in formalin solution 10%. These fragments were deposited in cassettes and then placed in a dehydration machine (Leica TP1020). Histological sections were prepared with a Leica-type microtome, and then subjected to a deparaffinization phase. The histological sections were then rehydrated for the detection of hepatocytes, placed in a hematoxylin and eosin baths to stain the nuclei and the cytoplasm, respectively. A microscopic examination was carried out for the detection of inflammatory infiltrates (immune cells).

Therapeutic application of Z. lotus and R. chalepensis phenolic extracts

In vivo anti-Salmonella enterica subsp. arizonae effect

For the *in vivo* therapeutic application of *Z. lotus* and *R. chalepensis* PPE in the treatment of gastroenteritis induced by *S. enterica* subsp. *arizonae*, 25 Wistar rats weighted 200 ± 5 g b.w. were used (n = 5 per test group). The animals were divided into seven groups as mentioned in the experimental design. The NCG that received 10 mL/kg of normal saline water: placebo effect solution, whereas the PCG was given 2 mL of *S. enterica* subsp. *arizonae* suspension, prepared previously in sterile physiological water (0.9%) and of 4 x 10^6 CFU/mL concentrations in exponential phase.

Wistar rats in the test groups C, D, E, F and G were firstly administered 10 mL/kg of *S. enterica* subsp. *arizonae* induced gastroenteritis. Subsequently, after 3 days of an incubation period of the MDR isolate of *Salmonella* to induce its pathogenicity at the level of the intestine, the Wistar rats in group C were treated with an antibiotic therapy: neomycin$^{200\,mg/kg}$, While, Wistar rats in group D, E, F and G were daily treated by oral administration of a single dose (400 mg/kg) of $ZL^{MeOH.E}$, $ZL^{Aq.E}$, $RC^{MeOH.E}$ and $RC^{Aq.E}$, respectively for 7 consecutive days. Clinical symptoms were determined in Wistar rats of all test groups, and the body weight evolutions were reported.

At the end of the experiments, the animals were anesthetized and sacrificed to complete the study. Internal organs were removed, and their weights were determined. Blood samples were collected in EDTA-type anticoagulant and sodium citrate blood specimen collection tubes for further analysis.

Diarrheal parameters determination

Faecal water content

The percentage of Wistar rats that responded to diarrhea, the latency period: the time between the administration of *S. enterica* subsp. *arizonae* and the appearance of the first diarrheal drop, and faecal water content (FW) were recorded. The stool samples (M_1 = 1 g) taken from animals in the different test groups were transported immediately to the laboratory, dried for 18 h and reweighed (M_2). The fecal water content (FWC) was calculated as follows (Murugesan et al. 2000):

$$FWC = (M_1 - M_2)\ g.$$

Anti-enteropooling activity

Enteropooling which is defined as the accumulation of intraluminal fluid was determined as described by Islam et al. (2013). For this evaluation, Wistar rats deprived of food and water for 18 h were selected in 7 groups as mentioned previously: NCG, PCG, ITG^{N}, $ITG^{ZLMeOH.E}$, $ITG^{ZLAq.E}$, $ITG^{RCMeOH.E}$, and $ITG^{RCAq.E}$. These different groups received both plants extracts at 400 mg/kg b.w. and the standard antibiotic: neomycin at 200 mg/kg b.w. by gastric gavage. Thirteen minutes after the treatment, the Wistar rats were given 10 mL/kg b.w. of *S. enterica* subsp. *arizonae* 4 × 10^6 CFU/mL suspension. Thus, 3 days after the administration of the infectious germ, each Wistar rat was sacrificed and the ends of the small intestine were attached, at the level of the pylorus and the caecum. This section was dissected for recovery of intestinal contents. The intestinal contents were collected in pre-weighed graduated tubes (m_0) and the new weight (m_1) was measured. The intraluminal fluid mass was calculated as $m_1 - m_0$ (g).

The average percentage inhibition of intestinal liquid content weight (IILC) was determined using the following formula (Mamza et al. 2014):

$$IILC = [(A-B) / A] \times 100$$

Where, A is the average weight of the intestinal contents of the PCG, and B is the average weight of the intestinal contents of the test groups.

Detection of Salmonella enterica subsp. arizonae in fecal flora: Stool examination

The stool examination was carried out with the aim of detecting the MDR *S. enterica* subsp. *arizonae* in the faecal flora by its isolation on selective Salmonella Shigella (SS) Agar medium (Kubab et al. 2006). The stool samples (1 g) taken in aseptic conditions were immediately transported to the Laboratory of Bioconversion, Microbiological Engineering and Health Safety of Mascara University for microbiological analysis. Decimal dilutions were prepared to achieve the 10^{-4} dilution and volumes of 100 µL were inoculated in Petri dishes of SS agar medium. Enumeration of the viable *S. enterica* subsp. *arizonae* expressed in Log CFU/g was made on colorless colonies and identification was carried out using biochemical tests of API 20E system (bioMerieux).

Hematological analysis: Blood Cells Count (CBC)

The hematological analysis was carried out by Hemogram or Complete Blood Count (CBC). Various hematological parameters including White Blood Cells (WBC), Red Blood Cells (RBC), Granulocytes (GRA), Hematocrit (HT), Platelets (PLT), Hemoglobin (HB), Mid-range percent (MID) including basophils, eosinophils and monocytes, Lymphocytes (LYM), Mean Corpuscular Hemoglobin Content (MCHCt), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) were analyzed and quantified using DIATRON automation hematology (Abacus 380).

Determination of lipid parameters

A volume of blood for each Wistar rat in the ITG^N was collected in heparinized tubes for the determination of an important lipid parameter. An observation of an important and large mass of fat after dissection of animals in the infected treated group with neomycin led us to analyze the blood triglycerides (TG), to confirm any hypercholesterolemia, using commercial kits (Hospitex Diagnostics, Germany).

Determination of inflammation and infection markers

The inflammation and infection markers including Alanine Aminotransferse (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), C-reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR) were analyzed in the serum of blood samples using commercial kits (Hospitex Diagnostics, Germany).

Detection of bacteremia and bacterial translocation

Rigorous injection of blood sterile tubes containing a broth for blood culture was carried out under aseptic conditions. The tubes were incubated at 37 °C under aerobiosis. Blood cultures were examined daily for 15 days before returning a negative result.

The bacterial translocation (BT) from the gastrointestinal microbiota to internal sterile organs was done by counting in selective agar mediums, the viable bacteria in the liver, spleen, and lung sample solutions (1 g), previously prepared in sterile saline water 0.9%. This experiment allowed us to detect bacteria with high pathogenicity frequency, expressed by its ability to cross intestinal defense barriers, passing through the blood or lymphatic systems to other sterile organs.

Statistical analysis

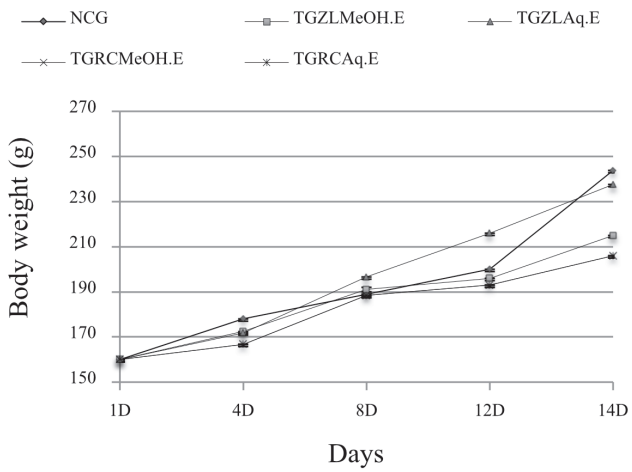
Replicates were prepared for all experiments (n = 5) and results were given as means and their standard deviations (means ± SD). The statistical analyses were realized using the SPSS software for comparing between the averages using the one-way and multivariate analysis of variance

Table 2. Clinical signs resulting from *Z. lotus* and *R. chalepensis* phenolic extracts administration.

Toxicity signs	Wistar rats (n = 5)				
	NCG	$TG^{ZLMeOH.E}$	$TG^{ZLAq.E}$	$TG^{RCMeOH.E}$	$TG^{RCAq.E}$
Mobility	N	RM	N	RM	N
Aggressiveness	N	N	N	N	N
Somnolence	A_{bs}	A_{bs}	A_{bs}	P_{rs}	A_{bs}
Stool aspect and color	N	N	N	N	N
Urine aspect and color	N	N	N	N	N
Anxiety state	N	N	N	A_b	N
Vomiting	A_{bs}	A_{bs}	A_{bs}	A_{bs}	A_{bs}
Fever	A_{bs}	A_{bs}	A_{bs}	A_{bs}	A_{bs}
Diarrhea	A_{bs}	A_{bs}	A_{bs}	A_{bs}	A_{bs}
Mortality	A_{bs}	A_{bs}	A_{bs}	A_{bs}	A_{bs}

NCG: Negative Control Group; TG: Treated Group; RM: Reduced Mobility; N: Normal; A_b: Abnormal; P_{rs}: Presence; A_{bs}: Absence.

Figure 2. Body weight evolution of Wistar rats treated with *Z. lotus* and *R. chalepensis* hydromethanolic and aqueous extracts ($p < 0.05$).



(ANOVA). Significant differences were also mentioned ($p < 0.05$).

Results and discussions

Many herbal medicines cause serious side effects for their users (Aguianaga et al. 2014; Naidu et al. 2014). Although the pharmacological properties of medicinal plant extracts are continually being established, their safety needs to be carefully studied to maximize their health benefits (Ebelle Etame et al. 2017).

In this study, the polyphenolic extracts from *Z. lotus* and *R. chalepensis* have been the subject of toxicological studies to minimize the risks of using these plants at various doses. For this purpose, an acute oral toxicity study of both plants PPE was carried out on Wistar rats, with a body weight of 150 to 200 g and receiving a high dose, 5000 mg/kg b.w. for each PPE of each plant. The present study was therefore designed to investigate the safety of the hydromethanolic and aqueous extracts of *Z. lotus* leaves and the flowering aerial part of *R. chalepensis*. Furthermore, the antimicrobial effect of phenolic extracts of *Z. lotus* leaves and *R. chalepensis* aerial parts against enteric pathogenic bacteria, *S. enterica* subsp. *arizonae*, through *in vitro* and *in vivo* experiments were also investigated.

Clinical signs and body weight evolution

Oral administration of hydromethanolic and aqueous extracts of *Z. lotus* and *R. chalepensis* by gastric gavage, at a concentration of 5000 mg/kg did not cause any mortality, as well as no clinical sign was observed during the 14 days of the experiment. The absence of mortality in different test groups, and the non-detection of adverse side effects indicated that both plants PPE were not toxic in a single stronger dose administration, therefore the LD_{50} was greater than 5000 mg/kg ($LD_{50} > 5000$ mg/kg).

Results of the various clinical signs, behavior, and the body weight evolution of Wistar rats are mentioned in Table 2 and Figure 2. Our results were revealed in agreement with those of Gonzalez et al. (2006) who determined an LD_{50} greater than 5000 mg/kg for *R. chalepensis* extracts. The LD_{50} of *Z. lotus* determined during this study was consistent with the results of Sirreratawong et al. (2012). However, Borgi et al. (2007) showed that the extracts of this plant are toxic by intraperitoneal administration, at a dose of 1000 mg/kg b.w. in albino mice.

A normal increase in body weight was observed in all treated Wistar rats compared to animals in the control group (Fig. 2). Treatment with hydromethanolic and aqueous extracts of *Z. lotus* and *R. chalepensis* did not result in any significant changes in body weight. In a previous study, it was demonstrated that the administration of an HFADP diet (HFAD+ZL pulp) led to a significant decrease in the body weight of rats (Ghalem et al. 2018).

However, undesirable side effects were observed during the first 3 h after treatment, including reduced mobility, anxiety, and somnolence in the animals treated with the hydromethanolic extracts of *R. chalepensis* (Table 2). These effects began to disappear 18 h after treatment.

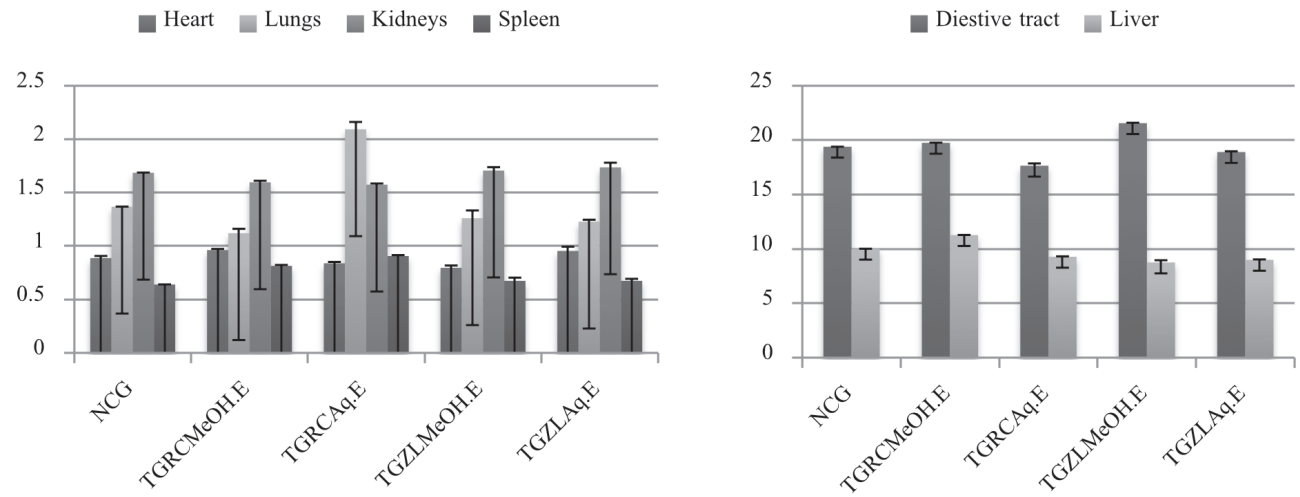
The side effects of treatment with both plants PPEs have been documented in a table that briefly mentions various clinical signs. In human medicine, this table is of paramount importance as it contains the most precise possible list of symptoms or signs of clinically observable pathological conditions.

The decrease in locomotor activity may reflect the sedative effect of *Z. lotus* and *R. chalepensis* PPE, while the somnolence effect could be attributed to the stimulation of melatonin synthesis (N-acetyl-5-methoxytryptamine), often referred to as the sleep hormone. This stimulation occurs following the administration of *R. chalepensis* hydromethanolic extracts ($RC^{MeOH.E}$). This phenolic extract could induce the abundant secretion of a neurotransmitter, serotonin (serotonin N-acetyltransferase), by the pineal gland in the brain. The latter catalyzes an N-acetylation reaction, leading to the production of N-acetylserotonin, which is subsequently transformed into melatonin by the enzyme acetylserotonin O-methyltransferase enzyme (ASMT). Consequently, somnolence developed in Wistar rats (Altun and Ugur-Altun 2007). This side effect disappeared a few days after the treatment.

Organ's weight

The organ weights of Wistar rats treated with both plants PPEs are depicted in Figure 3. Oral ingestion of *Z. lotus* and *R. chalepensis* PPEs did not lead to any significant

Figure 3. Animals organs weight after treatment with *Z. lotus* and *R. chalepensis* polyphenolic extracts.



changes in organ weight, including the digestive tract, liver, kidneys, heart, lungs, and spleen (Fig. 3).

A decrease in the relative weight of the liver was observed at a dose of 5000 mg/kg b.w. This decrease could be attributed to a potential hepatoprotective activity of both plant PPEs (Ozturk et al. 2009). However, an increase in liver weight was recorded in Wistar rats administered the hydromethanolic extract of *R. chalepensis*, suggesting a possible hepatotoxic activity (Fig. 3). Additionally, during the removal of internal organs, an abnormal appearance of the livers of rats treated with this extract was noted, characterized by the presence of black spots and an abundance of blood vessels. These findings led us to infer that the $RC^{MeOH.E}$ extract may be hepatotoxic. Further analysis of hepatic biochemical parameters and histological studies confirmed the presence of anomalies, lesions, and hepatic inflammation.

We also observed ulcerations in the stomach and intestines of animals treated with the $RC^{MeOH.E}$ extract. These gastric mucosal lesions were distributed along the digestive tract, indicating a disruption of the protective layer of the mucous membranes at the sites of ulcer formation. This damage can be attributed to the treatment with the hydromethanolic extract of *R. chalepensis*, which suggests that the extract penetrated the intestinal wall and caused cellular damage.

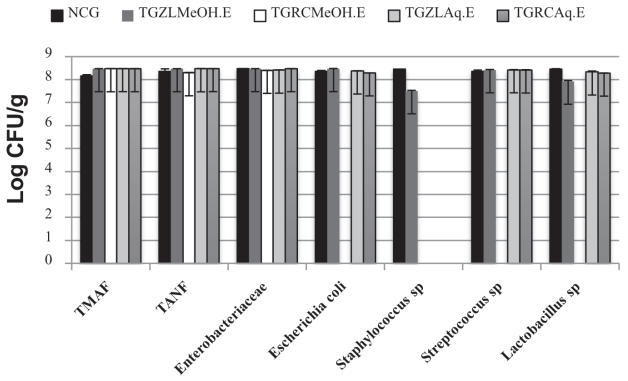
Effects of *Z. lotus* and *R. chalepensis* extracts on gastrointestinal microbiota

These experiments were conducted to assess the impact of plant extracts on key bacteria within the native gastrointestinal tract microbiota (GM). GM plays a crucial role in maintaining the integrity of the intestinal mucosa, acting as a barrier against various pathogenic microorganisms and contributing to immune system development. Our objective was to explore antibiotic alternatives that do not negatively affect GM, in contrast to antibiotic therapy, which often leads to adverse side effects. Additionally, we conducted an evaluation of the in vivo antimicrobial activity against Lactobacillus, a bacterium that promotes the growth of beneficial microorganisms and inhibits pathogenic microorganisms through the production of specific substances, contributing to probiotic effects.

The results of GM enumeration in the ileum and colon of both treated and control animals are presented in Figures 4 and 5 as Log N CFU/g (colony-forming units per gram of tissue). This analysis encompassed the enumeration of the total mesophilic aerobic flora, total anaerobic flora, Enterobacteriaceae, as well as Staphylococcaceae and Streptococcaceae.

The results revealed a significant increase ($p < 0.05$) in total mesophilic aerobic flora (TMAF) in the intestines of rats treated with *Z. lotus* and *R. chalepensis* extracts (Figs. 4-5). Similarly, higher levels ($p < 0.05$) of anaerobic bacteria (TANF) were detected in both parts of the intestine in treated animals when compared to the control group. In the control group, microbial counts for TMAF were determined to be 8.166 ± 0.05 Log CFU/g in the ileum and 8.179 ± 0.13 Log CFU/g in the colon. However, in the intestine of treated animals with both hydromethanolic and aqueous extracts, samples from the ileum recorded microbial counts of 8.476 ± 0.001, 8.470 ± 0.009, 8.476 ± 0.001, and 8.472 ± 0.007 Log CFU/g for animals treated with $ZL^{MeOH.E}$, $RC^{MeOH.E}$, $ZL^{Aq.E}$, and $RC^{Aq.E}$, respectively (Fig. 4). A similar trend was observed in the colon samples of the treated rats when compared to those of the control

Figure 4. *In vitro* evaluation of gastrointestinal microbiota (GM) in the ileum after treatment with *Z. lotus* and **R. chalepensis** phenolic extracts ($p < 0.05$). NCG: Negative Control Group; $TG^{ZLMeOH.E}$: Treated Group with *Z. lotus* Methanolic Extract; $TG^{ZLAq.E}$: Treated Group with *Z. lotus* Aqueous Extract; $TG^{RCMeOH.}$E: Treated Group with *R. chalepensis* Methanolic Extract; $TG^{RCAq.E}$: Treated Group with *R. chalepensis* Aqueous Extract.



group (Fig. 5). These results suggest a stimulating effect of *Z. lotus* and *R. chalepensis* phenolic extracts on bacterial growth in the intestine.

E. coli counts in the ileum and colon of the control group (CG) were 8.352 ± 0.046 and 8.364 ± 0.095 CFU/g, respectively. Additionally, representatives of *Staphylococcus*, *Streptococcus*, *Lactobacillus* genera, and strict anaerobic bacteria were also detected (Fig. 4-5). Abundant Enterobacteriaceae presence was observed in animals treated with plant phenolic extracts. However, after *R. chalepensis* hydromethanolic extract ($RC^{MeOH.E}$) treatment, no *E. coli* was cultured from the ileum and colon.

Staphylococcus was primarily detected in the ileum of the control group and with lower microbial counts in the ileum samples of *Z. lotus* hydromethanolic extract-treated animals. *Staphylococcus* was not detected in intestines of animals treated with other plant extracts, indicating their bactericidal effect on these bacteria.

Increased *Streptococcus* abundance was observed in ileum and colon samples of test groups compared to controls (Figs. 4-5). Streptococci were not detected in the colon of the control group but present in the ileum. $RC^{MeOH.E}$ eliminated *Streptococcus* in both parts of the intestine, while $RC^{Aq.E}$ led to high microbial counts. No impact for *Z. lotus* extracts on *Streptococcus* was observed. The search for *Clostridium* sulphite reducers yielded negative results for all animals in both the control and test groups. *Lactobacillus* sp. were cultured from the intestines of both control and treated animals. Microbial counts were similar but just slightly higher in test groups as in controls, suggesting the tolerance of lactobacilli to these plant extracts.

In summary, *R. chalepensis* hydromethanolic extract adversely affected bacterial composition, eliminating *E. coli*, *Streptococcus* sp., and *Lactobacillus* sp. However, *Z. lotus* and *R. chalepensis* phenolic extracts stimulated bacterial growth, including Enterobacteriaceae (e.g., *E. coli*), *Streptococcus* sp., and *Lactobacillus* sp.

According to Hale (2003), phenolic compounds are recognized as phytochemicals with prebiotic effects, promoting the growth of bacteria such as Enterobacteriaceae (*E. coli*), *Streptococcus* sp., and *Lactobacillus* sp. In a study by Yamakoshi et al. (2001), grape seed extracts were shown to increase *Bifidobacterium* populations while reducing Enterobacteriaceae. Additionally, Tzounis et al. (2011) observed a significant increase in *Bifidobacterium* and *Lactobacillus* populations due to the consumption of cocoa-derived flavonols. Furthermore, Wiciński et al. (2020) reported that bioactive compounds extracted from medicinal plants influenced the gastrointestinal microbiota by elevating the levels of probiotic genera *Bifidobacterium* and *Lactobacillus* while diminishing the presence of pathogenic microorganisms such as *Clostridium* sp.

Previous research has also explored the antimicrobial properties of *Z. lotus* and *R. chalepensis* extracts. The methanolic extract of *R. chalepensis* exhibited inhibition of bacterial (*E. coli* and *S. aureus*) and yeast (*Candida albicans*) growth. High concentrations of flavonoids in the methanolic extract are known for their potent antimicrobial activity (Al-Salmani et al. 2021; Bekkar et al. 2021a). Similarly, *Z. lotus* methanolic extract displayed inhibitory effects on the growth of *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *C. albicans* (Ait Abderrahim et al. 2019). Previous studies have also demonstrated the antimicrobial activity of *Ziziphus* fruits and leaves extracts against vari-

Figure 5. *In vitro* evaluation of gastrointestinal microbiota (GM) in the colon after treatment with *Z. lotus* and *R. chalepensis* phenolic extracts ($p < 0.05$). NCG: Negative Control Group; $TG^{ZLMeOH.E}$: Treated Group with *Z. lotus* Methanolic Extract; $TG^{ZLAq.E}$: Treated Group with *Z. lotus* Aqueous Extract; $TG^{RCMeOH.E}$: Treated Group with *R. chalepensis* Methanolic Extract; $TG^{RCAq.E}$: Treated Group with *R. chalepensis* Aqueous Extract.

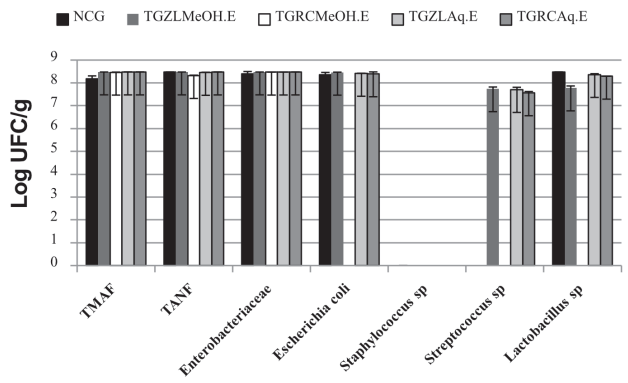


Table 3. Lipid profile (g/L) of Wistar rats after treatment with *Z. lotus* and *R. chalepensis* phenolic extracts. Results are expressed as means ± SD (n = 5). $p < 0.05$.

	TC	TG	HDL	LDL	G
CG	1.244 ± 0.03	0.544 ± 0.059	0.252 ± 0.022	0.914 ± 0.021	1.088 ± 0.139
$TGZL^{MeOH.E}$	1.19 ± 0.007	0.364 ± 0.045	0.414 ± 0.023	0.702 ± 0.015	0.872 ± 0.011
$TGZL^{Aq.E}$	1.066 ± 0.026	0.436 ± 0.029	0.394 ± 0.009	0.526 ± 0.018	1.032 ± 0.066
$TGRC^{MeOH.E}$	1.152 ± 0.019	0.98 ± 0.007	0.356 ± 0.034	0.616 ± 0.032	1.078 ± 0.022
$TGRC^{Aq.E}$	1.126 ± 0.011	0.48 ± 0.02	0.258 ± 0.023	0.796 ± 0.023	0.86 ± 0.077

TC: Total cholesterol; TG: Triglycerides; HDL: High density lipoprotein; LDL: Low density lipoprotein; G: Glucose.

ous bacteria, including *S. aureus*, *E. coli*, *Salmonella typhi*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Bacillus subtilis* (Abdulla et al. 2016). Fruits and leaves of *Z. lotus* were found to have higher polyphenol and flavonoid content compared to roots and seeds.

The antimicrobial effect of *Ziziphus* extracts may be attributed to their phytochemical components, particularly polyphenols (Abdoul-Azize 2016). Polyphenols are believed to exert harm on microorganisms by chelating essential metal ions required for microbial growth or through non-specific interactions with cell wall proteins or extracellular enzymes, ultimately leading to the inhibition of bacterial proliferation (Rsaissi et al. 2013).

The impact of *Z. lotus* and *R. chalepensis* extracts on blood biochemical parameters

Lipid and glucose profile

The mean concentrations of these parameters in the treated and control rats are summarized in Table 3. It was observed that the administration of different extracts led to significant changes in most serum endpoint levels in the treated groups compared to the control group (CG). Administration of $RC^{MeOH.E}$ increased the levels of triglycerides (TG; 0.98 ± 0.007 g/L) and high-density lipoprotein (HDL; 0.356 ± 0.034 g/L) while reducing the level of low-density lipoprotein (LDL; 0.616 ± 0.032 g/L). On the other hand, $ZL^{MeOH.E}$ lowered the levels of TG (0.364 ± 0.045 g/L) and LDL (0.414 ± 0.023 g/L) and elevated the HDL level (0.414 ± 0.023 g/L). Cholesterol levels in the treated groups did not differ significantly from the CG.

Saad et al. (2018) reported that the administration of *R. chalepensis* extracts did not affect serum cholesterol and triglyceride levels. However, it has been shown that rutin, a bioactive substance found in *Z. lotus* (Terkmane et al. 2017), can reduce serum cholesterol levels (Ziaee et al. 2009) and TG levels (Ganeshpurkar and Saluja 2017).

In comparison to the CG, no significant changes in glucose levels were observed among the treated groups. It is worth noting that *R. chalepensis* has been known to decrease streptozotocin-induced hyperglycemia in rats (Hamdiken et al. 2017).

In an experimental protocol conducted on a streptozotocin-induced hyperglycemia Wistar rat model (Abdel-Zaher et al. 2005), the aqueous extracts of roots and leaves of *Z. lotus* demonstrated efficient hypoglycemic activities (Benammar et al. 2014). The significant presence of vitamin A in the leaves and roots of *Z. lotus* may be associated with this beneficial impact (Abdoul-Azize 2016). Furthermore, Marmouzi et al. (2019) demonstrated that the aqueous extract of *Z. lotus* has an antidiabetic effect by inhibiting α-glucosidase and α-amylase in vitro, with a more potent impact than the common medication acarbose.

In their study, Bencheikh et al. (2021a) revealed that the administration of *Z. lotus* aqueous extract to mice subjected to a chronic high-fat diet resulted in reduced plasma triglyceride levels. This finding suggests that this plant extract may restore triglyceride catabolism in mice on a high-fat diet. Improved removal of triglyceride-rich lipoproteins by lipase lipoproteins and enhanced uptake of triglycerides carried in very low-density lipoprotein (VLDL) by peripheral organs may contribute to this effect (Jawed et al. 2019).

Pancreatic lipase, which catalyzes the hydrolysis of triglycerides into mono- and diglycerides that are absorbed by intestinal cells, may also play a role in regulating intestinal uptake of TG (Kamoun et al. 2018). Although the direct contribution of TG to atherosclerosis has not been firmly established, lowering blood TG levels has been suggested as an effective treatment for heart disease and prevention of hyperlipidemia (Olkkonen et al. 2018).

It's worth noting that medicinal plants are rich in several secondary metabolites, including polyphenols and flavonoids, which are known to have important anti-cholesterolemic and anti-hypertriglyceridic activities (Dahlia et al. 2020).

Table 4. Hepatic profile of Wistar rats treated with *Z. lotus* and *R. chalepensis* phenolic extracts. Results are expressed as means ± SD (n = 5). $p < 0.05$.

	AST (U/L)	ALT (U/L)	DB (mg/L)	TB (mg/L)	Pr (g/L)
CG	94 ± 3.240	100.2 ± 1.789	1.56 ± 0.035	8.938 ± 0.053	66.36 ± 0.907
$TGZL^{MeOH.E}$	85.4 ± 3.286	90.6 ± 0.894	1.634 ± 0.022	8.324 ± 0.015	63.18 ± 0.61
$TGZL^{Aq.E}$	85.2 ± 2.28	91.4 ± 1.517	1.65 ± 0.051	9.262 ± 0.020	66.38 ± 0.421
$TGRC^{MeOH.E}$	119 ± 1	123.4 ± 2.302	2.466 ± 0.03	11.7 ± 0.071	67.28 ± 0.396
$TGRC^{Aq.E}$	83.2 ± 0.47	111 ± 2	1.84 ± 0.033	9.608 ± 0.034	57.02 ± 0.597

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; DB: Direct bilirubin; TB: Total bilirubin; Pr: Protidemia.

Hepatic profile

The mean concentrations of the biochemical parameters related to liver function in the treated groups and the control group (CG) are presented in Table 4.

Compared to the CG, the $RC^{MeOH.E}$ significantly increased ($p < 0.001$) the activity of transaminases (aspartate aminotransferase, AST; alanine aminotransferase, ALT) in the treated groups, with AST and ALT levels of 119 ± 1 U/L and 123.4 ± 2.302 U/L, respectively. In contrast, AST and ALT levels were slightly decreased in the other test groups.

Transaminases are used as clinical markers to diagnose and assess liver disease. Elevated levels of these biomarkers in the blood indicate liver damage. ALT is a more specific marker for liver cell damage, primarily occurring in the liver, while AST is also found in other tissues like the heart, skeletal muscle, kidneys, brain, pancreas, and blood (Touiti et al. 2020). The increased serum ALT and AST activities in rats treated with the methanolic extract of *R. chalepensis* may be indicative of initial liver damage.

Bilirubin is a biomarker directly related to the extent of liver damage and toxicity. It is produced during the breakdown of aged or damaged red blood cells, releasing heme and globin (Basso et al. 1992). Bilirubin is processed in the liver before being excreted in bile. In this study, total and direct bilirubin levels in the group treated with RCEM were increased ($p < 0.05$) compared to the CG.

Bencheikh et al. (2019) demonstrated that the aqueous extract of *Z. lotus* fruit has a hepatoprotective effect against hepatic lesions induced by CCL4 in rats. After the administration of two different doses of the extract, plasma levels of ALT, AST, and ALP decreased, and the concentrations of bilirubin (both direct and total) in the plasma were also reduced.

Table 5. Renal profile. $p < 0.05$.

	BU (g/L)	BC (mg/L)
CG	0.176 ± 0.011	7.22 ± 0.228
G_{TrRCEM}	0.154 ± 0.011	6.9 ± 0.141
G_{TrRCEA}	0.204 ± 0.002	7.68 ± 0.217
G_{TrZLEM}	0.206 ±0.002	7.44 ± 0.503
G_{TrZLEA}	0.21 ±0.005	6.6 ± 0.324

BU: Blood urea; BC: Blood creatinine.

Kidney profile

Table 5 presents the plasma biomarkers of renal function in both control and treated rats. Results indicate that the administration of *Z. lotus* and *R. chalepensis* extracts to Wistar rats resulted in elevated urea level in the $TGRC^{Aq.E}$, $TGZL^{MeOH.E}$ and $TGZL^{Aq.E}$ groups compared to the CG. However, the levels of creatinine remained unchanged in all groups.

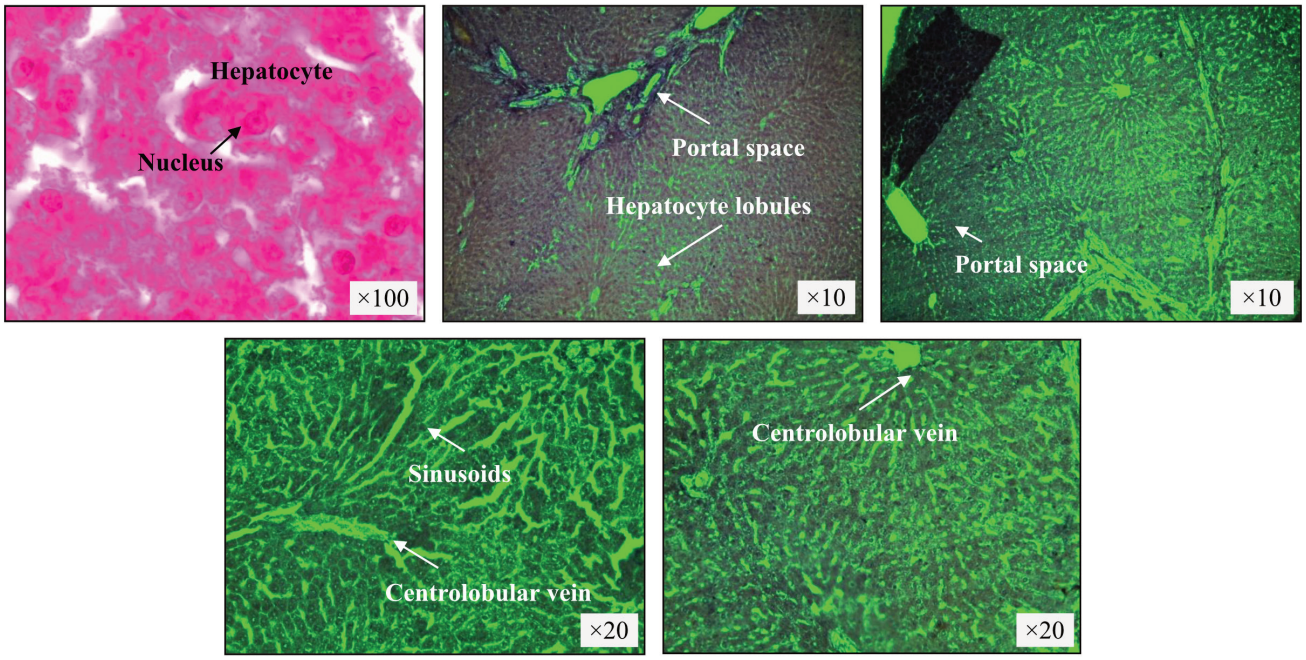
The kidneys play a central role in drug excretion and are therefore a critical target for assessing drug toxicity. Blood urea and creatinine levels in experimental animals are crucial parameters for evaluating renal function and glomerular filtration (Amadi et al. 2013). In line with our findings, Bencheikh et al. (2021b) reported that the *Z. lotus* extract improved altered parameters in nephrotoxicity induced by gentamicin. Specifically, they observed significant reductions in urea, acid uric and creatinine levels in treated rats.

Evaluation of liver histopathological changes

Liver has a crucial role in metabolizing xenobiotics, making it a primary target for toxic compounds. Hepatotoxicity related to xenobiotics, including drugs, and naturally occurring substances, can lead to liver damage (Le Daré et al. 2021).

Hematoxylin and eosin staining revealed that the liver exhibited a normal histological appearance in all groups (Fig. 6). Both the treated and control rats displayed normal hepatic parenchyma, composed of lobules with a portal space at the apex. The lobules were centered around a centrilobular vein, and normal hepatocytes were arranged in bays and separated by sinusoids. No elemental lesions were observed, indicating normal liver histology in both control and treated rats, with an absence of lymphocyte

Figure 6. Microscopic examination (X100, W10, X20) of liver histology in rats treated with *Z. lotus* and *R. chalepensis* hydromethanolic and aqueous extracts.



inflammatory infiltrate. The histological appearance of the liver in the control rats was consistent with that of the test groups. These results indicate the absence of inflammation following the oral administration of a high dose of *Z. lotus* and *R. chalepensis* extracts at 5000 mg/kg b.w., demonstrating that the use of these natural drugs does not induce hepatotoxicity.

In the case of rats treated with the hydromethanolic extract of *R. chalepensis*, despite recording higher liver blood parameters compared to the control group, the anatomopathological study revealed a normal liver histological appearance. This could be explained by two factors: either the histological section was not taken from the part of the organ where inflammation was present, or inflammation had not been well established, and hepatotoxicity was not clearly manifested within the organ.

In vivo phytotherapeutic treatment of gastroenteritis induced by *S. enterica* subsp. *Arizonae*

Clinical symptoms, body, and organ weights evolution

Table 6 provides an overview of clinical symptoms observed in rats across different test and control groups, while Figure 7 illustrates body weight changes.

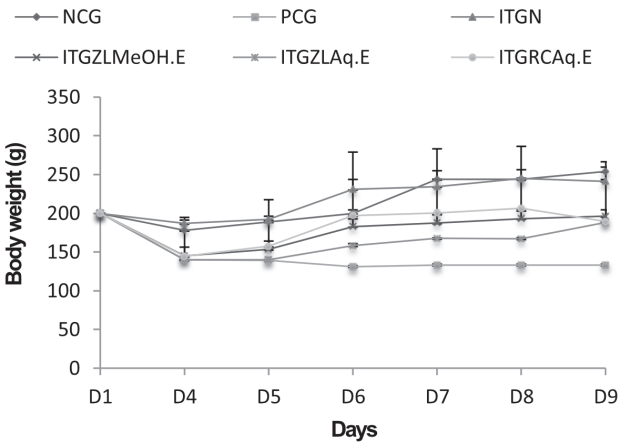
Following induction of enteric infection with *S. enterica* subsp. *arizonae* and a 3-day incubation period, untreated infected rats exhibited progressive diarrhea, ranging from mild to severe. This diarrhea was characterized by liquid, bloody stools with mucus. Infected rats had blackish-tarry or dark green feces with a foul odor, reduced mobility, loss of appetite, and weight loss. Painful defecation and colon inflammation were also evident.

Concerning body weight changes, a significant and consistent weight gain ($p < 0.05$) was observed after 9 days in the control group and rats treated with a dose of 400 mg/kg b.w. of both plant polyphenolic extracts (PPE). Weight gain was positive from the 4th to the 9th day of the experiment (Fig. 7).

However, differences in body weight were noted among different treatment groups. Animals treated with the hydromethanolic extract of *Z. lotus* and the aqueous extract of *R. chalepensis* maintained higher body weights than those treated with the aqueous extract of *Z. lotus* (Fig. 7). Consequently, rats treated with both plant extracts had higher body weights than infected untreated animals (positive control group), which showed a significant decrease in body weight (Fig. 7). These results indicate the absence of toxicity signs in rats treated with both plant PPE and their tolerance for the employed phytotherapeutic treatment.

Clinical symptoms began to subside after the 2nd day of treatment with both plant extracts (from day "D5"). A change in the appearance and color of feces was noted, resembling that of rats in the negative control group (uninfected rats). The unpleasant odor disappeared, and the color changed from tarry black or dark green to light or dark brown. However, rats treated with antibiotics

Figure 7. Body weight evolution ($p < 0.05$).



exhibited a noticeable loss of appetite, suggesting an influence on host physiology.

Rats treated with *Z. lotus* and *R. chalepensis* PPE displayed reduced colon inflammation, normal appetite, water intake, and mobility. In contrast, untreated infected rats (PCG) and infected rats treated with neomycin experienced exacerbated clinical symptoms, including increased mortality, compared to the other test groups (Table 6). The results indicate that both plant extracts have no toxic effects when used as a treatment for gastroenteritis, as their daily administration at a concentration of 400 mg/kg b.w. for 7 days did not result in mortality.

Extensive intestinal inflammation was observed in animals infected with *S. enterica* subsp. *arizonae*, along with frequent ulcers in some rats. These ulcers were distributed throughout the intestine, with a higher prevalence in the duodenum and jejuno-ileum. Necrotizing enterocolitis, extensive ulcerations, and necrosis of the jejuno-ileum and colon were also noted, attributed to inflammation induced by the pathogen, leading to the release of inflammatory mediators and dysregulation of cell growth factors, resulting in intestinal lesions.

In the ITG^{N} group, a reduction in intestinal inflammation and mesentery inflammation was observed, with the absence of intestinal ulcers. However, swelling of the cecum was reported, accompanied by significant fatty tissue accumulation around the digestive tract.

Diarrheal parameters assessments

Faecal water content and anti-enteropooling activity

Following the induction of enteric infection with *S. enterica* subsp. *arizonae*, all rats developed diarrhea within a latency period of 48 to 72 h. To assess diarrheal parameters, fecal water content and the percent inhibition of intestinal fluid accumulation were measured, and the results are presented in Figure 8.

In the gastrointestinal enteropooling test, rats pretreated with *Z. lotus* and *R. chalepensis* PPE exhibited a significant decrease ($p < 0.05$) in fecal water content and intestinal fluid accumulation, in a dose-dependent manner at 400 mg/kg, compared to the positive control group (Fig. 8). The fecal water content in treated rats was notably lower than in untreated animals (PCG). Between the 4^{th} and 6^{th} days of the experiment, a significant reduction in fecal water content was observed in all the rats in the test groups compared to those in the PCG (Fig. 8).

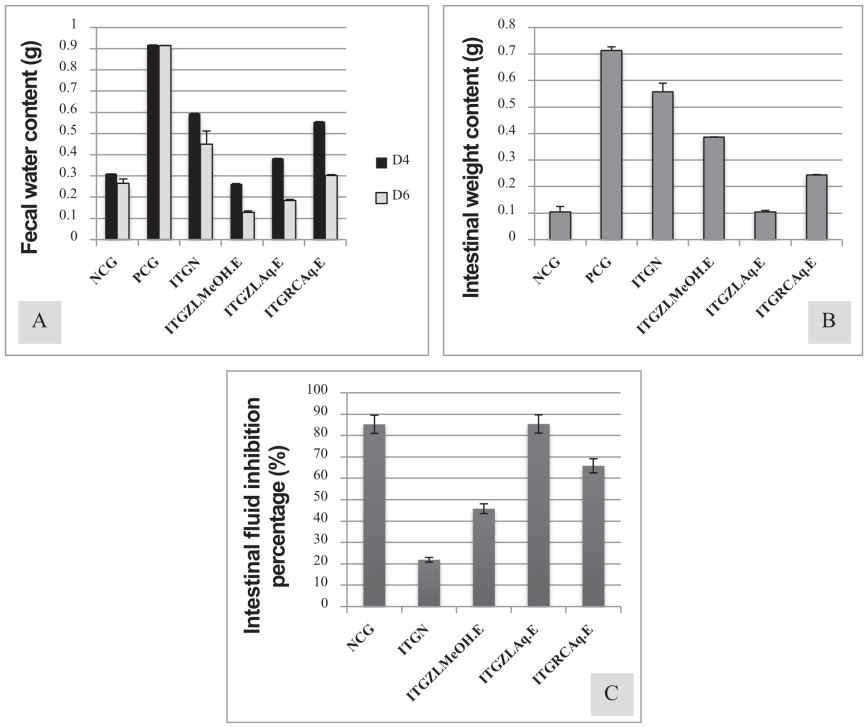
In diarrheal conditions, a high fecal water content indicates an excess of gastrointestinal fluid compared to the norm. According to the results of this study, the highest percentage of inhibition of intestinal fluid accumulation was observed in the group of infected rats treated with the aqueous extract of *Z. lotus*, reaching 85.41% (Fig. 8C). Thus, *Z. lotus* and *R. chalepensis* PPE exhibited more significant inhibition of intestinal fluid accumulation compared to neomycin-treated rats (21.88%). This suggests that the

Table 6. Clinical signs observed in infected untreated and infected rats treated with phenolic extracts of *Z. lotus* and *R. chalepensis*.

Clinical signs	NCG	PCG	ITG^{N}	$ITG^{ZLMeOH.E}$	$ITG^{ZLAq.E}$	$ITG^{RCAq.E}$
N°	05	05	05	05	05	05
Mobility	N	R	R	N	N	N
Stool states	N	A_b	A_b	N	N	N
Vomiting	A_{bs}	A_{bs}	A_{bs}	A_{bs}	A_{bs}	A_{bs}
Fever	A_{bs}	P_r	P_r	A_{bs}	A_{bs}	A_{bs}
Loss of appetite	A_{bs}	P_r	P_r	P_r	P_r	A_{bs}
Diarrhea	A_{bs}	P_r	P_r	A_{bs}	A_{bs}	A_{bs}
Mortality	A_{bs}	2/5	1/5	A_{bs}	A_{bs}	A_{bs}

ITG^{N}: Infected treated group with neomycin; $ITG^{ZLMeOH.E/ITGZLAq.E}$: Infected treated group with *Z. lotus* hydromethanolic extract/aqueous extract; $ITG^{RCAq.E}$: Infected treated group with *R. chalepensis* aqueous extract.
N: Normal; R: Reduced; A_b: Abnormal; A_{bs}: Absence; P_r: Presence.

Figure 8. Effect of phytotherapeutic treatment with *Z. lotus* and *R. chalepensis* phenolic extracts on faecal water content (A), enteropooling (B) and intestinal fluid inhibition (C).



antibiotic used in this study reduced diarrhea but did not eliminate it entirely.

The mechanism involved in this process is associated with a dual effect on gastrointestinal motility, water, and electrolyte transport (reduced absorption of Na^+ and K^+) through the intestinal mucosa. *Z. lotus* and *R. chalepensis* PPE reduced diarrhea by increasing the reabsorption of electrolytes and water while inhibiting the accumulation of intestinal fluid after inhibiting bacterial growth and eradicating the infectious germ (Gaginella and Phillips 1975).

The antidiarrheal activity of some medicinal plants can be attributed to the presence of phenols, tannins, flavonoids, saponins, and terpenoids (Havagiray et al. 2004). Accordingly, numerous studies have demonstrated the effectiveness of phytotherapeutic treatments in reducing intraluminal fluid and treating diarrheal diseases (Teke et al. 2010; Saheed et Omotayo 2016; Degu et al. 2016).

Detection of S. enterica subsp. arizonae in faecal flora

After detecting *S. enterica* subsp. *arizonae* in the faecal microbiota of rats in the untreated infected group (PCG), we conducted stool examinations for animals in the infected groups treated with the antibiotic (ITG^N) and those treated with *Z. lotus* and *R. chalepensis* PPE. The results, presented in Figure 9, show the enumeration of *S. enterica* subsp. *arizonae* on Salmonella-Shigella (SS) agar as Log CFU/g of faecal specimens.

The stool samples from rats treated with *Z. lotus* PPE exhibited a significant reduction ($p < 0.05$) in pathogen count compared to untreated infected rats, where the pathogen count was considerably higher (Fig. 9). More-

Figure 9. Effect of antibiotic and phenolic extracts therapy against colonizing multidrug resistant *S. enterica* subsp. *arizonae* strain ($p < 0.001$).

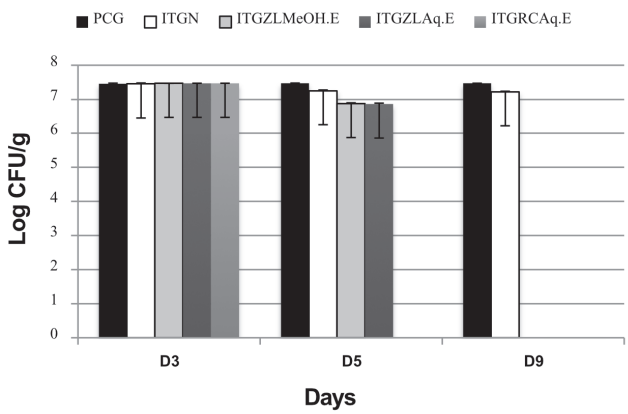
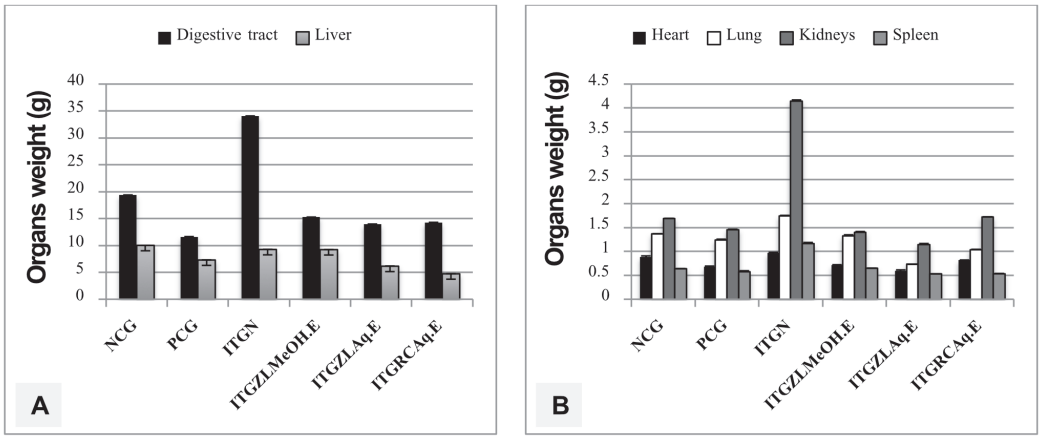


Figure 10. Organ's weight of different control and treated groups ($p < 0.05$).



over, rats treated with plant extracts showed a slightly lower pathogen count compared to those treated with neomycin, underscoring the effectiveness of this plant-based treatment.

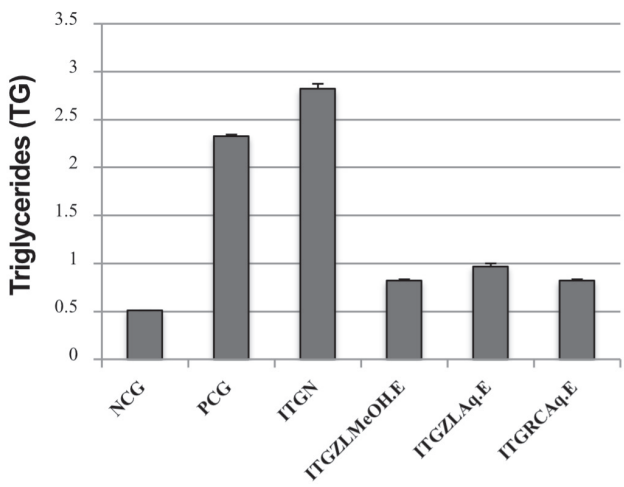
On the 5th day of the experiment, the stool specimens of rats treated with the antibiotic showed a 7.254 ± 0.023 Log CFU/g value. In contrast, microbial counts of 6.877 ± 0.02 and 6.862 ± 0.025 Log CFU/g were recorded in the stool samples of animals treated with $ZL^{MeOH.E}$ and $ZL^{Aq.E}$ extracts, respectively (Fig. 9). An absence of the infectious pathogen in the faecal flora of rats treated with the aqueous extract of *R. chalepensis* was observed from the early days of treatment (D^5), as no characteristic Salmonella colonies were detected or identified. The complete eradication of *Salmonella* in rats treated with *Z. lotus* was reported after seven days of treatment (D^9). The absence of *Salmonella* in the faecal flora of rats after long term treatment with *Z. lotus* and *R. chalepensis* extracts confirms the effective antimicrobial properties of these plant extracts.

Recent studies have provided substantial evidence supporting the antimicrobial efficacy of *Z. lotus* and *R. chalepensis* as natural sources (Abderrahim et al. 2019; Boughendjiou 2019). Yahia et al. (2020) further highlighted the robust antimicrobial potential of bioactive compounds derived from *Z. lotus*, showcasing inhibition against *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli*. Additionally, Degu et al. (2020) documented significant antidiarrheal activity with *R. chalepensis* at a dosage of 400 mg/kg.

Organ weight

Organ weight results are depicted in Figure 10. During the examination of rats from various groups, a notable accumulation of fat covering all internal organs was observed in animals treated with neomycin at a dose of 200 mg/kg, resulting in an increase in their organ weights (Fig. 10AB). This increase is particularly pronounced in the case of the digestive tract and kidneys when compared to the results of untreated animals (NCG) (Fig. 10AB). The substantial presence of fat raises suspicions of hypercholesterolemia (elevated blood cholesterol levels), a suspicion confirmed by the analysis of a key lipid parameter (triglyceridemia).

Figure 11. Results of triglycerides dosage ($p < 0.05$).



Dosage of blood triglycerides

The results of the blood triglyceride test (TG) are presented in Figure 11. Significantly different levels ($p < 0.05$) of triglycerides were observed in different groups of animals. Untreated infected rats and those infected and treated with neomycin exhibited elevated triglyceride levels, measuring 2.33 ± 0.014 and 2.83 ± 0.049 g/L, respectively (Fig. 11). These triglyceride levels were

Table 7. Complete Blood Count (CBC) determination (n = 2), p<0.05.

	NCG	PCG	ITG^{N}	ITG$^{ZLMeOH.E}$	ITG$^{ZLAq.E}$	ITG$^{RCAq.E}$
WBC (x 10^9/L)	5.665 ± 0.021	9.035 ±0.021	9.915 ± 0.007	8.6 ± 0.014	6.475 ± 0.021	6.645 ± 0.04
LYM (%)	46 ± 0	72.1 ± 0	60.4 ± 0	42 ± 0	52.5 ± 0.14	59.3 ± 0.14
MID (%)	10.2 ± 0.14	9.7 ± 0.14	11.65 ± 0.21	14.55 ± 0.07	8.8 ± 0	16.6 ± 0.14
GRA (%)	43.55 ± 0.21	18 ± 0	28.05 ± 0.07	43.4 ± 0	38.75 ± 0.07	23.9 ± 0
RBC (x 10^9/L)	8.85 ± 0.07	8.505 ± 0.021	8.3 ± 0.014	7.365 ± 0.007	7.43 ± 0.014	8.63 ± 0
HB (g/dL)	16.25 ± 0.21	13.35 ± 0.21	14.05 ± 0.07	13.7 ± 0	12.1 ± 0.14	14.8 ± 0.14
HT (%)	46.9 ± 0.14	40.035 ± 0.049	39.95 ± 0.014	40.205 ± 0.007	36.085 ± 0.02	41.675 ± 0.049
MCV (fL)	53 ± 0	47 ± 0	48 ± 0	51.5 ± 0.71	48.5 ± 0.71	48 ± 0
MCHCt (pg)	17.3 ± 0.28	15.5 ± 0.57	17.05 ± 0.07	17.3 ± 0.14	16.5 ± 0.14	17.2 ± 0.14
MCHC (g/dL)	32.9 ± 0	33.85 ± 0.07	35.35 ± 0.07	34.05 ± 0.07	33.9 ± 0	36 ± 0.14
PLT (x 10^9/L)	574.5 ± 2.12	706.5 ± 2.12	646 ± 1.41	531 ± 1.41	523.5 ± 2.12	622 ± 0

significantly higher compared to the other animal groups and the negative control group (NCG) where triglyceride levels were 0.51 ± 0 g/L. On the other hand, rats treated with *Z. lotus* and *R. chalepensis* PPE exhibited normal triglyceride levels, indicating that the phytotherapeutic treatment using these extracts did not have an adverse effect on blood fat levels.

Complete Blood Count (CBC)

Hematological and biochemical characterization of animals is a vital aspect in the diagnosis of various diseases (Obi and Oduye 1985; Popoff 1981). In this study, we investigated the impact of inducing diarrhea with *S. enterica* subsp. *arizonae* and the daily oral administration of *Z. lotus* and *R. chalepensis* PPE at a concentration of 400 mg/kg b.w. on blood parameters, and the results are summarized in Table 7.

Our findings suggest that both the hydromethanolic and aqueous extracts of *Z. lotus* and *R. chalepensis* did not have significant effects on circulating blood cells or their production. Nevertheless, a noteworthy increase ($p < 0.05$) in the percentages of white blood cells (WBC) and lymphocytes (LYM) was observed in all rats in the test groups, except for those treated with ZL$^{MeOH.E}$, where the LYM percentage was closer (42%) to that of the negative control group (NCG) (46%) (Table 7). The untreated infected animals exhibited a LYM percentage of 72.1% in their blood samples (Table 7).

The elevated WBC levels indicate an activation of the immune system in response to the treatment of enteric infection induced by the pathogen. Additionally, the increase in the total number of leukocytes (MID) suggests that *Z. lotus* and *R. chalepensis* phenolic extracts may contain bioactive compounds capable of modulating the immune system by augmenting the count of defensive white blood cells, which could potentially act against *Salmonella enterica* subsp. *arizonae* at the intestinal level.

Determination of inflammation and infection markers

This analysis aimed to identify variations in specific serum parameters that serve as biomarkers for inflammation and infection. These parameters include transaminases (AST and ALT), alkaline phosphatase (ALP), Erythrocyte Sedimentation Rate (ESR), and C-reactive protein (CRP). Each parameter plays a crucial role in indicating inflammatory and infectious states. The results of these analyses are comprehensively illustrated in Figures 12 and 13.

The study involved the oral administration of both plants extracts at a dose of 400 mg/kg body weight, administered daily for 7 days, for treating gastroenteritis. This treatment led to significant modifications ($p < 0.05$) of liver marker enzymes (ALP, AST, and ALT) compared

Figure 12. Analysis of hepatic toxicity (transaminases dosage) after daily oral administration of *Z. lotus* and *R. chalepensis* phenolic extracts and standards treatments: neomycin for seven consecutive days ($p < 0.05$).

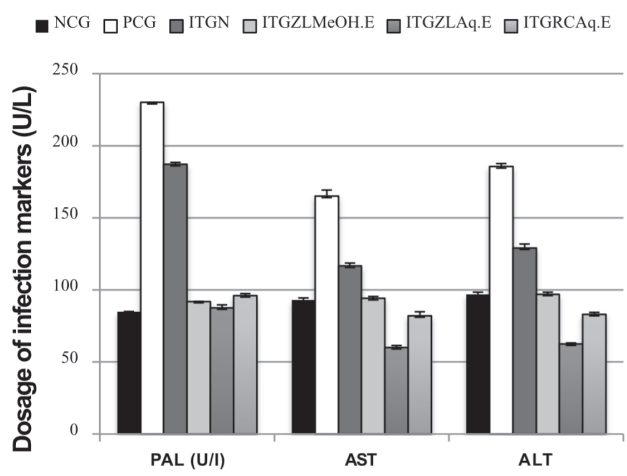
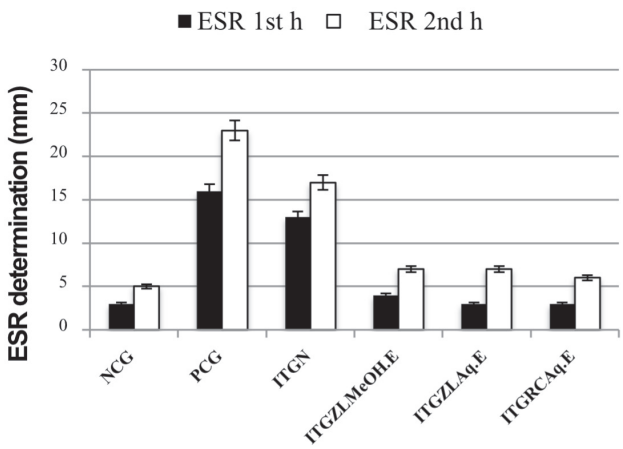


Figure 13. Results of Erythrocyte Sedimentation Rate (ESR) determination ($p < 0.05$).



to the normal control group (NCG).

Alkaline phosphatases, a family of enzymes, hydrolyze phosphoric esters, releasing mineral phosphates. ALP is heterogeneous, originating from various tissue sources, with bone contributing 50%, and liver, intestine, kidney, and placenta also being significant sources (Renier 1982). In the study, ALP activity showed a significant increase ($p < 0.05$) in the infected rats without treatment (PCG): 230 ± 0 U/L, and in those receiving the antibiotic standard treatment: 187 ± 1.41 U/L, compared to the NCG: 85 ± 0 U/L (Fig. 14). Notably, serum alkaline phosphatase levels were reduced in rats treated with the phenolic extracts of both plants compared to other test groups.

Further, a significant decrease ($p < 0.05$) in AST and ALT enzymes was observed 6 days post-administration of the plants' polyphenolic extracts (PPEs). The highest levels of transaminases were recorded in untreated infected rats: 165 ± 4.24 U/L for AST and 185.5 ± 2.12 U/L for ALT, and in rats treated with neomycin: 116.5 ± 2.12 U/L for AST and 129 ± 2.82 U/L for ALT (Fig. 12).

These results suggest that the induced infection and antibiotic treatment cause liver damage, evident from the elevated levels of liver enzymes. Conversely, the treatment with *Z. lotus* and *R. chalepensis* PPEs led to a significant decrease ($p < 0.05$) in these markers (Fig. 12), indicating the potential hepatoprotective activity of these plant extracts at a dose of 400 mg/kg b.w.

Abnormal values of the sedimentation rate (ESR) of RBCs, an important infection indicator, were also recorded. ESR values greater than 10 mm after the first hour of the test were noted in untreated infected rats (PCG), and those receiving the antibiotic (Fig. 13). The high ESR values indicated persistent and aggravated infection in these animals. However, a significant decrease in ESR was observed in rats treated with both plant PPEs (Fig. 13).

These results demonstrate the effectiveness of *Z. lotus* and *R. chalepensis* extracts in treating enteric infection, as evidenced by lower ESR values compared to untreated infected animals.

Regarding the CRP assay, the analysis was conducted qualitatively using the LATEX agglutination test to confirm inflammation and bacterial infection. Positive agglutination was observed in blood samples from untreated infected rats, while samples from other test groups showed negative detection.

Detection of bacteremia and bacterial translocation

A positive blood culture was recorded in infected untreated rats and infected rats treated with neomycin, which required identification of the germ responsible of sepsis.

Bacterial cultures on selective agar were realised to complete the study. Bacteria present in blood samples from tubes representing a positive blood culture were isolated on SS agar Hektoen and EMB agar, especially for the detection of *S. enterica* subsp. *arizonae*.

After the incubation period (24h), we detected the presence of colorless colonies on SS agar, the appearance of orange and green colonies on Hecktoen agar, as well as purple colonies with a metallic appearance, and other transparent amber colonies without a metallic appearance on EMB agar. The use of miniaturized galleries, API 20E allowed us to identify two translocate germs, *E. coli* and *S. enterica* subsp. *arizonae*.

Table 8 summarizes the macroscopic appearance of the colonies that appeared on selective agar following a positive blood culture.

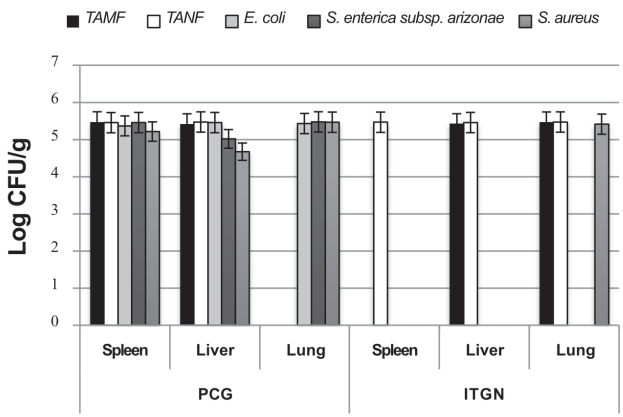
The blood culture was negative for the different groups of infected rats treated with *Z. lotus* and *R. chalepensis*

Table 8. Detection of bacterial blood translocation: septicemia and bacterial translocation to sterile internal organs.

Test groups	NCG	PCG	ITG^{N}	$ITG^{ZLMeOH.E}$	$ITG^{ZLAq.E}$	$ITG^{RCAq.E}$
Septicemia	A_{bs}	P_r	P_r	A_{bs}	A_{bs}	A_{bs}
BT	A_{bs}	P_r	P_r	A_{bs}	A_{bs}	A_{bs}

BT: Bacterial translocation; A_{bs}: Absence; P_r: Presence.

Figure 14. Results of quantitative analysis of bacterial translocation to sterile internal organs (spleen, liver and lungs).



PPEs, which indicates that no bacterial translocation from digestive tract to blood system was induced. According to these results, most of the experiments have shown that the treatment of *S. enterica* subsp. *arizonae* infection using neomycin remains ineffective and represents an increased risk in the complication of pathologies, to aggravate the physiological and health status of the host organism by comparing with the phytotherapeutic treatment, using the polyphenolic extracts of *Z. lotus* and *R. chalepensis*.

Besides, this experiment allowed us to detect bacterial translocation defined as the passage of germs to the sterile internal organs through the bloodstream. The figure 16 represents the bacterial translocation germs count (Log CFU/g) at the levels of three organs: spleen, liver and lungs. Moreover, positive bacterial translocation was detected in rats of the PCG and ITG^{N} groups (Fig. 14).

Bacterial translocation was very abundant in spleen, liver, and lungs with quantification of total aerobic mesophilic and anaerobic flora, *E. coli*, *S. enterica* subsp. *arizonae* and *S. aureus*. For the group of untreated infected rats, *S. aureus* was detected in the spleen, liver and lungs compared to the group of animals treated with the antibiotic, whose growth in this germ was recorded only in the lungs with a concentration of 5.415 Log CFU/g. No growth of *S. enterica* and *E. coli* was recorded in the organs of the animals treated with the antibiotic, whereas an abundant presence was determined in the untreated rats (PCG).

Concentrations of 5.456 Log CFU/g, 5.017 Log CFU/g and 5.474 Log CFU/g in *S. enterica* subsp. *arizonae* were quantified in the spleen, the liver and the lungs, respectively of untreated infected rats (PCG) (Fig. 14).

No germs were detected in the organs of animals in the other test groups, which mean a negative bacterial translocation.

The detection of a bacterial translocation in the PCG allowed us to conclude that the *in vivo* induction of *S. enterica* subsp. *arizonae* infection caused an imbalance of the intestinal microbiota, and subsequently the physiological state of the host, which causes a dysfunction of the immune system and therefore the neutralization of the infectious germ will be limited, which allows the latter with other germs to cross the barriers and escape the defense of the immune system. Subsequently, the infectious germ is therefore in the mesenteric lymph nodes, reaching the bloodstream causing sepsis and subsequently, manages to colonize various sterile internal organs of the organism.

Thus, the antibiotic treatment did not lead to a complete elimination of the germ, the concentration decreased slightly, but the germ still persists in the intestine, which allows it to cross the intestinal defense barriers. The bacterial translocation is inhibited using phytotherapeutic treatment with both plants PPEs. It can therefore be concluded that *Z. lotus* and *R. chalepensis* are more effective in the treatment of *Salmonella* gastroenteritis, the growth of the germ is inhibited at the intestinal level by using both plants extracts, and therefore, it can reach neither the blood circulation nor the organs.

Conclusion

This study highlights the efficacy of *Z. lotus* and *R. chalepensis* phenolic extracts in treating gastrointestinal infections in Wistar rats, underscoring the value of medicinal plants in traditional medicine and conservation efforts. An acute oral toxicity study confirmed the non-toxicity of these plant extracts, with LD50 values over 5000 mg/kg body weight, aligning with our goal to identify safe and effective bioactive compounds.

The phenolic extracts did not negatively impact gastrointestinal microbiota, maintaining consistent levels of various bacterial groups, including beneficial probiotic bacteria like *Streptococcus* and *Lactobacillus*. This indicates that even at high doses, these extracts are safe for intestinal flora.

Additionally, the extracts effectively treated intestinal *Salmonella enterica* subsp. *arizonae* infections, outperforming traditional antibiotics. A significant germ reduction was observed within two days, leading to complete eradication after seven days.

In summary, *Zizyphus lotus* and *Ruta chalepensis* extracts demonstrate significant potential as safe, natural antimicrobial agents for treating severe microbial infections in humans.

Acknowledgments

The authors wish to express their sincere gratitude to the University of Mascara, Algeria, for the financial support granted to facilitate this research. Special thanks are also extended to the Laboratory of Bioconversion, Microbiological Engineering, and Health Safety at Mascara University for their invaluable assistance in the execution of this study.

References

Abdel Zaher AO, Salim SY, Assaf MH, Abdel Hady RH (2005) Antidiabetic activity and toxicity of *Zizyphus spina-christi* leaves. J Ethnopharmacol 101(1-3):129-138.

Abderrahim LA, Taibi K, Abderrahim AC (2019) Assessment of the antimicrobial and antioxidant activities of *Zizyphus lotus* and *Peganum harmala*. Iran J Sci Technol Trans A Sci 43:409-414.

Abdoul Azize S (2016) Potential benefits of jujube (*Zizyphus lotus* L.) bioactive compounds for nutrition and health. J Nutr Metab 2016:2867470.

Abdulla G, Abdel Samie MA, Zaki D (2016) Evaluation of the antioxidant and antimicrobial effects of *Ziziphus* leaves extract in sausage during cold storage. Pakistan J Food Sci 26(1):10-20.

Ait Abderrahim L, Taïbi K, Ait Abderrahim C (2019) Assessment of the antimicrobial and antioxidant activities of *Ziziphus lotus* and *Peganum harmala*. Iran J Sci Technol Trans Sci 43:409-414.

Al-Ismail K, Al-Atewi R, Al-Dabbas M, Ajo R (2022) Antioxidant and α-amylase inhibition activity of *Ruta chalepensis* L extracts. Carpathian J Food Sci Tech 14(1):110-117.

Al Salmani MS, Yahya HN, Oohayyed NA, Alsaeed AA (2021) Antimicrobial and immunoprotective potentials of *Ruta chalpensis* methanolic extract: in vitro and in vivo studies. Int J Drug Delivery Technol 11(3):1027-1030.

Althaher AR, Oran SA, Bustanji YK (2020) Phytochemical analysis, *in vitro* assessment of antioxidant properties and cytotoxic potential of *Ruta chalepensis* L. J Essent Oil Bear Plants 23(6):1409-1421.

Altun A, Ugur Altun B (2007) Melatonin: therapeutic and clinical utilization. Int J Clin Pract 61(5):835-845.

Amadi BA, Agomuo EN, Duru MKC (2013) Toxicological studies of *Asmina triloba* leaves on haematology, liver, kidney using rat model. Int Sci Res J 4(2):11-17.

Anguinaga YJ, Claudiano SG, Marcusso FP, Ikefuti C, Ortega GG, Eto FS, da Cruz C, Moraes JRE, Moraes FR, Fernandes JBK (2014) Acute toxicity and determination of the active constituents of aqueous extract of *Uncaria tomentosa* bark in *Hyphessobrycon eques*. J Toxicol 2014: 412437.

Archana H, Geetha V (2022) Evaluation of phytoconstituents from selected medicinal plants and its synergistic antimicrobial activity. Chemosphere 287(4):132-276.

Basso D, Fabris C, Plebani M, Del Favero G, Muraca M, Vilei MT, Panozzo MP, Meggiato T, Fogar P, Burlina A, Naccarato R (1992) Alterations in bilirubin metabolism during extra-and intrahepatic cholestasis. Clin Investig 70(1):49-54.

Bekkar NEH, Meddah B, Cakmak YS, Keskin B (2021a) Phenolic composition, antioxidant and antimicrobial activities of *Zizyphus lotus* L. and *Ruta chalepensis* L. growing in Mascara (western Algeria). J Microbiol Biotechnol Food Sci 10(5):e3004-e3004.

Bekkar NEH, Meddah B, Keskin B, Sonnet P (2021b) Oral acute toxicity, influence on the gastrointestinal microbiota and in vivo anti-salmonellosis effect of *Zizyphus lotus* (L.) and *Ruta chalepensis* (L.) essential oils. J Appl Biotechnol Rep 8(1):13-26.

Belkaid Y, Hand TW (2014) Role of the microbiota in immunity and inflammation. Cell 157(1):121-141.

Benammar C, Baghdad C, Belarbi M, Subramaniam S, Hichami A, Khan NA (2014) Antidiabetic and antioxidant activities of *Zizyphus lotus* L aqueous extracts in Wistar rats. J Nutr Food Sci S8:004).

Bencheikh N, Bouhrim M, Kharchoufa L, Choukri M, Bnouham M, Elachouri M (2019) Protective effect of *Zizyphus lotus* L. (Desf.) fruit against CCl4-induced acute liver injury in rat. Evid-based Complement Altern Med 2019:6161593.

Bencheikh N, Bouhrim M, Merrouni I-A, Boutahiri S, Kharchoufa L, Addi M, Elachouri M (2021a) Antihyperlipidemic and antioxidant activities of flavonoid-rich extract of *Ziziphus lotus* (L.) lam. fruits. Appl Sci 11(17):7788.

Bencheikh N, Bouhrim M, Kharchoufa L, Al Kamaly OM, Mechchate H, Es Safi I, Elachouri M (2021b) The nephroprotective effect of *Zizyphus lotus* L.(Desf.) fruits in a gentamicin-induced acute kidney injury model in rats: a biochemical and histopathological investigation. Molecules 26(16):4806.

Beraud J (2001) The Technician Biological Analyzes. Theoretical and Practical Guide. Tec and Doc Lavoisier, Paris. 988-990.

Borgi W, Bouraoui A, Chouchane N (2007) Antiulcerogenic activity of *Zizyphus lotus* (L.) extracts. J Ethnopharmacol 12:228-231.

Boughendjiou A (2019) Yield, chemical composition and antibacterial activity of *Ruta chalepensis* L. essential oil growing spontaneously in Algeria. Pharm Pharmacol Int J 7(1):33-36.

Can F, Yilmaz Z, Demirbilek M, Bilezikci B, Kunefeci G, Atac FB (2005) Diagnosis of *Helicobacter pylori* infection and determination of clarithromycin resistance by fluorescence in situ hybridization from formalin-fixed,

paraffin-embedded gastric biopsy specimens. Can J Microbiol 51(7):569-573.
Chavan UD, Shahidi F, Naczk M (2001) Extraction of condensed tannins from beach pea (*Lathyrus maritimus* L.) as affected by different solvents. J Food Chem 75(4):509-512.
Chouaibi M, Mahfoudhi N, Rezig L, Donsì F, Ferrari G, Hamdi S (2012) Nutritional composition of *Zizyphus lotus* L. seeds. J Sci Food Agric 92(6):1171-1177.
Coimbra A, Miguel S, Ribeiro M, Coutinho P, Silva L, Duarte AP, Ferreira S (2022) *Thymus zygis* essential oil: phytochemical characterization, bioactivity evaluation and synergistic effect with antibiotics against *Staphylococcus aureus*. Antibiotics 11:146.
Crowe S-E (2019) *Helicobacter pylori* infection. N Engl J Med 380(12):1158-1165.
Dahlia F, Barouagui S, Hemida H, Bousaadia D, Rahmoune B (2020) Influence of environment variations on antiglycaemic, anti-cholesterolemic, antioxidant and antimicrobial activities of natural wild fruits of *Ziziphus lotus* (L.). S Afr J Bot 132:215-225.
Degu A, Kefale B, Alemayehu D, Temesgen TG (2020) Evaluation of the antidiarrheal activity of hydromethanol crude extracts of *Ruta chalepensis* and *Vernonia amygdalina* in mice. Evid Based Complementary Altern Med 2020:8318713.
Dhibi M, Amri Z, Bhouri A, Hammami S, Hammami M (2022) Comparative study of the phenolic profile and antioxidant activities of moringa (*Moringa oleifera* Lam.) and jujube (*Ziziphus lotus* Linn.) leaf extracts and their protective effects in frying stability of corn oil. Measurment: Food 7:100045.
Ebelle Etame RM, Mouokeu RS, Ngono Ngane RA, Assam Assam JP, Masoohe AM, Tientcheu R, Hopogap ML, Etoa FX (2017) Acute and sub-acute toxicity of *Harungana madagascariensis* LAM (*Hypericaceae*) stem bark methanol extract. J Appl Pharm Sci 7(03):160-167.
FMS-AC/EUCAST: French Society of Microbiology-Antibiogram Committee/European Committee on Antimicrobial Susceptibility Testing (2020) Antibiogram committee of the French Society of Microbiology. Available: www.sfm-microbiologie.org.
Gaginella TS, Phillips SF (1975) Ricinoleic acid: current view of an ancient oil. Am J Dig Dis 20:1171-1177.
Ganeshpurkar A, Saluja AK (2017) The pharmacological potential of rutin. Saudi Pharm J 25(2):149-164.
Ghalem M, Murtaza B, Belarbi M, Akhtar Khan N, Hichami A (2018) Antiinflammatory and antioxidant activities of a polyphenol-rich extract from *Zizyphus lotus* L fruit pulp play a protective role against obesity. J Food Biochem 42(6):e12689.
Gonzalez Trujano ME, Carrera D, Ventura Martinez A, Cedillo Portugal E, Navarrete A (2006) Neuropharmacological profile of an ethanol extract *Ruta chalepensis* L. in mice. J Ethnopharmacol 106:129-135.
Hale AL (2003) Screening potato genotypes for antioxidant activity, identification of the responsible compounds, and differentiating Russet Norkotah strains using AFLP and microsatellite marker analysis. PhD Thesis. Texas A&M University, Texas.
Hamdiken M, Bouhalit S, Kechrid Z (2017) Effect of *Ruta chalepensis* on zinc, lipid profile and antioxidant levels in the blood and tissue of streptozotocin-induced diabetes in rats fed zinc-deficient diets. Can J Diabetes 42(4):356-364.
Hani A-M, Zaouani M, Mimoune N, Ainouz L, Djellout B, Remichi H, Boudjellaba S, Bouchoucha A (2020) Evaluation of anti-inflammatory and antidiarrheal activity of leaf aqueous extracts of *Zizyphus lotus* (L) in albino Wistar rats. Vet Med 77(1):53-60.
Havagiray R, Ramesh C, Sadhna K (2004) Study of antidiarrhoeal activity of *Calotropis gigantea* R.Br. in experimental animals. J Pharm Sci 7:70-75.
Hawaz E (2014) Isolation and identification of probiotic lactic acid bacteria from curd and *in vitro* evaluation of its growth inhibition activities against pathogenic bacteria. Afr J Microbiol Res 8(13):1419-1425.
Islam AM, Uddin ME, Chowdhury AU, Rahman M, Habib R, Rahman A (2013) *In vivo* antidiarrheal and cytotoxic potential of different fractions of *Pandanus foetidus* leaves. Am J Biomed Sci 5(3):208-216.
Jawed A, Singh G, Kohli S, Sumera A, Haque S, Prasad R, Paul D (2019) Therapeutic role of lipases and lipase inhibitors derived from natural resources for remedies against metabolic disorders and lifestyle diseases. S Afr J Bot 120:25-32.
Kamoun J, Rahier R, Sellami M, Koubaa I, Mansuelle P, Lebrun R, Aloulou A (2019) Identification of a new natural gastric lipase inhibitor from star anise. Food Funct 10(1):469-478.
Kim S, Covington A, Pamer EG (2017) The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. Immunol Rev 279(1):90-105.
Kubab N, Hakawati I, Alajati-Kubab S (2006) Guide of biological examinations, 4th Ed., Lamarre, France.
Le Daré B, Ferron PR, Gicquel T (2021) Once upon a time hepatotoxicity. EDP Sci 37(3):235-241.
Letaief T, Garzoli S, Masci VL, Mejri J, Abderraba M, Tiezzi A, Ovidi E (2021) Chemical composition and biological activities of Tunisian *Ziziphus lotus* extracts: evaluation of drying effect, solvent extraction, and extracted plant parts. Plants 10(12):10.3390.
Mamza UT, Sodipo OA, Khan IZ, Wampana B (2014) Evaluation of antidiarrhoeal activity of ethanolic extract from the leaves of *Phyllanthus amarus* (Euphorbiacea). Int J Green Herb Chem 3(1):171-178.
Marmouzi I, Kharbach M, El Jemli M, Bouyahya A, Cher-

rah Y, Bouklouze A, Faouzi MEA (2019) Antidiabetic, dermatoprotective, antioxidant and chemical functionalities in *Zizyphus lotus* leaves and fruits. Ind Crops Prod 132:134-139.

Mohammed A, Ahmed H, Goji AD, Okpanachi AO, Ezekiel I, Tanko Y (2009) Preliminary anti-diarrhoeal activity of hydromethanolic extract of aerial part of *Indigofera pulchra* in rodents. Asian J Med Sci 1(2):22-25.

Mujumdar AM, Misar AV, Salaskar MV, Upadhye AS (2001) Antidiarrhoeal effect of an isolated fraction (JC) of *Jatropha curcas* roots in mice. J Nat Remedies 1(2):89-93.

Murugesan T, Lopamudra G, Kakali M, Das J, Pal M (2000) Evaluation of antidiarrhoeal profile of *Jussiaea suffruiticosa* Linn- extracts in rats. Phytother Res 14:381-383.

Nahar L, El-Seedi HR, Khalifa SAM, Hosseini MM, Sarker SD (2021) *Ruta* essential oils: composition and bioactivities. Molecules 26(16):4766.

Naidu RJ, Ismail R, Sasidharan S (2014) Acute oral toxicity and brine shrimp lethality of methanol extract of *Mentha spicata* l (Lamiaceae). Trop J Pharm Res 13(1):101-107.

Nsofor CA, Sarah U, Chinyere U (2014) Isolation and characterization of lactic acid bacteria from ogi sold in Elele Nigeria. J Biol Food Sci Res 3:19-22.

Obi TU, Oduye OO (1985) Haematological changes in natural and experimental peste des petits ruminants virus infection in goats. Rev Elev Med Vét Pays Trop38:11-15.

OECD: Organization for Economic Cooperation and Development (2008) Repeated dose oral toxicity test method? OECD/OCDE Guidelines for Testing of Chemicals, Ed. OECD, Paris France.

Olkkonen VM, Sinisalo J, Jauhiainen M (2018) New medications targeting triglyceride-rich lipoproteins: Can inhibition of ANGPTL3 or apoC-III reduce the residual cardiovascular risk. Atherosclerosis 272:27-32.

Ozturk N, Lee J, Gaddameedhi S, Sancar A (2009) Loss of crytochrome reduces cancer risk in p53 mutant mice. PNAS 106(8):2841-2846.

Popoff M (1981) Données biochimiques chez les ovins : application au diagnostic différentiel de quelques maladies métaboliques. Bull Soc Vét Prat France 9:695-706. [in French]

Quetin Leclercq J (2002) The unusual journey from plant to medicine. J Pharm Belgium 56(4):95-104.

Renier J-C (1982) Lettre ouverte aux biologistes où les phosphatases alcalines et leurs unités dites internationales. New Presse Med 11:1032. [in French]

Romani A, Pinelli P, Cantini C, Cimato A, Heimler D (2006) Characterization of Violetto di Toscana, a typical Italian variety of artichoke *(Cynara scolymus* L.). J Food Chem 95:221-225.

Rsaissi N, Kamili EL, Bencharki B, Hillali L, Bouhache M (2013) Antimicrobial activity of fruits extracts of the wild jujube *Ziziphus lotus* (L.) Desf. Int J Sci Eng Res 4(9):1521-1528.

Saad SE, Ahmed AF, Fayed MS, Elhamili A, Sughir AA (2018) Studying the effects of *Ruta chalepensis* on blood glucose, cholesterol and triglycerides levels in rats. Lebda Med J 4(1):132-136.

Saheed S, Omotayo TAA (2016) Antimicrobial and antidiarrheal activities of *Pelargonium luridum* (Andrews) sweet root extracts. Pharmacol 7:202-210.

Shakya SS, Bhandari M, Thapa SR, Shrestha R, Poudyal R, Purbey B, Gurung RB (2016) Medication adherence pattern and factors affecting adherence in *Helicobacter pylori* eradication therapy. Kathmandu Univ Med J (KUMJ)14:58-64.

Shokryazdan P, Sieo CC, Kalavathy R, Liang JB, Alitheen NB, Faseleh Jahromi M, Ho YW (2014) Probiotic potential of *Lactobacillus* strains with antimicrobial activity against some human pathogenic strains. Biomed Res 2014:927268.

Sirreratawong S, Vannasiri S, Nanna U, Singhalak T, Jaijoy K (2012) Evaluation of acute and chronic toxicities of the water extract from *Ziziphus attopensis* Pierre. Pharmacol 2012:789651.

Szewczyk A, Marino A, Molinari J, Ekiert H, Miceli N (2022) Phytochemical characterization, and antioxidant and antimicrobial properties of agitated cultures of three rue species: *Ruta chalepensis*, *Ruta corsica*, and *Ruta graveolens*. Antioxidants 11(3), 592

Teke NG, Kuiate JR, Kueté V, Teponno RB, Tapondjou LA, Vilarem G (2010) Antidiarrheal activity of extracts and compound from *Trilepisium madagascariense* stem bark. Indian J Pharmacol 42(3):157-163.

Terkmane S, Gali L, Bourrebaba L, Shoji K, Legembre P, Konstantia G, Ioanna C, Bedjou F (2017) Chemical composition, antioxidant, and anticancer effect of *Ruta chalepensis*'s extracts against human leukemic cells. Phytothérapie 16(S1):S225-S236.

Touiti N, Bousta D, Boukhira S, Chebaibi M, Achour S (2020) Phytochemical screening, acute and sub-acute toxicity of aqueous extract from a mixture of some recipe of *Herniaria glabra* L., *Opuntia ficusindica*, *Zea mays* L. and *Zizyphus lotus* L. used traditionally against renal lithiases. Phcog Res 12(1):60-64.

Tzounis X, Rodriguez Mateos A, Vulevic J, Gibson GR, Kwik Uribe C, Spencer JP (2011) Prebiotic evaluation of cocoa derived flavanols in healthy humans by using a randomized, controlled, double blind, crossover intervention study. Am J Clin Nutr 93(1):62-72.

Velàzquez C, Calzada F, Torres J, Gonzalez F, Ceballos G (2006) Antisecretory activity of plants used to treat gastrointestinal disorders in Mexico. J Ethnopharmacol 103(1):66-70.

WHO (2017) Diarrhoeal disease. Available: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease.

Wiciński M, Gębalski J, Mazurek E, Podhorecka M, Śniegocki M, Szychta P, Sawicka E, Malinowski B (2020) The influence of polyphenol compounds on human gastrointestinal tract microbiota. Nutrients 12(2):E350.

Yahia M, Benabderrahim MA, Tlili N, Bagues M, Nagaz K (2020) Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two *Ziziphus* Mill. species. PLoS ONE 15(5):e0232599.

Yamakoshi J, Tokutake S, Kikuchi M (2001) Effect of proanthocyanidin rich extract from grape seeds on human fecal flora and fecal odor. Microb Ecol Health Dis 13:25-31.

Zazouli S, Chigr M, Ramos PAB, Rosa D, Castro MM, Jouaiti A, Duarte MF, Santos SAO, Silvestre AGD (2022) Chemical profile of lipophilic fractions of different parts of *Zizyphus lotus* L. by GC-MS and evaluation of their antiproliferative and antibacterial activities. Molecules 27(2):10.3390.

Ziaee A, Zamansoltani F, Nassiri M, Abbasi E (2009) Effects of rutin on lipid profile in hypercholesterolaemic rats. Basic Clin Pharmacol Toxicol 104(3):253-258.