International Journal of Secondary Metabolite
2022, Vol. 9, No. 4, 397–414
https://dx.doi.org/10.21448/ijsm.1106820
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

Mineral contents, antimicrobial profile, acute and chronic toxicity of the aqueous extract of Moroccan *Thymus vulgaris* in rodents

Nidal Naceiri Mrabti[1], Hanae Naceiri Mrabti[2], Latifa Doudach[3], Zineb Khalil[4], Mohamed Reda Kachmar[5], Mouna Mekkaoui[6], Ryan A. Sheikh[7], Emad M. Abdallah[8], Gokhan Zengin[9,*], Samiah Hamad Al-Mijalli[10], Abdelhakim Bouyahya[11], Moulay El Abbes Faouzi[2], Menana Elhallaoui[1]

[1]Modeling and Environmental Engineering, LIMME, Faculty of Sciences Dhar El Mehraz, Sidi Mohammed Ben Abdellah University, USMBA, BP.1796, 30000, Atlas, Fez. Morocco
[2]Laboratory of Pharmacology and Toxicology, Bio-Pharmaceutical and Toxicological Analysis Research Team, Faculty of Med. and Phar., Mohammed V Uni. in Rabat, Rabat, Morocco
[3]Biomedical Eng. Dep., National School of Arts and Crafts Rabat Mohammed V Univ. in Rabat, Rabat, Morocco
[4]Lab. of Med. Chem., Drug Sciences Res. Center, Fac. of Med. and Phar., Mohammed V University in Rabat, Rabat, Morocco
[5]Fac. of Sci., Health and Envir. Lab., Plant Protection Team, Moulay Ismail Univ., Meknes, Zitoun, Morocco
[6]Phar. Res. Team, Laboratory of Phar. and Tox., Fac. of Med. and Phar., Uni. Mohammed V in Rabat, Morocco
[7] Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[8]Dep. of Science Laboratories, College of Science and Arts, Qassim University, ArRass, 51921 Saudi Arabia
[9]Department of Biology, Science Faculty, Selcuk University, Konya, Türkiye
[10]Dep. of Bio., College of Sciences, Princess Nourah Bint Abdulrahman University, Riyadh 11671, Saudi Arabia
[11]Lab. of Human Pathologies Biology, Dep. of Bio., Fac. of Sciences, Mohammed V University in Rabat, Morocco

Abstract: Moroccan flora is rich in plants used in traditional medicine, but the further scientific investigation is necessary. The aim of the research was to evaluate the nutritional content and antimicrobial activity of Moroccan *Thymus vulgaris*, as well as its possible acute and chronic toxicological effects on rodents. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to determine the mineral content. The antimicrobial activity was determined using a well-diffusion test, a minimum inhibitory concentration (MIC), and a minimum bactericidal/fungicidal concentration (MBC/MFC) assay. Acute and chronic toxicity studies were conducted *in vivo* on mice and rats, respectively. Following that, haematological, serum-biochemistry, and histological investigations were performed. Moroccan Thyme was shown to be a source of numerous minerals which are necessary for health promotion. All antimicrobial testing, disc diffusion, MIC, and MBC tests revealed that thyme had potent antibacterial activity against all microorganisms tested. *Staphylococcus aureus* was the most susceptible bacterium, followed by *Salmonella enterica* and *Escherichia coli*. Additionally, thyme exhibited great antifungal efficacy against *Candida albicans*. The acute toxicity results indicated that the aqueous extract of *T. vulgaris* is almost non-toxic when taken orally. According to the chronic toxicity study, the extract is generally safe when taken orally over an extended period of time. The biochemical and haematological characteristics of the serum and blood were within acceptable limits, and histological examination revealed no abnormalities. In conclusion, the findings of this investigation, confirm the antimicrobial efficacy of the aqueous extract of Moroccan *T. vulgaris* and its safety for experimental animals.

ARTICLE HISTORY
Received: Apr. 21, 2022
Revised: Sep. 06, 2022
Accepted: Oct. 17, 2022

KEYWORDS
Thymus vulgaris,
Mineral analysis,
Toxicity,
Antimicrobial,
Rodents.

*CONTACT: Gokhan Zengin ✉ gokhanzengin@selcuk.edu.tr Department of Biology, Science Faculty, Selcuk University, Konya, Türkiye

ISSN-e: 2148-6905 / **© IJSM 2022**

1. INTRODUCTION

Since ancient times, natural products have been the main source of the most active ingredients in drugs and remedies (Mrabti *et al.*, 2018, Bouyahya *et al.,* 2020a; Bouyahya *et al*., 2021a; Bouyahya *et al.*, 2021b; Bouyahya *et al.,* 2021c). Despite the dominance of synthetic chemical drugs in the current modern era, natural products still receive great attention from the scientific community and the population. About 80% of the World's inhabitants rely on these products for primary healthcare, and almost half of the new medications approved since 1994 are based on natural products (Harvey, 2008). Recently, a new concept has emerged and is widely spread all over the globe, which is "functional food" or nutraceuticals. These are foods rich in nutrients and associated with a number of powerful health benefits, such as berries, some vegetables, green tea, fruits, nuts, and many more (Gupta & Mishra, 2020).

Antimicrobial resistance, according to the World Health Organization, is a worldwide public health concern that must be addressed with the greatest seriousness (Mendelson & Matsoso, 2015). The issue is exacerbated by the rise of emerging infectious diseases, with 335 new infectious diseases identified between 1940 and 2004 (Jones *et al*., 2008). Antibiotic abuse hastened the emergence of antibiotic-resistant bacteria and antibiotic resistant genes, significantly lowering their therapeutic value against human and animal infections (Bouyahya, *et al.,* 2017c; Qiao *et al.,* 2018). So, there is a real need for new antimicrobial drugs, and medicinal plants could be a good source for novel antimicrobial agents (Abdallah, 2011; Bouyahya *et al.,* 2017b; Bouyahya *et al.*, 2019). Secondary metabolites (phytochemical compounds) are produced by plants that are not necessary for regular growth or development but are vital for reproduction and defense mechanisms against herbivors, fungi, bacteria and viruses, among others. These substances include terpenoids, flavonoids, phenolic acids, and other groups and have a high potential for acting as medicines (Anand *et al.*, 2019; Bouyahya, *et al.*, 2020a; Chamkhi *et al.*, 2021, 2022; Salehi *et al.*, 2021; Sharifi-Rad *et al.,* 2021). Extensive studies have shown that numerous medicinal plants possess extraordinary antimicrobial, antioxidant, anticancer, and anti-inflammatory properties (Noumi *et al.,* 2020; Reddy *et al.*, 2020; Hajlaoui *et al.*, 2021; Khalfaoui *et al.*, 2021; Al Kaabi *et al.*, 2022), and large drug companies should investigate the prospect of using these plants in the development of novel antimicrobials (Cowan, 1999). Scientific research has proved the effectiveness of certain plants' extracts against a variety of microbes and their activities were competitive to antibiotics, including curry leaf plant (*Murraya koenigii*, Rutaceae) (Joshi *et al.,* 2018; Abuga *et al.,* 2020), ginger (*Zingiber officinale*, Zingiberaceae) (Zare-Shehneh *et al.*, 2014; Chakotiya *et al.*, 2017), coriander (*Coriandrum sativum*, Apiaceae) (Matasyoh *et al.*, 2009; Zare-Shehneh *et al.*, 2014), rosemary (*Salvia rosmarinus*, Lamiaceae) (Miraj & Kiani, 2016; Pieracci *et al.*, 2021) and black cumin (*Nigella sativa*, Ranunculaceae) (Salman *et al.*, 2008; Randhawa *et al.*, 2017).

Thymus vulgaris L. known as thyme, belongs to the Lamiaceae family. It is a perennial short-shrub (up to 25 cm high), with tiny oval to oblong leaves (6-12 mm) (Escobar *et al.,* 2020). This herb is a famous functional food, known since ancient civilizations, indigenous to the Mediterranean countries and Northern Africa (Oliviero *et al.,* 2016). It is a popular food in Morocco. Indeed, it has been used as a flavoring ingredient, cooking plant, and herbal medicine for ages (Mustafa *et al.,* 2020). *T. vulgaris* has a long history of usage in traditional medicine to cure a variety of ailments, such as respiratory disorders, toothaches, urinary tract diseases, gastrointestinal disorders, and microbial infections (Abdallah, 2016). In the last decades, various pharmacological investigations carried out on thyme revealed interesting biological activities. Some studies reported that it has antioxidant, anti-inflammatory, antiviral, insecticidal, and antibacterial properties (Prasanth *et al.*, 2014). *T. vulgaris* is a typical aromatic plant. Its essential oils reveale that it contains up to 47 compounds. Thymol, *p*-cymene, and carvacrol are the major constituents, together comprising about 79.2% of the essential oil

constituents (Rota *et al.,* 2008). Morocco is rich in diverse flora and herbs grown in this area are an integral part of Moroccan traditions and culture (Eddouks *et al.,* 2002a; Mrabti *et al.*, 2021). However, there is still a lot of this herbal wealth that needs scientific verification. Besides, the chemical composition of thyme and, subsequently, its biological activities might vary greatly from one region to another, and it is critical to figure out what the true composition of wild plants is in different localities (Guillén & Manzanos, 1998). Therefore, we have proposed in this study to evaluate the mineral contents, Acute and chronic toxicity of the aqueous extract of Moroccan thyme, besides the antimicrobial properties.

2. MATERIAL and METHODS

2.1. Plant Material Collection and Extraction

The aerial parts of *T. vulgaris* were collected from north-eastern Morocco, about 30 km away from Taza (Morocco) in March 2021. The plant material was authenticated by a botanist in the laboratory of botany at the Scientific Institute of Rabat/Morocco, and a voucher number, RAB-1201, was deposited in the herbarium for future reference. Plant material was dried in the shade at room temperature, powdered to achieve a mean particle size, and kept in the dark until future use. The powder was extracted by infusion (for toxicological assessment) and maceration with 80% methanol (for antimicrobial profile). Briefly, 50 g of plant powders were infused in 500 mL of distilled water for a period of 30 min, then filtered and evaporated under vacuum at 50 °C using a rotary evaporator. The recovered extract was frozen and lyophilized to remove all traces of water.

2.2. Minerals Content

The mineral content of *T. vulgaris* was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Tests were carried out on *T. vulgaris* powder using the method we used before (Zaazaa *et al.*, 2021).

2.3. Well-Diffusion Assay

The antibacterial activity of *T. vulgaris* was determined using an agar well-diffusion method, which was modified somewhat from a previously reported assay (Abdallah *et al.,* 2021) with minor modifications. 25 mL of an autoclaved molten nutrient agar (Oxoid Ltd, Ireland) for bacteria or Sabouraud dextrose agar (Thermo Fisher Scientific, USA) for yeast was poured in sterile plates (90 mm diameter) and allowed to solidify. Microorganisms were distributed over the appropriate agar plate (adjusted to 0.5 McFarland). Using a sterilized cork borer. On the inoculated plates, three wells (6 mm in diameter) were bored. 100 µL of thyme methanolic extract was added to these wells, except for one well in each plate that was filled with 100 µL of the reference antibiotic (2.5 mg/mL chloramphenicol for bacteria or 5 mg/mL clotrimazole for yeast). After 24 hours of incubation at 35 °C, all plates were inspected for inhibition zones, which were measured in millimeters (mm). The test was repeated three times and the mean was calculated.

2.4. Minimum Inhibitory Concentration Assay (MIC)

Microorganisms which showed sensitivity in the well-diffusion assay were subjected to MIC test, using the micro-dilution method (Gulluce *et al.*, 2006) with slight modifications. Briefly, tested microorganisms were sub-cultured for up to 12 hours, and inoculum was taken and adjusted to (0.5 McFarland standard). Thyme extract was diluted in two-fold serial dilution to get a concentration range from 3.9 to 250 mg/mL in nutrient broth for bacteria or Sabouraud dextrosebroth for yeast. The 96-well plates were prepared by loading 95 µL of double-strength nutrient broth and 5 µL of inoculum. The first well was filled with 100 µL of extract (250 mg/mL) and then, from their successive dilutions, 100 µL was put into seven wells in a row. As a negative control, the last well was filled with 195 µL of nutrient broth without extract. The

prepared micro-wells were loaded with 5 µL of the inoculum in the appropriate medium, according to the type of microbe. Plates were covered with sterile plate sealer (Bio-Rad Laboratories Inc. USA) and incubated overnight at 35 ºC with frequent gentle shaking. Determination of microbial growth in the 96-well plates containing the formulated media was performed using a microplate reader (Bio-Rad Laboratories Inc. USA) at 595 nm. The test was repeated three times to pinpoint the MIC value accurately.

2.5. Minimum Bactericidal/Fungicidal Concentration Assay

The minimum bactericidal concentration (MBC) for bacteria and minimum fungicidal concentration (MFC) for yeast were determined after the MIC test (Carolina et al., 2013). In brief, 50 µL was pipetted from each MIC tube and spotted over plates containing nutrient agar for bacteria or Sabouraud dextrose agar for yeast, then incubated at 35 ºC for 24 hours. After incubation, the plates were checked for microbial growth. MBC was defined as the lowest MIC that showed no observable growth. The MBC/MIC was also determined to be bacteriostatic or bactericidal.

2.6. Experimental Animals

Adult Swiss albino mice and Wistar rats were used for acute treatment and ninety-day toxicity studies, respectively. Animals were obtained from the animal colony of the Department of Pharmacology and Toxicology of the Faculty of Medicine and Pharmacy, Mohamed V University (Rabat-Morocco). Animals were kept in standard animal house conditions including a 12 h light/dark cycle and a room temperature of 20–22 °C. All experimental procedures were performed based on the guidelines of the Ethical Committee for the care and use of laboratory animals.

2.7. Acute Oral Toxicity Study

The acute toxicity of *T. vulgaris* extract was evaluated according to OECD standards. Organization for Economic Cooperation and Development (OECD) 423 (OECD, 2001). A total of 60 mice weighing 25-35 g were randomly divided into six experimental groups of 10 mice each (6 males and 6 females per group). After overnight fasting, an aqueous extract (dissolved in aqueous solution) was administered by oral gavage as a single dose of 500, 1000, 2000 and 5000 mg / kg body weight, respectively. After treatment, the animals were observed for 14 days while they had unrestricted access to water and food.

2.8. Chronic Toxicity Study

The chronic toxicity study was performed according to OECD Test Guidelines 408 for 90 days with certain modifications (El Kabbaoui *et al.,* 2017). A total of 48 male and female Wister rats weighing between 190 and 250 g were randomly divided into four groups (n = 6 males and 6 females/group). After conceiving the animals in 4 groups of 6 rats each, the treated group received daily gastric gavage at doses (500, 700, and 1000 mg/kg) of the aqueous extract tested at the time when the control group received physiological solution (vehicle) for 90 days. During the experimental period, the body weights of all groups were measured once a week. Animals were visually observed for mortality, behavioral changes, physical appearance changes, and signs of illness.

2.9. Hematology and Serum Biochemistry

For the hematological investigation, all animals were fasted overnight but were allowed access to water ad libitum. Rats were then anesthetized and blood samples were taken from the abdominal aorta. Whole blood was collected in EDTA tubes and processed immediately for hematologic analysis. The parameters measured were red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), white blood cell count (WBC),

neutrophils (NEU), eosinophils (EOS), basophils (BASO), lymphocytes (LYM), and monocytes (MONO), platelet count (PLT). The hematological analysis was performed using an automatic hematological analyzer (Sysmex XN-550). Biochemical tests are done by using dry tubes that have been drained of blood. They were centrifuged at 3000 rpm at 4 °C for 10 minutes to get the serum, which was used to figure out the total serum protein (Anand et al., 2019), the total bilirubin (T-BIL), the alkaline phosphatase (Major, 1954), the ALT, and the AST, as well as uric acid (URIC), urea, creatinine, total cholesterol (TC), triglycerides (TG), and glucose (GLU) using an auto-analyzer called the "Cobas c 502."

2.10. Histopathology

Rats were sacrificed by sodium pentobarbital overdose. Liver, kidney, and spleen samples were obtained immediately and fixed in 10% phosphate buffered formalin for further examination. Tissue samples were cut into 2–3 mm thick slices with a surgical blade. The samples were processed using a tissue processor and embedded in paraffin. Paraffin blocks were cut using a microtome cutter and stained with hematoxylin and eosin before observation under light microscopy.

2.11. Statistical Analysis

Data was expressed as the mean ± standard deviation (Mann *et al.,* 2000). Statistical significance between control and treated groups was determined by one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test. GraphPad Prism version 6.0 for Windows was used for statistical analysis. Data analyses from male and female groups were done separately, and the differences were considered statistically significant at $p<0.05$.

3. RESULTS

3.1. Mineral Composition

As shown in Table 1, the mineral contents of the edible aerial parts of Moroccan thyme have some important minerals including macro-elements (Ca, P, K, Mg, and Na), the microelements (S, Co, Fe, B, Cu, Zn, Mn, and V). The result of the analyses was established to give nutrient values per mg/kg of dried weight. According to our results, the contents of Calcium (Ca) and Potassium (K) were very high, with a concentration of 13 574.51 mg/kg and 10764.47mg/kg respectively, while elements such as Cobalt (Co) were the least abundant microelements with a concentration of 0.001 mg/kg.

Table 1. Levels of some mineral contents in the aerial parts of *T. vulgaris.*

Mineral	Content in mg/kg dw
Macro-elements	
K	10764.47
Mg	2476.26
Na	509.85
Ca	13574.51
P	1078.73
Micro-elements	
B	26.83
Cu	31.59
S	89.190
Mn	36.23
Fe	419.26
Zn	42.08
Co	0.001
Cr	1.224
V	5.881

3.2. Antimicrobial Activity

The antimicrobial activity of *T. vulgaris* was screened by the agar well-diffusion assay. The methanolic extract of *T. vulgaris* exhibited noticeable antimicrobial activity against all tested microorganisms (Figure 1). The statistical analysis revealed that Thyme has significant antimicrobial activity against all tested microorganisms at (P 0.05, one-way ANOVA), as shown in (Table 2). For antibacterial activity, *Staphylococcus aureus* was found to be the most sensitive (32.5±07 mm), *Salmonella enterica* came next (18.5±0.7 mm), followed by *Escherichia coli* (13.5±0.7 mm). Some bacteria were comparable in sensitivity against the extract to the antibiotic, such as *Staphylococcus aureus* (Figure 1 and Table 2). For antifungal activity, *Candida albicans* exhibited significant sensitivity to the extract (25.5±0.7 mm) and was comparable to the antibiotic used. The MIC and MBC/MFC results are shown in (Table 3). Bacteriostatic or bactericidal activity was assessed by MBC/MIC. When the MBC/MIC or MFC/MIC ratio is less than or equal to 4, the extract is considered bactericidal/fungicidal, whereas if the ratio is higher than 4, the extract is bacteriostatic/fungistatic (Abdallah, 2016). Accordingly, methanol extract of thyme was highly fungicidal to *Candida albicans* (at <3.9 mg/mL), followed by other bacteria, where it was highly bactericidal to *Staphylococcus aureus* (15.6 mg/mL) and *Escherichia coli* (31.2 mg/mL), and to a lesser degree with *Salmonella enterica* (7.8 mg/mL).

Figure 1. The inhibition zone (mm) of *T. vulgaris* against tested microorganisms. (Ext.) represent the methanolic crude extract (500 mg/ml), (Chl.) chloramphenicol (2.5 mg/ml), (Clo.) clotrimazole (5 mg/ml).

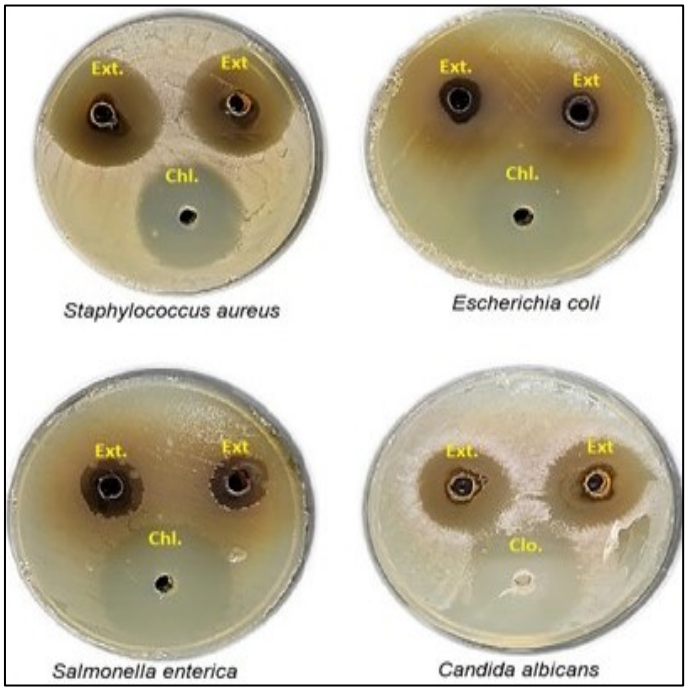


Table 2. Antimicrobial activity of methanol extract of thyme against tested microorganisms

Tested microorganisms	Mean inhibition zone diameter (mm)		
	T. vulgaris (500 mg/mL)	Chloramphenicol (2.5 mg/mL)	Clotrimazole (5 mg/mL)
Staphylococcus aureus	32.5±0.7[a]	32.5±0.7[a]	-
Salmonella enterica	18.5±0.7[b]	27.5±0.7[b]	-
Escherichia coli	13.5±0.7[c]	24.5±0.7[c]	-
Candida albicans	25.5±0.7[b]	-	31.5±2.1[a]

*Mean±Standard deviation, Different letters (a-c) indicate significantly differences (p<0.05).

Table 3. MIC, MBC and MBC/MIC rations of thyme methanol extract against tested microorganisms.

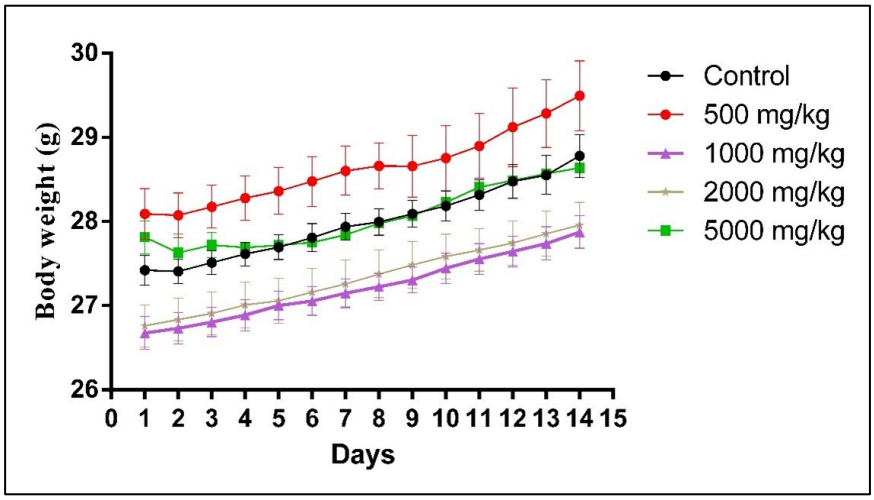
Microorganisms	Methanol extract of thyme (mg/mL)		
	MIC	MBC or MFC*	MBC/MIC
Staphylococcus aureus	15.6	15.6	1.0
Salmonella enterica	7.8	31.2	4.0
Escherichia coli	31.2	31.2	1
Candida albicans	< 3.9	< 3.9	0

*MFC test for the yeast *Candida albicans*, MBC for other tested bacteria.

3.3. Acute Toxicity Study

Regarding acute toxicity evaluation of thyme aqueous extract, administration of different 500 mg/kg,1000 mg/kg, 2000 mg/kg and 5000 mg/kg doses of aqueous extracts of the aerial parts of *T. vulgaris* did not show any signs of toxicity like restlessness, motor activity, breathing, and diarrhea, etc. There was no difference in body weight gained compared to the witness. As it is shown in (Figure 2), in other words, all mice survived for the first 24 h and up to 14 days of cage side follow-up. This implies that, the median lethal dose (LD_{50}) of the extract causing 50% of the deaths of the animals is said to be greater than 5000 mg/kg, suggesting a good safety margin.

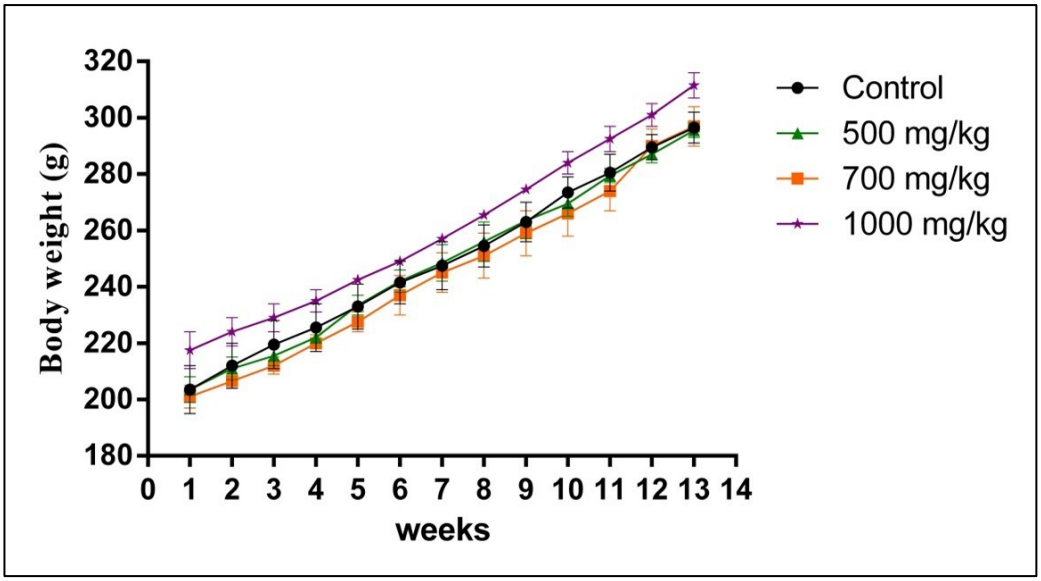
Figure 2. Acute toxicity of *T. vulgaris* aqueous extract.



3.4. Sub-Chronic Toxicity Study

Since body weight is the best indicator of good health and efficient metabolic homeostasis. Throughout the study period, we found no signs of toxicity or deaths of animals at any dose up to the maximum of 1000 mg/kg during the 13 weeks of repeated oral treatment. No statistically significant difference in body weight was noted between the control group and the treated group, either females or males. As it is shown in (Figure 3), which allows us to deduce the absence of signs of toxicity in rats treated with the aqueous extract of the plant studied and the tolerance of this extract in rats.

Figure 3. Chronic toxicity of *T. vulgaris* aqueous extract.



3.5. Hematology Analysis

The hematological parameters of male and female rats after 90 days of treatment with aqueous extract of *T. vulgaris* are shown in (Tables 4 and 5). The effect of sub-chronic oral administration of different doses of 500, 700 and 1000 mg/kg of extract showed no significant difference from the control group. All hematological parameters were within normal limits.

Table 4. Hematological parameters of male's rats after 90 days of treatment with *T. vulgaris* extract.

Parameters	Control	*T. vulgaris* (mg/kg B.W)		
		500	700	1000
WBC (10^3/mm^3)	9.41 ± 0.60	9.24 ± 0.81	9.92 ± 0.63	9.19 ± 0.92
RBC (10^6/mm^3)	7.53 ± 0.29	7.96 ± 0.25	8.19 ± 0.62	8.26 ± 0.45
HCT (%)	38.92 ± 2.02	42.38 ± 4.39	42.56 ± 5.43	39.71 ± 2.29
HGB (g/dL)	13.64 ± 0.69	13.74 ± 0.79	13.16 ± 0.89	14.21 ± 0.54
MCV (µm^3/red cell)	58.02 ± 2.04	57.85 ± 2.22	57.71 ± 3.72	56.37 ± 2.28
MCH (pg/ red cell)	17.07 ± 0.12	17.27 ± 1.03	16.91 ± 0.05	16.83 ± 0.02
MCHC (g/dL)	33.24 ± 1.02	32.82 ± 0.01	33.07 ± 0.09	33.13 ± 0.01
PLT (10^3 cells/mm^3)	892.13 ± 107.11	884.72 ± 103.07	878.03 ± 105.06	863.03 ± 99.01
LYM (%)	73.11 ± 4.06	72.02 ± 4.04	71.57 ± 5.01	71.83 ± 4.21
NEU (%)	24.02 ± 2.09	24.50 ± 0.11	24.22 ± 1.03	24.87 ± 1.05
MONO (%)	3.48 ± 0.02	3.67 ± 0.06	3.35 ± 0.02	3.73 ± 0.04
BASO (%)	0.05 ± 0.01	0.05 ± 0.10	0.06 ± 0.02	0.06 ± 0.04
EOS (%)	1.57 ± 0.01	1.52 ± 0.04	1.49 ± 0.07	1.3 ± 0.03

Values are expressed as mean ± SD, n = 6.

Table 5. Hematological parameters of female rats after 90 days of treatment with *T. vulgaris* extract.

Parameters	Control	*T. vulgaris* (mg/kg B.W)		
		500	700	1000
WBC (10^3/mm^3)	8.46 ± 0.57	9.11 ± 0.02	9.21 ± 0.09	9.24 ± 0.03
RBC (10^6/mm^3)	8.32 ± 0.61	8.35 ± 0.04	8.56 ± 0.07	8.42 ± 0.06
HCT (%)	41.12 ± 0.05	39.15 ± 0.08	39.82 ± 0.02	40.16 ± 0.09
HGB (g/dL)	13.41 ± 0.01	13.37 ± 0.09	13.72 ± 0.08	13.42 ± 0.08
MCV (µm^3/red cell)	54.22 ± 0.06	55.33 ± 0.01	53.22 ± 0.09	53.45 ± 0.03
MCH (pg/ red cell)	16.71 ± 0.03	16.48 ± 0.09	16.57 ± 0.01	16.37 ± 0.05
MCHC (g/dL)	33.12 ± 2.04	32.87 ± 1.05	32.91 ± 0.72	33.08 ± 2.01
PLT (10^3 cells/mm^3)	871.37 ± 1.64	873.13 ± 1.53	901.75 ± 1.01	904.17 ± 0.08
LYM (%)	71.51 ± 0.04	70.63 ± 0.09	70.82 ± 0.07	71.51 ± 0.02
NEU (%)	23.13 ± 1.09	23.22 ± 0.02	23.81 ± 0.08	23.79 ± 1.08
MONO (%)	3.21 ± 0.02	3.14 ± 0.04	3.61 ± 0.05	3.47 ± 0.09
BASO (%)	0.01 ± 0.02	0.01 ± 0.04	0.02 ± 0.01	0.02± 0.03
EOS (%)	1.63 ± 0.08	1.65 ± 0.06	1.62 ± 0.07	1.60 ± 0.09

Values are expressed as mean ± SD, n = 6.

3.6. Clinical Biochemistry Analysis

Tables 6 and 7 summarize the levels or activities of biochemical parameters in male and female rats. All the tested doses of *T. vulgaris* extract did not induce a significant change in the concentration of ALT, AST, total bilirubin, total protein, albumin, creatinine, urea and uric acid. However, the extracts studied at doses of 700 and 1000 mg / kg caused a significant reduction in the level of total cholesterol, triglycerides, in both groups of rats compared to the control groups. Although *Thymus* extract induced various significant decreases (p<0.05 and p<0.01) in the fasting glucose levels of the treated groups of rats, which was labeled at the dose of 1000 mg / kg, (p<0.01).

Table 6. Biochemical parameters of male rats after 90 days of treatment with *T. vulgaris* extract.

Parameters	Control	*T. vulgaris* (mg/kg B.W)		
		500	700	1000
AST (UI/L)	145.72 ± 1.03	145.83 ± 1.04	146.15 ± 1.19	146.33 ± 1.02
ALT (UI/L)	56.32 ± 0.05	52.80 ± 0.03	49.97 ± 0.08	54.45 ± 0.03
ALP (UI/L)	185.07 ± 1.04	181.45 ± 1.03	179.22 ± 1.05	182.31 ± 1.02
Total bilirubin (mg/L)	0.49 ± 0.02	0.48 ± 0.07	0.51 ± 0.04	0.52 ± 0.01
Total proteins (g/L)	68.17 ± 1.01	69.25 ± 0.02	67.23 ± 0.08	67.85 ± 1.06
Total cholesterol (g/L)	0.64 ± 0.06	0.61 ± 0.03	0.52 ± 0.02*	0.51 ± 0.01**
Triglycerides (g/L) HDL	0.70 ± 0.03	0.62 ± 0.02*	0.58 ± 0.02**	0.58 ± 0.02**
Glucose (g/L)	1.09 ± 0.16	0.93 ± 0.03*	0.90 ± 0.05**	0.88 ± 0.05**
Urea (g/L)	0.35 ± 0.03	0.36 ± 0.04	0.36 ± 0.03	0.36 ± 0.05
Creatinine (mg/L)	4.17 ± 0.09	3.81 ± 0.02	4.12 ± 0.09	4.22 ± 0.02
Uric acid (mg/L)	11.25 ± 1.04	11.57 ± 1.33	11.83 ± 0.75	11.97 ± 1.02

Values are expressed as mean ± SD, n = 6. Compared to control group (one-way ANOVA followed by Dunnet's post-hoc test). $^*p<0.05$ compared to control group (one-way ANOVA followed by Dunnet's post-hoc test). $^{**}p<0.01$ compared to control group (one-way ANOVA followed by Dunnet's post-hoc test).

Table 7. Biochemical parameters of female rats after 90 days of treatment with *Thymus* extracts.

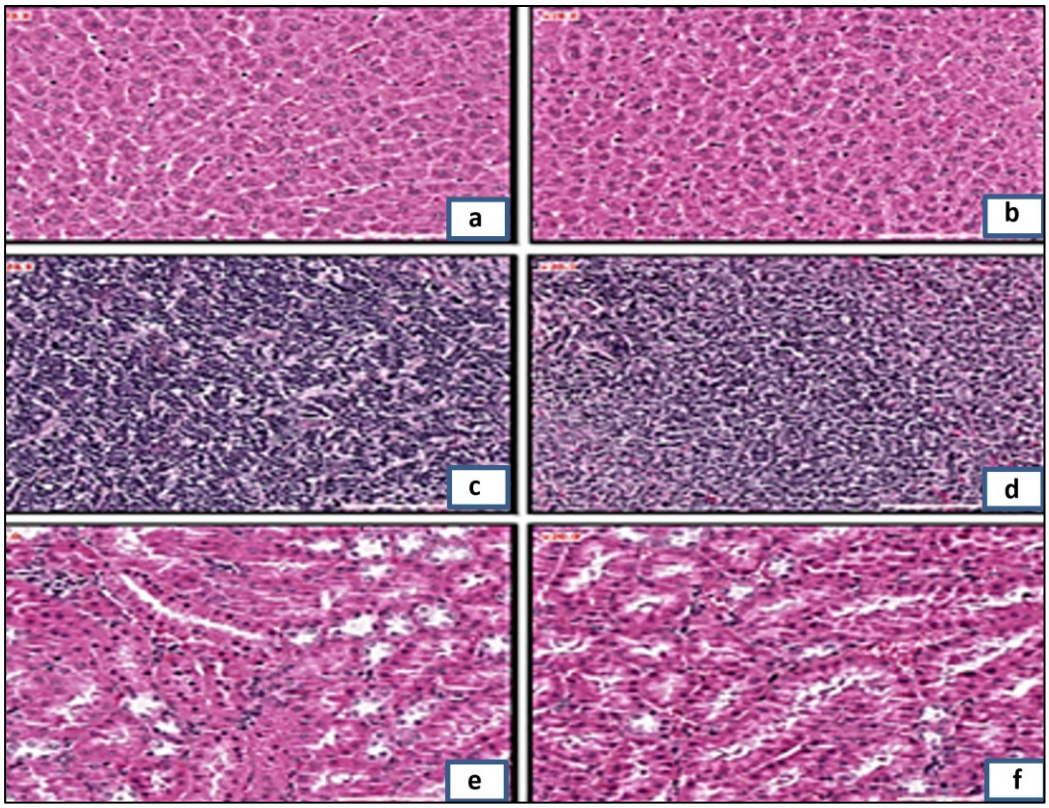
Parameters	Control	*T. vulgaris* (mg/kg B.W)		
		500	700	1000
AST (UI/L)	148.27 ± 1.01	148.83 ± 1.02	147.15 ± 1.03	148.33 ± 1.09
ALT (UI/L)	54.12 ± 0.03	53.80 ± 0.01	54.27 ± 0.02	54.15 ± 0.08
ALP (UI/L)	195.11 ± 0.04	191.18 ± 0.03	194.16± 0.04	192.21 ± 0.03
Total bilirubin (mg/L)	0.47 ± 0.14	0.47 ± 0.25	0.49 ± 0.21	0.48 ± 0.31
Total proteins (g/L)	67.05 ± 0.02	67.75 ± 0.01	68.33 ± 0.03	67.39 ± 0.04
Total cholesterol (g/L)	0.61 ± 0.06	0.53 ± 0.08	0.49 ± 0.04**	0.48 ± 0.04**
Triglycerides (g/L) HDL	0.69 ± 0.01	0.50 ± 0.01	0.47 ± 0.03**	0.43 ± 0.02**
Glucose (g/L)	0.99 ± 0.13	0.84 ± 0.04*	0.80 ± 0.06**	0.80 ± 0.05**
Urea (g/L)	0.36 ± 0.01	0.36 ± 0.02	0.35 ± 0.01	0.35 ± 0.03
Creatinine (mg/L)	3.87 ± 0.05	3.91 ± 0.01	4.01 ± 0.05	4.11 ± 0.03
Uric acid (mg/L)	12.78 ± 0.01	12.98 ± 0.03	13.03 ± 0.05	13.07 ± 0.02

Values are expressed as mean ± SD, n = 6. *$p<0.05$ (one-way ANOVA followed by Dunnet's post-hoc test). **$p<0.01$ (one-way ANOVA followed by Dunnet's post-hoc test).

3.7. Histological Analysis

Microscopic examination of liver, spleen, and kidney tissues was performed on rats that were either untreated (a, c, e) or treated with 1000 mg/kg of *T. vulgaris* aqueous extract (b, d, f, respectively). Demonstrate a normal morphology comparable to that of the control group, indicating that repeated oral administration of the aqueous extract of *T. vulgaris* for 13 weeks by gavage generated no detrimental modifications or morphological abnormalities (Figure 4).

Figure 4. Chronic toxicity (tissue histology). There no difference between treated and control group. On the right, a (1000 mg/kg), b (700 mg/kg) and c (500 mg/kg) represent treated groups. On the left, a (1000 mg/kg), b (700 mg/kg) and c (500 mg/kg) represent no treated groups.



4. DISCUSSION

Calcium is the most abundant mineral in this plant, owing to its role in the formation and strength of bones and teeth. Additionally, it is involved in a broad variety of biological processes, including muscular contraction and nerve impulse transmission (Vannucci *et al.*, 2018). Our results also suggested that Moroccan thyme was rich in macro- and micro-elements, which are necessary for health maintenance through supporting metabolism, energy generation, growth, and healing (Soni *et al.*, 2010). Our findings corroborated previous studies on the mineral content of *T. vulgaris*, as the Turkish variety of this plant also included highest concentrations of Ca, Fe, K, Mg, and S, when compared to thirty-two Turkish medicinal herbs (Özcan, 2004).

Other studies revealed also the richness of *Thymus* species in mineral compounds Indeed, according to study of Ouknin *et al.* (2018), it was revealed that *T. zygis* subsp. gracilis, *T. pallidus*, *T. willdenowii* containing numerous mineral compounds with certain variability depending on plant species and plant parts (Ouknin *et al.,* 2018). Moreover, other species belonging to *Thymus* genus such as *T. capitatus* and *T. broussonetii* containing also different mineral compounds. The variability of these components depends to different factors such as the genetic of specie, plant parts used, and climatic changes (Bouyahya *et al.,* 2020c; Naceiri Mrabti *et al.,* 2021).

Additionally, it was found that species belonging to the genus *Thymus* have a wide variety of chemical constituents; this diversity can be explained by both endogenous (plant varieties, organ tested and vegetative state) and exogenous factors such as soil characteristics, seasons and climatic features (Mseddi *et al.,* 2020).

The findings of this study also revealed that *T. vulgaris* showed good antimicrobial activity against all tested microorganisms to varied degrees using the well diffusion method. The well-diffusion assay is a simple and effective method to determine the possible presence of antimicrobial agents in tested plant extracts (Savaroğlu *et al.*, 2011). Previous studies have been done on thyme grown in various regions worldwide and showed antimicrobial potential of *T. vulgarus* (Al-Bayati, 2008; Jafari *et al.,* 2020).

Indeed, it was reported that thyme essential oils showed various degrees of antibacterial activity, but higher antifungal activity was recorded against *C. albicans*. Tural & Turhan (2017) mentioned that thyme essential oils have remarkable antibacterial effects, against *S. aureus*, *E. coli* and *L. monocytogenes*. Moreover, the current study exhibited that, based on the MBC/MIC values, *T. vulgaris* has potent fungicidal (against *C. albicans*) and bactericidal effects especially against *S. aureus* and *E. coli*. Previous published studies confirmed the thyme essential oils recorded remarkable MIC values, which reflect its effectiveness as a potent antimicrobial against different microorganisms *(*Borugă *et al.,* 2014; Fournomiti *et al.,* 2015; Imelouane *et al.,* 2011). Therefore, the aqueous extract of thyme has an effective antimicrobial properties. There is a robust relationship between the phytochemical structures of thyme and its antimicrobial activity, which could be related to terpenes present in thyme, and this compound is thought to cause rupture of the microbial membrane by a lipophilic mechanism (Imelouane *et al.,* 2011).

T. vulgaris, on the other hand, is regularly consumed by Moroccans with no obvious toxic effects documented (Eddouks *et al.,* 2002b). The scientific assessment of this reputation needs an examination of the plant's long- or medium-term effect on the organism. To the best of our knowledge, no study assessing the safety of the aqueous extract of *T. vulgaris* has been conducted in Morocco. As a result, the oral route was selected for the acute toxicity investigation since it is the most often utilized route in people under normal settings. The results obtained indicated that there were no indicators of toxicity and no death in mice during a 14-

day period with any of the dosages examined, indicating that its usage is safe and without harmful consequences.

Concerning chronic toxicity, the male and female rats administered with the aqueous extract of *T. vulgaris* exhibited a normal body weight evolution, indicating the lack of toxicity. Hematological examination revealed no significant changes in the parameters measured in rats treated with *T. vulgaris* aqueous extract. The aqueous extract also did not influence biochemical parameters at any of the dosages studied. However, this extract resulted in a considerable decrease in total cholesterol and triglycerides.

In agreement with our results, another study found that supplementing drinking water with thyme extract reduces plasma triglycerides and total cholesterol (Abdulkarimi *et al.,* 2011). Thus, this extract resulted in a considerable drop in blood glucose levels throughout therapy, indicating that it is suitable for use in the treatment of diabetes (Tuama, 2016). A similar study conducted in Peru showed no evidence of toxicity in acute and repeated 28-day oral dosage toxicity tests of *T. vulgaris* essential oil in rats (Rojas-Armas *et al.,* 2019).

During the experiment, oral administration of the aqueous extract of *T. vulgaris* caused no detrimental alterations or morphological abnormalities in the tissues examined and this was also in harmoney with previous studies (Benourad *et al.*, 2014). On the contrary, one study revealed that it had a healing impact on liver tissues when rabbits were exposed to methotrexate (MTX)-induced toxicity (Swayeh *et al.,* 2014).

Acknowledgments

Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2022R158) Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship contribution statement

Nidal Naceiri Mrabti: Resources, Software, Formal Analysis, and Writing-original draft original draft. **Hanae Naceiri Mrabti**: Resources, Software, Formal Analysis, and Writing-original draft original draft. **Latifa Doudach**: Resources, Software, Formal Analysis, and Writing-original draft original draft. **Zineb Khalil**: Resources, Software, Formal Analysis, and Writing-original draft original draft. **Mohamed Reda Kachmar**: Resources, Software, Formal Analysis, and Writing-original draft original draft. **Mouna Mekkaoui**: Resources, Software, Formal Analysis, and Writing-original draft original draft. **Ryan A. Sheikh**: Investigation, Visualization, and Editing. **Emad M. Abdallah**: Investigation, Visualization, and Editing. **Gokhan Zengin**: Investigation, Visualization, and Editing. **Samiah Hamad Al-Mijalli**: Investigation, Visualization, and Editing. **Abdelhakim Bouyahya**: Methodology, Supervision, and Validation. **Moulay El Abbes Faouzi**: Methodology, Supervision, and Validation. **Menana Elhallaoui**: Methodology, Supervision, and Validation.

Orcid

Nidal Naceiri Mrabti https://orcid.org/0000-0001-6389-7300
Hanae Naceiri Mrabti https://orcid.org/0000-0002-8145-2572
Latifa Doudach https://orcid.org/0000-0002-4687-0066
Zineb Khalil https://orcid.org/0000-0003-0389-4081
Mohamed Reda Kachmar https://orcid.org/0000-0001-8191-1129
Mouna Mekkaoui https://orcid.org/0000-0003-4821-6145
Ryan A. Sheikh https://orcid.org/0000-0003-3275-0861

Emad M. Abdallah https://orcid.org/0000-0003-0549-249X
Gokhan Zengin https://orcid.org/0000-0001-6548-7823
Samiah Hamad Al-Mijalli https://orcid.org/0000-0003-3160-6008
Abdelhakim Bouyahya https://orcid.org/0000-0001-9317-1631
Moulay El Abbes Faouzi https://orcid.org/0000-0003-3863-4677
Menana Elhallaoui https://orcid.org/0000-0002-9863-6298

REFERENCES

Abdallah, E.M. (2011). Plants: An alternative source for antimicrobials. *Journal of Applied Pharmaceutical Science*, *1*(6), 16–20.

Abdallah, E.M. (2016). Antibacterial activity of *Hibiscus sabdariffa* L. calyces against hospital isolates of multidrug resistant Acinetobacter baumannii. Journal of Acute Disease, *5*(6), 512–516. https://doi.org/10.1016/j.joad.2016.08.024

Abdallah, E.M., Mujawah, A.A.H., & Al-Mijalli, S.H. (2021). GC-MS and Antibacterial potential of methanolic extract hyphaene Thebaica L. Fruit pulp against antibiotics-resistant pathogens. *Journal of Pure and Applied Microbiology*, *15*(3), 1655–1664. https://doi.org/10.22207/jpam.15.3.62

Abdulkarimi, R., Daneshyar, M., & Aghazadeh, A. (2011). Thyme (*Thymus vulgaris*) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights
in broiler chickens. *Italian Journal of Animal Science*, *10*(2), e20. https://doi.org/10.4081/ijas.2011.e20

Abuga, I., Sulaiman, S.F., Wahab, R.A., Ooi, K.L., & Rasad, M.S.B.A. (2020). In vitro antibacterial effect of the leaf extract of *Murraya koenigii* on cell membrane destruction against pathogenic bacteria and phenolic compounds identification. *European Journal of Integrative Medicine*, *33*, 101010.

Al Kaabi, S.K.S., Senthilkumar, A., Kizhakkayil, J., Alyafei, M.A.M., Kurup, S.S., Al Dhaheri, A.S., & Jaleel, A. (2022). Anticancer activity of *Moringa peregrina* (Forssk.) Fiori.: A Native plant in traditional herbal medicine of the United Arab Emirates. *Horticulturae*, *8*(1), 37.

Al-Bayati, F.A. (2008). Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. *Journal of Ethnopharmacology*, *116*(3), 403–406.

Anand, U., Jacobo-Herrera, N., Altemimi, A., & Lakhssassi, N. (2019). A comprehensive review on medicinal plants as antimicrobial therapeutics: Potential avenues of biocompatible drug discovery. *Metabolites*, *9*(11), 258.

Benourad, F., Kahvecioglu, Z., Youcef-Benkada, M., & Colet, J.-M. (2014). Prospective evaluation of potential toxicity of repeated doses of *Thymus vulgaris* L. extracts in rats by means of clinical chemistry, histopathology and NMR-based metabonomic approach. *Drug Testing and Analysis*, *6*(10), 1069–1075.

Borugă, O., Jianu, C., Mişcă, C., Goleţ, I., Gruia, A.T., & Horhat, F.G. (2014). *Thymus vulgaris* essential oil: Chemical composition and antimicrobial activity. *Journal of Medicine and Life*, *7*(Spec Iss 3), 56–60.

Bouyahya, A., Dakka, N., Et-Touys, A., Abrini, J., & Bakri, Y. (2017a). Medicinal plant products targeting quorum sensing for combating bacterial infections. *Asian Pacific Journal of Tropical Medicine*, *10*(8), 729–743. https://doi.org/10.1016/j.apjtm.2017.07.021

Bouyahya, A., Et-Touys, A., Bakri, Y., Talbaui, A., Fellah, H., Abrini, J., & Dakka, N. (2017b). Chemical composition of *Mentha pulegium* and *Rosmarinus officinalis* essential oils and their antileishmanial, antibacterial and antioxidant activities. *Microbial Pathogenesis*, *111*, 41–49.

Bouyahya, A., Dakka, N., Talbaoui, A., Et-Touys, A., El-Boury, H., Abrini, J., & Bakri, Y. (2017c). Correlation between phenological changes, chemical composition and biological activities of the essential oil from Moroccan endemic Oregano (*Origanum compactum* Benth). *Industrial Crops and Products*, *108*, 729-737. https://doi.org/10.1016/j.indcrop.2017.07.033

Bouyahya, A., Abrini, J., Dakka, N., & Bakri, Y. (2019). Essential oils of *Origanum compactum* increase membrane permeability, disturb cell membrane integrity, and suppress quorum-sensing phenotype in bacteria. *Journal of Pharmaceutical Analysis*, *9*(5), 301-311. https://doi.org/10.1016/j.jpha.2019.03.001

Bouyahya, A., El Omari, N., Elmenyiy, N., Guaouguaou, F.-E., Balahbib, A., El-Shazly, M., & Chamkhi, I. (2020a). Ethnomedicinal use, phytochemistry, pharmacology, and toxicology of *Ajuga iva* (L.,) Schreb. *Journal of Ethnopharmacology*, *258*, 112875.

Bouyahya, A., Belmehdi, O., Benjouad, A., El Hassani, R. A., Amzazi, S., Dakka, N., & Bakri, Y. (2020b). Pharmacological properties and mechanism insights of Moroccan anticancer medicinal plants: What are the next steps? *Industrial Crops and Products*, *147*, 112198.

Bouyahya, A., Chamkhi, I., Guaouguaou, F.-E., Benali, T., Balahbib, A., El Omari, N., Taha, D., El-Shazly, M., & El Menyiy, N. (2020c). Ethnomedicinal use, phytochemistry, pharmacology, and food benefits of *Thymus capitatus*. *Journal of Ethnopharmacology*, *259*, 112925. https://doi.org/10.1016/j.jep.2020.112925

Bouyahya, A., El Menyiy, N., Oumeslakht, L., El Allam, A., Balahbib, A., Rauf, A., Muhammad, N., Kuznetsova, E., Derkho, M., & Thiruvengadam, M. (2021a). Preclinical and clinical antioxidant effects of natural compounds against oxidative stress-induced epigenetic instability in tumor cells. *Antioxidants*, *10*(10), 1553.

Bouyahya, A., El Omari, N., Elmenyiy, N., Guaouguaou, F.-E., Balahbib, A., Belmehdi, O., Salhi, N., Imtara, H., Mrabti, H. N., & El-Shazly, M. (2021b). Moroccan antidiabetic medicinal plants: Ethnobotanical studies, phytochemical bioactive compounds, preclinical investigations, toxicological validations and clinical evidences; challenges, guidance and perspectives for future management of diabetes worldwide. *Trends in Food Science & Technology*, *115*, 147–254.

Bouyahya, A., Guaouguaou, F.-E., El Omari, N., El Menyiy, N., Balahbib, A., El-Shazly, M., & Bakri, Y. (2021c). Anti-inflammatory and analgesic properties of moroccan medicinal plants: Phytochemistry, in vitro and in vivo investigations, mechanism insights, clinical evidences and perspectives. *Journal of Pharmaceutical Analysis*, *12*, 35–37.

Carolina, A., Santana, E.F. De, Marcos, A., Coutinho, F.N., Henrique, R., Castro, A. De, & Nelly, M. (2013). Which approach is more effective in the selection of drugs. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 2005–2010.

Chakotiya, A.S., Tanwar, A., Narula, A., & Sharma, R. K. (2017). *Zingiber officinale*: Its antibacterial activity on Pseudomonas aeruginosa and mode of action evaluated by flow cytometry. *Microbial Pathogenesis*, *107*, 254–260.

Chamkhi, I., Benali, T., Aanniz, T., El Menyiy, N., Guaouguaou, F.-E., El Omari, N., El-Shazly, M., Zengin, G., & Bouyahya, A. (2021). Plant-microbial interaction: The mechanism and the application of microbial elicitor induced secondary metabolites biosynthesis in medicinal plants. *Plant Physiology and Biochemistry*, *167*, 269–295.

Chamkhi, I., Charfi, S., Hachlafi, N.E., Mechchate, H., Guaouguaou, F.-E., El Omari, N., Bakrim, S., Balahbib, A., Zengin, G., & Bouyahya, A. (2022). Genetic diversity, antimicrobial, nutritional, and phytochemical properties of *Chenopodium album:* A comprehensive review. *Food Research International*, *154*, 110979.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*(4), 564–582.

Eddouks, M., Maghrani, M., Lemhadri, A., Ouahidi, M.-L., & Jouad, H. (2002a). Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). *Journal of Ethnopharmacology*, *82*(2), 97–103. https://doi.org/10.1016/S0378-8741(02)00164-2

Eddouks, M., Maghrani, M., Lemhadri, A., Ouahidi, M.-L., & Jouad, H. (2002b). Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). *Journal of Ethnopharmacology*, *82*(2–3), 97–103.

El Kabbaoui, M., Chda, A., El-Akhal, J., Azdad, O., Mejrhit, N., Aarab, L., Bencheikh, R., & Tazi, A. (2017). Acute and sub-chronic toxicity studies of the aqueous extract from leaves of *Cistus ladaniferus* L. in mice and rats. *Journal of Ethnopharmacology*, *209*(April), 147–156. https://doi.org/10.1016/j.jep.2017.07.029

Escobar, A., Pérez, M., Romanelli, G., & Blustein, G. (2020). Thymol bioactivity: A review focusing on practical applications. *Arabian Journal of Chemistry*, *13*(12), 9243–9269. https://doi.org/10.1016/j.arabjc.2020.11.009

Fournomiti, M., Kimbaris, A., Mantzourani, I., Plessas, S., Theodoridou, I., Papaemmanouil, V., Kapsiotis, I., Panopoulou, M., Stavropoulou, E., Bezirtzoglou, E. E., & Alexopoulos, A. (2015). Antimicrobial activity of essential oils of cultivated oregano (*Origanum vulgare*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*) against clinical isolates of *Escherichia coli* , *Klebsiella oxytoca*, and *Klebsiella pneumoniae*. *Microbial Ecology in Health & Disease*, *26*(0). https://doi.org/10.3402/mehd.v26.23289

Guillén, M.D., & Manzanos, M.J. (1998). Study of the composition of the different parts of a Spanish thymus vulgaris L. plant. *Food Chemistry*, *63*(3), 373-383. https://doi.org/10.1016/S0308-8146(98)00013-2

Gulluce, M., Aslan, A., Sokmen, M., Sahin, F., Adiguzel, A., Agar, G., & Sokmen, A. (2006). Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca*, *Ramalina pollinaria, Ramalina polymorpha* and *Umbilicaria nylanderiana*. *Phytomedicine*, *13*(7), 515-521. https://doi.org/10.1016/j.phymed.2005.09.008

Gupta, E., & Mishra, P. (2020). Functional Food with Some Health Benefits, So Called Superfood: A Review. *Current Nutrition & Food Science*, *17*(2), 144-166. https://doi.org/10.2174/1573401316999200717171048

Hajlaoui, H., Arraouadi, S., Noumi, E., Aouadi, K., Adnan, M., Khan, M.A., Kadri, A., & Snoussi, M. (2021). Antimicrobial, antioxidant, anti-acetylcholinesterase, antidiabetic, and pharmacokinetic properties of *Carum carvi* L. and *Coriandrum sativum* L. essential oils alone and in combination. *Molecules*, *26*(12), 3625.

Harvey, A.L. (2008). Natural products drug discovery. *Drug Discovery Today*, *13*(19–20), 894–901. https://doi.org/10.1016/j.drudis.2008.07.004

Imelouane, B., Amhamdi, H., Whathelet, J., Ankit, M., Khedid, K., & EL Bachiri, A. (2011). Chemical composition and antimicrobial activity of essential oil of *Thymus vulgaris* from Yemen. *Turkish Journal of Biochemistry*, *36*(4), 342–349.

Jafari, B., Fatemi, S., Pashazadeh, M., Al-Snafi, A.E., & Shariat, A. (2020). Antibacterial effects of *Thymus vulgaris, Mentha pulegium, Crocus sativus* and *Salvia officinalis* on pathogenic bacteria: A brief review study based on gram-positive and gram-negative bacteria. *Jorjani Biomedicine Journal*, *8*(3), 58–74.

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, *451*(7181), 990–993.

Joshi, T., Jain, T., Mahar, R., Singh, S.K., Srivastava, P., Shukla, S.K., Mishra, D.K., Bhatta, R.S., Banerjee, D., & Kanojiya, S. (2018). Pyranocarbazoles from *Murraya koenigii* (L.) Spreng. As antimicrobial agents. *Natural Product Research*, *32*(4), 430–434.

Khalfaoui, A., Noumi, E., Belaabed, S., Aouadi, K., Lamjed, B., Adnan, M., Defant, A., Kadri, A., Snoussi, M., & Khan, M.A. (2021). LC-ESI/MS-Phytochemical Profiling with antioxidant, antibacterial, antifungal, antiviral and in silico pharmacological properties of Algerian *Asphodelus tenuifolius* (Cav.) organic extracts. *Antioxidants*, *10*(4), 628.

Major, R.H. (1954). A history of medicine. In *A history of medicine* (pp. 563–563).

Mann, C.M., Cox, S.D., & Markham, J.L. (2000). The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Letters in Applied Microbiology*, *30*(4), 294–297.

Matasyoh, J.C., Maiyo, Z.C., Ngure, R.M., & Chepkorir, R. (2009). Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum. Food Chemistry*, *113*(2), 526–529.

Mendelson, M., & Matsoso, M. P. (2015). The World Health Organization global action plan for antimicrobial resistance. *SAMJ: South African Medical Journal*, *105*(5), 325–325.

Miraj, S., & Kiani, S. (2016). A review study of therapeutic effects of *Salvia officinalis* L. *Der Pharmacia Lettre*, *8*(6), 299-303.

Mrabti, H.N., Bouyahya, A., Ed-Dra, A., Kachmar, M.R., Mrabti, N.N., Benali, T., Shariati, M.A., Ouahbi, A., Doudach, L., & Faouzi, M.E.A. (2021). Polyphenolic profile and biological properties of Arbutus unedo root extracts. *European Journal of Integrative Medicine*, *42*(September 2020), 101266. https://doi.org/10.1016/j.eujim.2020.101266

Mrabti, H.N., Jaradat, N., Fichtali, I., Ouedrhiri, W., Jodeh, S., Ayesh, S., Cherrah, Y., & Faouzi, M.E.A. (2018). Separation, identification, and antidiabetic activity of catechin isolated from *Arbutus unedo* L. Plant roots. *Plants*, *7*(2), 31. https://doi.org/10.3390/plants 7020031

Mseddi, K., Alimi, F., Noumi, E., Veettil, V.N., Deshpande, S., Adnan, M., Hamdi, A., Elkahoui, S., Alghamdi, A., & Kadri, A. (2020). *Thymus musilii* Velen. as a promising source of potent bioactive compounds with its pharmacological properties: In vitro and in silico analysis. *Arabian Journal of Chemistry*, *13*(8), 6782–6801.

Mustafa, S., Hina, S., Mahmood, S., Mueen-ud-din, G., & Alam, M.Q. (2020). Exploring phytochemical potential of nature' s bliss *Thymus vulgaris* L. Mini review. *International Journal of Botany Studies*, *5*(3), 493–496.

Naceiri Mrabti, H., Doudach, L., El Menyiy, N., Bourhia, M., Mohammad Salamatullah, A., Reda Kachmar, M., Belmehdi, O., El Moudden, H., Naceiri Mrabti, N., & Harhar, H. (2021). Phytochemistry and Pharmacology of *Thymus broussonetii* Boiss. *Evidence-Based Complementary and Alternative Medicine*, *2021*, Article ID 6350035, 15 pages

Noumi, E., Snoussi, M., Anouar, E.H., Alreshidi, M., Veettil, V.N., Elkahoui, S., Adnan, M., Patel, M., Kadri, A., & Aouadi, K. (2020). HR-LCMS-based metabolite profiling, antioxidant, and anticancer properties of *Teucrium polium* L. methanolic extract: Computational and in vitro study. *Antioxidants*, *9*(11), 1089.

OECD (2001). Acute Oral Toxicity – Fixed Dose Procedure (chptr). In *Guidelines for the Testing of Chemicals* (pp. 1–14).

Oliviero, M., Romilde, I., Beatrice, M.M., Matteo, V., Giovanna, N., Consuelo, A., Claudio, C., Giorgio, S., Maggi, F., & Massimo, N. (2016). Evaluations of thyme extract effects in human normal bronchial and tracheal epithelial cell lines and in human lung cancer cell line. *Chemico-Biological Interactions*, *256*, 125–133. https://doi.org/10.1016/j.cbi.2016.06.024

Ouknin, M., Romane, A., Arjouni, M.Y., & Majidi, L. (2018). Mineral composition, multivariate analysis of some oligo-elements and heavy metals in some species of genus *Thymus*. *Journal of Materials and Environmental Science*, *9*(3), 980–985.

Özcan, M. (2004). Mineral contents of some plants used as condiments in Turkey. *Food Chemistry*, *84*(3), 437–440.

Pieracci, Y., Ciccarelli, D., Giovanelli, S., Pistelli, L., Flamini, G., Cervelli, C., Mancianti, F., Nardoni, S., Bertelloni, F., & Ebani, V.V. (2021). Antimicrobial activity and composition of five *Rosmarinus* (Now *Salvia* spp. and varieties) essential oils. *Antibiotics*, *10*(9), 1090. https://doi.org/10.3390/antibiotics10091090

Prasanth, R., Kandisa, R.V., Varsha, P., & Satyam, S. (2014). Review on *Thymus vulgaris* traditional ues and pharmacological properties. *Medicinal & Aromatic Plants*, *03*(04), 3–5. https://doi.org/10.4172/2167-0412.1000167

Qiao, M., Ying, G.-G., Singer, A.C., & Zhu, Y.-G. (2018). Review of antibiotic resistance in China and its environment. *Environment International*, *110*, 160–172.

Randhawa, M.A., Alenazy, A.K., Alrowaili, M.G., & Basha, J. (2017). An active principle of *Nigella sativa* L., thymoquinone, showing significant antimicrobial activity against anaerobic bacteria. *Journal of Intercultural Ethnopharmacology*, *6*(1), 97-101. https://doi.org/10.5455/jice.20161018021238

Reddy, M.N., Adnan, M., Alreshidi, M.M., Saeed, M., & Patel, M. (2020). Evaluation of anticancer, antibacterial and antioxidant properties of a medicinally treasured fern *Tectaria coadunata* with its phytoconstituents analysis by HR-LCMS. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *20*(15), 1845–1856.

Rojas-Armas, J., Arroyo-Acevedo, J., Ortiz-Sánchez, M., Palomino-Pacheco, M., Castro-Luna, A., Ramos-Cevallos, N., Justil-Guerrero, H., Hilario-Vargas, J., & Herrera-Calderón, O. (2019). Acute and repeated 28-day oral dose toxicity studies of *Thymus vulgaris* L. essential oil in rats. *Toxicological Research*, *35*(3), 225–232.

Rota, M.C., Herrera, A., Martínez, R.M., Sotomayor, J.A., & Jordán, M.J. (2008). Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and Thymus hyemalis essential oils. *Food Control*, *19*(7), 681-687. https://doi.org/10.1016/j.foodcont.2007.07.007

Salehi, B., Quispe, C., Chamkhi, I., El Omari, N., Balahbib, A., Sharifi-Rad, J., Bouyahya, A., Akram, M, Iqbal, M., Docea, A.O., Caruntu, C., Leyva-Gómez, G., Dey, A, Martorell, M., Calina, D., López, V., & Les, F. (2021) Pharmacological Properties of Chalcones: A Review of Preclinical Including Molecular Mechanisms and Clinical Evidence. *Frontiers in Pharmacology*, *11,* 592654. https://doi.org/10.3389/fphar.2020.592654

Salman, M.T., Khan, R.A., & Shukla, I. (2008). Antimicrobial activity of *Nigella sativa* Linn. Seed oilagainst multi-drug resistant bacteria from clinical isolates. *Natural Product Radiance, 7*(1), 10-14.

Savaroğlu, F., İşçen, C.F., Vatan, A.P., Kabadere, O., & Sİlhan, S. (2011). Determination of antimicrobial and antiproliferative activities of the aquatic moss *Fontinalis antipyretica* Hedw. *Turkish Journal of Biology*, *35*(3), 361–369.

Sharifi-Rad, J., Dey, A., Koirala, N., Shaheen, S., El Omari, N., Salehi, B., Goloshvili, T., Silva, N.C.C., Bouyahya, A., & Vitalini, S. (2021). *Cinnamomum* species: Bridging phytochemistry knowledge, pharmacological properties and toxicological safety for health benefits. *Frontiers in Pharmacology*, *11*(12), 1541. https://doi.org/10.3390/plants11121541

Soni, M.G., Thurmond, T.S., Miller III, E.R., Spriggs, T., Bendich, A., & Omaye, S.T. (2010). Safety of vitamins and minerals: Controversies and perspective. *Toxicological Sciences*, *118*(2), 348–355.

Swayeh, N.H., Abu-Raghif, A.R., Qasim, B.J., & Sahib, H.B. (2014). The protective effects of Thymus Vulgaris aqueous extract against Methotrexate-induced hepatic toxicity in rabbits. *International Journal of Pharmaceutical Sciences Review and Research*, *29*, 187–193.

Tuama, R.J. (2016). Effect of thyme (*Thymus vulgaris* L.) oil on some biochemical parameters of diabetic female rats. *World Journal of Pharmaceutical Research, 4*(6), 320–325. https://doi.org/10.13140/RG.2.2.24589.49122

Tural, S., & Turhan, S. (2017). antimicrobial and antioxidant properties of thyme (*Thymus vulgaris* L.), Rosemary (*Rosmarinus officinalis* L.) and laurel *(Lauris nobilis* L.) essential oils and their mixtures. *The Journal of Food*, *42*(5), 588-596. https://doi.org/10.15237/gida.gd17030

Vannucci, L., Fossi, C., Quattrini, S., Guasti, L., Pampaloni, B., Gronchi, G., Giusti, F., Romagnoli, C., Cianferotti, L., Marcucci, G., & Brandi, M.L. (2018). Calcium Intake in bone health: A focus on calcium-rich mineral waters. *Nutrients*, *10*(12), 1-12. https://doi.org/10.3390/nu10121930

Zaazaa, L., Naceiri Mrabti, H., Ed-Dra, A., Bendahbia, K., Hami, H., Soulaymani, A., & Ibriz, M. (2021). Determination of Mineral Composition and Phenolic Content and Investigation of Antioxidant, Antidiabetic, and Antibacterial Activities of Crocus sativus L. Aqueous Stigmas Extracts. *Advances in Pharmacological and Pharmaceutical Sciences*, *2021,* Article ID 7533938, 7 pages. https://doi.org/10.1155/2021/7533938

Zare-Shehneh, M., Askarfarashah, M., Ebrahimi, L., Kor, N.M., Zare-Zardini, H., Soltaninejad, H., Hashemian, Z., & Jabinian, F. (2014). Biological activities of a new antimicrobial peptide from *Coriandrum sativum*. *International Journal of Biosciences, 4*(6), 89–99.