Trop J Nat Prod Res, February 2024 2024; 8(2):6423-6432 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of Polyphenolic Content, Antioxidant and Antimicrobial Activities, and Toxicity Study of *Ferula communis* L. Fruits

Imad Ed-Dahmani[1]*, Mohammed Kara[2,]*, Aziza Lfitat[1], Hanane Touijer[3], Fatima Z. Bousraf[1], Meryem Slighoua[4], Ghizlane Nouioura[5], Yassine El Atki[6], Mohamed El Fadili[7], Mustapha Taleb[1], Abdelfattah Abdellaoui[1]

[1]*Laboratory of Engineering, Electrochemistry, Modeling and Environment, Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco;*
[2]*Laboratory of Biotechnology, Conservation and Valorisation of Natural Resources (LBCVNR), Faculty of Sciences Dhar El Mehraz, Sidi Mohamed Ben Abdallah University, Fez 30000, Morocco;*
[3]*Laboratory of Biotechnology, Environment, Agri-food and Health, Faculty of Sciences Dhar E Mahraz, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco ;*
[4]*Laboratory of Biotechnology, Environment, Agri-Food, and Health, Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco;*
[5]*Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life (SNAMOPEQ), Faculty of Sciences Dhar El-Mehraz, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco;*
[6]*High Institute of Nursing Professions and Health Techniques Errachidia, Morocco;*
[7]*LIMAS Laboratory, Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, 30000 Fez, Morocco.*

ARTICLE INFO

Article history:
Received 11 October 2023
Revised 08 February 2024
Accepted 12 February 2024
Published online 01 March 2024

ABSTRACT

Many species of the genus Ferula, including *Ferula communis* are used medicinally to treat various diseases. Consumption of *Ferula communis* has been reported to have toxic effect. The study aims to evaluate the polyphenolic content, antioxidant, antimicrobial, and toxicological activities of the methanol and aqueous extracts of the edible fruit (MEFE and AEFE) and the fruit at the advanced stage of flowering (AEFAF and MEFAF) of *Ferula communis*. The polyphenolic (total phenolic and flavonoid) contents were evaluated using standard procedures. The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging, Ferric Reducing Antioxidant Power (FRAP), and Total Antioxidant Capacity (TAC) assays. The antimicrobial activity was evaluated against selected bacteria and fungal organisms using the disk diffusion method. Acute and sub-acute toxicity of the hydroethanol extract of the edible fruit (HEFE) was evaluated in mice according to standard procedures. The results indicate that the AEFE has higher phenolic and flavonoid content than the MEFE. Whereas, the MEFAF showed a very high amount of phenols and flavonoids compared to AEFAF. AEFAF displayed a higher antioxidant activity in the DPPH and FRAP assays, whereas MEFE showed higher antioxidant activity in the ABTS assay. AEEF and MEEF showed remarkable antibacterial action. Acute and sub-acute oral administration of HEFE did not cause any toxic effect in mice. On the basis of these findings, *Ferula communis* fruit could serve as a potential antioxidant against oxidative stress, and a potential antibacterial agent against pathogenic bacterial infection.

Keywords: *Ferula communis*, Phytochemicals, Antioxidant activity, Antimicrobial activity, Toxicity.

Introduction

Oxidative stress has been implicated in many human illnesses such as neurodegenerative diseases, cancers, cardiovascular diseases, diabetes, and inflammation.[1] Oxidative stress occurs as a result of free radical attack on various biomolecules, particularly lipids, proteins, and DNA, ultimately resulting in cell degradation and death.[2] Various synthetic antioxidants like butylated hydroxytoluene and butylated hydroxy anisole may be unsuitable for chronic use, as recent reports have stated their probable toxic effect on human health and the environment.[3]

*Corresponding author. E mail: imad.edhmani@gmail.com; mohammed.kara@usmba.ac.ma
Tel: 00212600778751; 002120658742494

Citation: Ed-Dahmani I, Kara M, Lfitat A, Touijer H, Bousraf FZ, Slighoua M, Nouioura G, El Atki Y, El Fadili M, Taleb M, Abdellaoui A. Evaluation of Polyphenolic Content, Antioxidant and Antimicrobial Activities, and Toxicity Study of *Ferula communis* L. FruitsTrop J Nat Prod Res. 2024; 8(2):6423-6432. http://www.doi.org/10.26538/tjnpr/v8i2.34

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Resistance to antibiotics by pathogenic microorganisms is a public health burden.[3] Despite the advances in modern medicine, medicinal plants are still being widely used for therapeutic purposes due to their effectiveness and safety.[4] The interesting pharmacological and therapeutic properties of medicinal plants have continued to attract the attention of researchers.[5–8] Therefore, interest in natural (non-toxic) antioxidants and antimicrobials, especially those of plant origin, has increased dramatically in recent years.[9] Antibacterial agents such as antibiotics and other anti-infectives have been available for decades. Today, multidrug-resistant (MDR) human pathogens are recognized as one of the world's most important health challenges.[10] Application of novel natural antibacterials, for example plants derived natural products are now under increasing threat due to the emergence of MDR bacteria, hence the need for more active treatments that act by new mechanisms that evades microbial resistance.[11]

The Ferula genus, which is part of the Apiaceae family, consists of about 170 species.[12] These species are widely distributed and cultivated across several regions in the world, from northern Africa to central Asia, and the western countries.[12] The genus Ferula is currently represented in Morocco by six species,[13] which have more or less extensive distribution. The most widespread species, *Ferula communis* L., is very

polymorphic and occupies a wide territory except for the desert and very arid regions.[14] Many species of this genus are used medicinally to treat various diseases. Among the Ferula species that have been used as natural remedies are *F. assa-foetida* (used as a diuretic, anticonvulsant, antispasmodic, carminative, aphrodisiac, antihelmintic, etc.), *F. badrakema* and *F. gummosa* (both used as anticonvulsant, tonic, antihysteric, decongestant, treatment of neurological disorders, and stomachache), and *F. persica* (used as antihysteric, carminative, laxative, treatment of lumbago, diabetes, backache and rheumatism) are the most famous.[15-17] It has long been known that consumption of *F. communis* can cause an often fatal disease known as ferulosis in cattle and cases of human intoxication through ingestion of *F. communis* have also been reported.[18] *F. communis* intoxication causes symptoms in cattle similar to those described for intoxication by fermented melilot, and it has been reported that the extract of this plant contains antithrombotic coumarin compounds.[19,20]
The toxic and non-toxic varieties of *F. communis* vary not only in the content of sesquiterpene esters or prenylated coumarins, but also in other classes of compounds, including phenylpropanoids and volatile terpenoids.[21] To the best of our knowledge, there is paucity of data on the antioxidant, antimicrobial, and toxicological activities of the Moroccan *F. communis* fruit. Therefore, the aim of the present study is to evaluate the antioxidant, and antimicrobial activities, as well as the acute and sub-acute toxicity of the fruit extracts of *F. communis*.

Materials and Methods

Collection and identification of plant material
F. communis fruits were harvested in the month of February, 2022 from Taounate, North of Morocco (34°08'64" N, 4°59'15" W). The plant material was identified by a taxonomist in the Department of Biology, Faculty of Science, Sidi Mohamed Ben Abdellah University, Fez, Morocco. A herbarium specimen with voucher number 2299/4-16-1/taw was deposited.
The edible fruits (EF) and the fruits at the advanced stage of flowering (FAF) were sorted, dried, and reduced to fine powder. The powdered fruits were stored in air-tight containers at room temperature (25.00 ± 2.00°C) away from light until when needed.

Extraction of plant material
The powdered samples (18 g each) of EF and FAF of *F. communis* were macerated separately with 150 mL of methanol, distilled water, and hydroethanol for 24 h. The resulting extracts were filtered, and concentrated at 40°C using a rotary evaporator. The concentrated extracts were stored in sterilized Eppendorf tubes and refrigerated at 4°C.
The percentage yields of the extracts were determined using the following formula:

$Y\ (\%) = (M_1/M_0) \times 100$
Where; M_0 is the mass (g) of the powdered plant material, and M_1 is the mass of the dry extract (g).

Determination of Total Phenolic Content (TPC)
The TPC of the methanol and aqueous extracts of *F. communis* fruits were determined by the Folin-Ciocalteu method.[22] Briefly, 2.5 mL of 10% Folin-Ciocalteu solution was added to 0.5 mL of extract and 2 mL of 7% sodium carbonate solution. The reaction mixture was left to stand for 2 h at room temperature in the dark. The absorbance of the reaction mixture was then measured at 760 nm using a spectrophotometer. A calibration curve was constructed using gallic acid. The TPC was expressed as milligrams of gallic acid equivalents per grams of dry-weight of extract (mg GAE/g Dw).

Determination of Total Flavonoid Content (TFC)
The TFC of the methanol and aqueous extracts of *F. communis* fruits were determined using aluminum chloride colorimetric method.[23] Briefly, 500 µL of sample or reference standard (quercetin) was added to 500 µL of 20% aluminum chloride. After 1 h of reaction at room temperature in the dark, the absorbance was measured at 420 nm. The total flavonoids content was in expressed as milligrams of quercetin equivalents per grams of dry-weight of extract (mg QE/g Dw).[23]

Determination of Antioxidant Activity
2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay
The DPPH radical scavenging activity of the methanol and aqueous extracts of *F. communis* fruits was evaluated using the method previously described by Wu *et al.* (2003).[24] Briefly, 1.5 mL of 0.1 mmol DPPH solution in methanol was added to 0.1 mL of the sample or standard solution at different concentrations. The mixture was incubated at room temperature in the dark for 30 min. Thereafter, the absorbance of the mixture was measured at 517 nm. butylated hydroxytoluene (BHT) was used as the positive control. The percentage inhibition of DPPH radical was calculated using the following formula:

$I\ (\%) = (1-(As/A_0)) \times 100$
Where; A_0 is the absorbance of the negative control, and As is the absorbance of the test sample or standard.

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Scavenging Assay
The ABTS radical scavenging activity was evaluated according to the procedure described by Re *et al*. (1999).[25] The $ABTS^{\cdot+}$ cationic radical was obtained by mixing 100 µL of 70 mM potassium persulfate ($K_2S_2O_8$) and 10 mL of 2 mM ABTS diammonium salt. The resulting mixture was incubated at room temperature in the dark for 24 h. The $ABTS^{\cdot+}$ cationic radical solution (2850 µL) was then mixed with 150 µL of the extract or Trolox (positive control). The absorbance of the resulting mixture was read at 734 nm following a 30-min incubation in the dark. The antiradical activity of the samples was expressed as percentage inhibition of the $ABTS^{\cdot+}$ radical according to the following formula:

Percentage ABTS radical inhibition (%) = [As–Ac] / Ac x 100
Where; As is the absorbance of the extracts and Ac is the absorbance of the control.
IC_{50} value was calculated as the concentrations resulting in 50% inhibition of the initial $ABTS^{\cdot+}$ radical.[26]

Ferric Reducing Antioxidant Power (FRAP) Assay
The ferric reducing antioxidant power of the methanol and aqueous extracts of *F. communis* fruits was evaluated using the method previously described by Oyaizu (1986).[27] To 200 µL of the extract solution was added 500 µL of 0.2 M phosphate buffer (pH 6.6), followed by 500 µL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). After incubating the mixture at 50°C for 20 min, 500 µL of 10% Trichloroacetic acid (TCA) was added, then centrifuged at 3000 rpm for 10 min. To 2.5 mL of the supernatant was added 100 µL of 0.1% $FeCl_3$ and 500 µL of distilled water. The absorbance of the resulting mixture was measured at 700 nm. Quercetin was used as the reference standard.

Total Antioxidant Capacity Assay
The determination of the total antioxidant capacity was based on the reduction of Mo(VI) to Mo(V) and the subsequent formation of a green phosphate Mo(V) complex in an acidic pH.[28] Briefly, 25 µL of the extract solution at different concentration was added to 1 mL of reagent solution (0.6 mol/L sulfuric acid, 4 mmol/L ammonium molybdate, and 28 mmol/L sodium phosphate). The mixture was incubated at 95°C at room temperature for 90 min. Then the absorbance was measured at 695 nm. Total antioxidant capacity was expressed as milligram vitamin C equivalence per gram of extract dry weight (mg vit C E/g Dw).

Determination of Antimicrobial Activity
Microbiological Strains
The antimicrobial activity of *F. communis* fruit extracts was assessed against four microbial strains; *Candida albicans* ATCC10231, *Staphylococcus aureus* ATCC6633; *Escherichia coli* K12, and *Bacillus subtilis* DSM 6333. All the microbial strains were provided by the Laboratory of Biotechnology, Environment, Agri-food, and Health, Faculty of Sciences Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, Fez, Morocco.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Evaluation of Antimicrobial Activity

The antimicrobial activity of methanol and aqueous extracts of *F. communis* fruits was assessed via the disk diffusion method.[29] Petri dishes containing Mueller–Hinton agar was used for the bacterial strains, while malt extract was used for the fungal strains. Inoculation was done by the double-layer technique from the freshly grown cultures in Mueller–Hinton broth or Malt extract. Serial dilutions were made in antiseptic physiological saline (NaCl 0.9%), and calibrated to a turbidity of 0.5 McFarland (10^6 - 10^8 CFU/mL) of which 100 µL was added to tubes containing 5 mL of agar culture media (0.5% of agar-agar). Then the inoculated tubes were spread in Petri dishes containing Mueller–Hinton or Malt extract medium. Sterile Whatman discs (6 mm diameter) were placed in the centre of the petri dish, and then impregnated with 20 µL of aqueous, and methanol extracts *F. communis* fruits at a concentration of 30 mg/mL in 10% DMSO. The inoculated petri dishes were incubated at 37°C for 24-48 h. Thereafter, the inhibition zone diameters were measured.[29-31]

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the methanol and aqueous extracts of *F. commuins* fruits against four microbial strains was determined using the microdilution method.[30] Briefly, sterile 96-well microplates were labelled, and 100 µL of *F. communis* extracts in 10% DMSO (v/v) were pipetted into the first row of the microplates. Then, 50 µL of sterile Mueller–Hinton or Malt extract was injected into each of the other wells. Using a multichannel pipette, serial dilutions were prepared, and then 30 µL of microbial solution of each strain was added to each well. The microplates were incubated at 37°C for 48 h (*C. albicans*) or 24 h (pathogenic bacteria).[30,32] The MIC was determined by the colorimetric method, using Resazurin.

Toxicity Studies

Acute and sub-acute toxicity were performed on forty (40) Swiss albino mice, male and female. The animals were provided by the Faculty of Science Dhar El Mehraz. The mice were aged between 4 and 6 weeks and weighed between 20 and 34 g. They were kept in cages under normal laboratory conditions.

Acute Toxicity Study

Acute toxicity was performed based on the protocol as stated by Costa-Silva *et al*. (2008),[33] following guideline No. 423. The mice were divided into four groups of 5 animals each, and were acclimatized for three days before beginning the experiment. The mice were fasted for 18 h prior to the administration of the various extracts. Group 1 (control) received distilled water, while groups 2, 3, and 4 received oral doses of the extract at 200, 300, and 400 mg/kg, respectively. Signs of toxicity (such as vomiting, diarrhoea, drowsiness, etc.), changes in general behaviour (aggressiveness and mobility), and body weights of mice in each group were monitored for 14 days.

Sub-acute Toxicity Study

Sub-acute toxicity was evaluated according to the method described by OECD (2008).[34] For this purpose, three groups of five mice each were used and treated as follows: group 1 (control) received distilled water orally, and groups 2, 3 and 4 received the extracts at 200, 300, and 400 mg/kg dose, respectively. The mice were treated daily for 28 days during which time signs of toxicity (such as vomiting, diarrhoea, drowsiness, etc.), changes in general behaviour (aggressiveness and mobility), and body weights were monitored daily. On the 29th day, blood samples were collected in dry tubes and used for biochemical analyses. The following hepatic and renal function parameters were analysed; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), urea, and creatinine. All the mice were anesthetized, and vital organs (liver, kidneys, and spleen) were harvested, weighed, and the kidneys and liver were used for histopathological examination.

Histopathological Examination

The harvested kidneys, livers, and spleens were preserved in 10% formalin solution until histological evaluation. Histopathological examination was done using the normal anatomic pathology procedures of fixation, dehydration, paraffin embedding, microtome sections, and hematoxylin-eosin-safran staining. A microscope was used for optical observation.

Statistical Analysis

Data were presented as means ± SD of triplicate experiments. The homogeneity of variances and normality were verified before deciding on the type of statistical analysis (parametric or non-parametric) to be used. Differences between means were analysed by one- and two-way analysis of variance (ANOVA) and Tukey's multiple range tests using the GraphPad Prism 8.0.2 software. $P < 0.05$ was regarded as significant.

Results and Discussion

Yields of F. Communis Extracts

The yields of the aqueous, methanol as well as the hydroethanol extracts of the fruits of *F. communis* are shown in Table 1. It was observed that the aqueous extracts had higher yields than the methanol and hydroethanol extracts. The highest yield (29.65 ± 0.05%) was obtained for the aqueous extract of the fruit at the advanced stage of flowering, followed by the aqueous extract of the edible fruit (Yield = 18.58 ± 0.10%). For the methanol extract, the fruit at the advanced stage of flowering had a higher yield (12.25 ± 0.05%) than the edible fruit (Yield = 10.50 ± 0.10%), while the hydroethanol extract of the edible fruit had the lowest yield (7.20 ± 0.10%). To the best of our knowledge, there are no studies on the yield of these fruits, the only study was that conducted on the roots where the yield of the ethanol extract of the root of *F. communis* was found to be 14.00%.[35]

Total Phenolic and Flavonoid Contents

The concentrations of bioactive compounds (phenolics, flavonoids, tannins, alkaloids, and saponins) has been shown to vary among various extracts depending on the extraction yields.[36] From the results obtained for the total phenolic content (Figure 1), it was observed that the total phenolic content of the aqueous extract of the edible fruit (0.49 ± 0.002 mg GAE/mg) was higher than that of the methanol extract. Whereas, for the fruit at the advanced stage of flowering, a very high total phenolic content was observed for the methanol extract (11.24 ± 11.77 mg GAE/g) compared to the aqueous extract with total phenolic content of 0.41 ± 0.03 mg GAE/g. The total flavonoid contents of the fruit extracts of *F. communis* are presented in Figure 2. It was observed that the flavonoid content of the methanol extract of FAF was higher than that of the aqueous extract (0.13 mg QE/g and 0.09 mg QE/g, respectively), whereas, for the EF, the aqueous extract had a higher total flavonoid content than the methanol extract (0.12 mg QE/g and 0.043 mg QE/g, respectively). When compared to the results of the study by Rahali *et al*. (2019),[37] who investigated the contents of polyphenols and flavonoids in the extracts of the flowers, fruits, and stem of *F. communis*, it was shown that the methanol extract of the fruits of *F. communis* had the highest content of polyphenol and flavonoids (422 mg GAE/g DW, and 425 mg QE/g DW, respectively) compared to the other parts of the plant. It has been reported that total phenol and flavonoid contents in a plant varies according to the plant part used.[38] Phenolics are strong antioxidant due to their reducing properties, they inhibit the oxidation of organic matter by transferring hydrogen ion from their hydroxyl group to free radicals that cause oxidation.[39]

Table 1: Yields of *F. communis* fruit extracts

Fruit sample	Yield (%)		
	ME	AE	HE
EF	10.50 ± 0.10^a	18.58 ± 0.10^b	7.20 ± 0.10^c
FAF	12.25 ± 0.05^a	29.65 ± 0.05^b	N. D

Value are Means ± SD, (n = 3). Values with different superscript letters in the same column are significantly different ($p < 0.05$). N.D: Not determined.
EF = Edible fruit, FAF = Fruit at the advanced stage of flowering, ME = Methanol extract, AE = Aqueous extract, HE = Hydroethanol extract

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

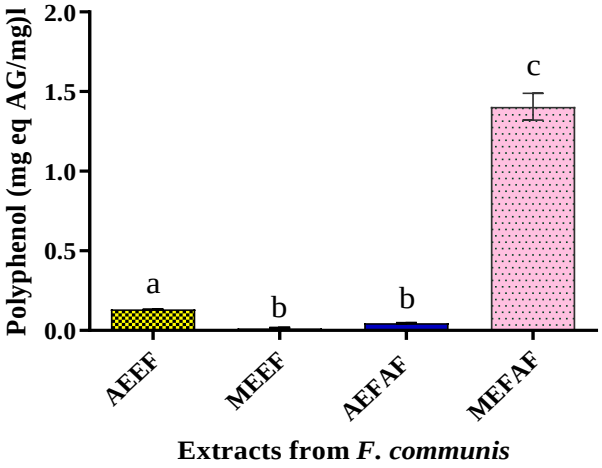

Figure 1: Total phenolic content of extracts of *F. communis*. Bars represent mean ± SD, (n = 3). Bars with different lower case letters are significantly different (P < 0.05).
AEEF = Aqueous extract of edible fruits, MEEF = Methanol extract of edible fruits, AEFAF = Aqueous extract of fruits at the advanced stage of flowering, MEFAF = Methanol extract of fruits at the advanced stage of flowering

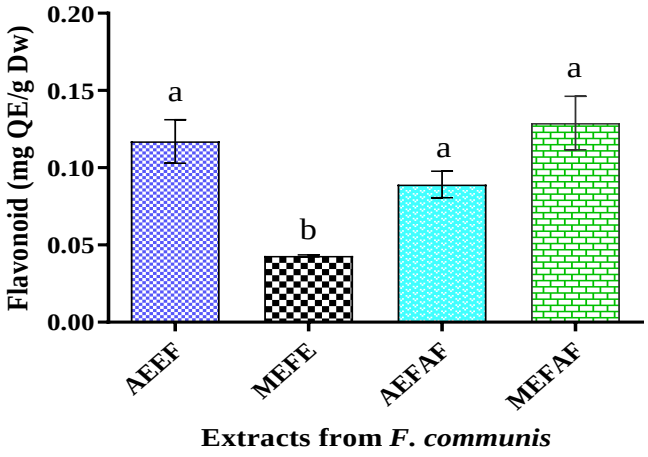

Figure 2: Total flavonoid content of extracts of *F. communis*. Bars represent mean ± SD, (n = 3). Bars with different lower case letters are significantly different (P < 0.05).
AEEF = Aqueous extract of edible fruits, MEFE = Methanol extract of edible fruits, AEFAF = Aqueous extract of fruits at the advanced stage of flowering, MEFAF = Methanol extract of fruits at the advanced stage of flowering

Table 2: DPPH free radical scavenging activity of *F. communis* fruits

Sample	IC_{50} value (mg/mL)	
	ME	AE
EF	0.076 ± 0.039[a]	0.26 ± 0.006[a]
FAF	0.075 ± 0.055[a]	0.85 ± 0.93[b]
BHT	0.12 ± 0.0001[b]	0.12 ± 0.0001[c]

Values are mean ± SD, (n = 3). Values with different lower case letters in the same column are significantly different (P < 0.05).
EF = Edible fruits, FAF = Fruits at the advanced stage of flowering, ME = Methanol extract, AE = Aqueous extract, BHT = Butylated hydroxytoluene

Polyphenolic content varies quantitatively from one plant to the other, this can be attributed to several factors, including climatic and environmental factors such as geographical location, drought, soil, attacks and diseases, the genetic heritage, the harvest period and the stage of plant development.[40]

Antioxidant Activity of F. communis Fruit Extracts
Assessment of oxidative stress (OS, *in vivo* oxidation) has become crucial as a result of their involvement in several disease conditions including rheumatoid arthritis, atherosclerosis, diabetes, aging and cancer.[41-43] Natural antioxidants present in plant extracts and essential oils can provide protection against OS by two main mechanisms, namely; scavenging reactive oxygen species (ROS) and inhibiting lipid peroxidation.[44 45]

DPPH Radical Scavenging Activity
The DPPH radical scavenging assay is a rapid, reliable, and reproducible method for evaluating antioxidant and free radical scavenging activity.[46] The antioxidant activity of the various extracts of *F. communis* fruits assessed by DPPH free radical scavenging assay is presented in Table 2. The results expressed as IC_{50} values showed that the inhibitory power of the methanol extracts of FAF and EF with IC_{50} of 0.075 ± 0.055 and 0.076 ± 0.039 mg/mL, respectively is greater compared to the aqueous extracts and BHT with IC_{50} values of 0.85 ± 0.93, 0.26 ± 0.006, and 0.12 ± 0.0001 mg/mL, for FAF, EF, and BHT, respectively.

ABTS Radical Scavenging Activity
ABTS is another synthetic radical which has the advantage of the working solution being soluble in aqueous and organic solvents over a large range of pH values, and the reaction time is shorter than the DPPH assay.[47] As shown in Table 3, the methanol extract of FAF (IC_{50} = 0.009 ± 0.00 mg/mL) had the highest scavenging effect against ABTS radical, followed by the aqueous extract of EF (IC_{50} = 0.022 ± 0.001 mg/mL) and the aqueous extract of FAF (IC_{50} = 0.25 ± 0.048 mg/mL). Rahali *et al*. (2019) investigated the antioxidant activity of the methanol extracts from the flowers, fruits, and the stem of *F. communis* by the ABTS radical scavenging method, their results showed that the fruits of *F. communis* have the highest antioxidant activity with IC_{50} value of 28.10 ± 0.52 µg/mL.[37]

Ferric Reducing Antioxidant Power (FRAP)
The ferric reducing antioxidant power of the various extracts of *F. communis* fruits were expressed as IC_{50} values (Table 4). The results showed that the aqueous extract of FAF (IC_{50} = 137.82 ± 1.41 mg/mL) demonstrated greater ferric reducing antioxidant power than the methanol and aqueous extracts of EF and the methanol extract of FAF with IC_{50} values of 202.71 ± 1.47, 208.04 ± 5.69, and 702.85 ± 0.85 mg/mL, respectively). However, the FRAP activity for all the extracts was lower compared to that of the reference standard - quercetin (IC_{50} = 0.033 mg/mL).

Total Antioxidant Capacity (TAC)
The results of the total antioxidant capacity of the different extracts of *F. communis* fruits (Table 5) showed that the methanol extract of EF had a significantly higher total antioxidant activity (2.7 ± 0.114 mg Vit C Eq/g) compared to the other extracts, while the aqueous extract of EF exhibited the lowest total antioxidant capacity (TAC = 0.143 ± 0.004 mg Vit C Eq/g extract) among all the extracts.
The antioxidant activity of plant extracts may be exhibited by different mechanisms, such as the prevention of lipid peroxidation, decomposition of peroxides, prevention of continuous hydrogen abstraction, free radical scavenging, reducing capacity, and chelation of transition metal ions.[48]
Based on the results obtained from the present study, it could be stated that the plant *F. communis* is rich in bioactive compounds, which confers it with a great antioxidant potential. This assertion is supported

by the findings of several other studies where it has been shown that the strong antioxidant activity of plant extracts and essential oils is attributed to their phenolic content,[49] with those having higher polyphenolic and flavonoid content possessing greater antioxidant activity.[50] A study by Kang *et al*. (2003) suggested that the presence of polar molecules in plant extracts contribute to their antiradical activity.[51]

Antimicrobial Activity

The antimicrobial activity (MIC and MBC) of *F. communis* fruits against the four microbial strains are summarized in Table 6 and Figure 3. Both the methanol and aqueous extracts of *F. communis* fruits exhibited low antibacterial activity compared to the control (Ampicillin). The methanol extract showed the highest inhibitory activity against *B. subtilis* with an inhibition zone diameter of 10.80 ± 0.20 mm, MIC of 25.00 ± 0.00 mg/mL and MBC of 12.50 µL/mL, followed by activity against *E. coli* with an inhibition zone diameter of 7.20 ± 0.20 mm, MIC of 25.00 ± 0.00 mg/mL and MBC of 25.00 ± 0.00 µL/mL, then *S. aureus* with an inhibition zone diameter of 6.07 ± 3.52 mm, MIC of 62.50 ± 0.00 mg/mL and MBC of 62.50 ± 0.00 µL/mL. However, *F. communis* methanol extract did not show any activity against *C. albicans*.

Table 3: ABTS free radical scavenging activity of *F. communis* fruits

Sample	IC_{50} value (mg/mL)	
	ME	**AE**
EF	0.25 ± 0.048[a]	0.022 ± 0.001[a]
FAF	0.009 ± 0.00[b]	0.040 ±0.016[b]
Trolox	0.01 ± 0.002[b]	0.01 ± 0.002[c]

Values are mean ± SD, (n = 3). Values with different lower case letters in the same column are significantly different (P < 0.05).
EF = Edible fruits, FAF = Fruits at the advanced stage of flowering, ME = Methanol extract, AE = Aqueous extract

Table 4: Ferric reduction antioxidant power (FRAP) of *F. communis* fruits

Sample	IC_{50} value (mg/mL)	
	ME	**AE**
EF	202.71 ± 1.47[a]	208.04 ± 5.69[a]
FAF	702.85 ± 0.85[b]	137.82 ± 1.413[b]
Quercetin	0.033 ± 0.0004[c]	0.033 ± 0.0004[c]

Values are mean ± SD, (n = 3). Values with different lower case letters in the same column are significantly different (P < 0.05).
EF = Edible fruits, FAF = Fruits at the advanced stage of flowering, ME = Methanol extract, AE = Aqueous extract

Table 5: Total antioxidant capacity (TAC) of *F. communis* fruits

Fruit sample	TAC (mg Vit C Eq/g Extract)	
	ME	**AE**
EF	2.70 ± 0.14[a]	0.143 ± 0.004[b]
FAF	0.406 ± 0.057[a]	0.492 ± 0.023[a]

Values are mean ± SD, (n = 3). Values with different lower case letters in the same column are significantly different (P < 0.05).
EF = Edible fruits, FAF = Fruits at the advanced stage of flowering, ME = Methanol extract, AE = Aqueous extract

On the other hand, the aqueous extract also showed the highest inhibitory activity against *B. subtilis* with an inhibition zone diameter of 9.00 ± 0.20 mm, MIC of 12.50 ± 0.00 mg/mL and MBC of 6.25 µL/mL, followed by activity against *E. coli* with an inhibition diameter of 8.00 ± 0.40 mm, MIC of 50.00 ± 0.00 mg/mL and MBC of 50.00 ± 0.00 µL/mL. However, *F. communis* fruit aqueous extract did not show any activity against *S. aureus*, and *C. albicans*.

The differences in the inhibition zone diameters *vis-à-vis* the antibacterial activity of the two extracts could be due mainly to the chemical composition of the different solvent (methanol and water) extracts of *F. communis* fruits. Previous report has indicated a positive correlation between antimicrobial activity and high phenolic content,[52] and presence of phenolic hydroxyl group contribute to an increase in antimicrobial effect.[49] Phenolic substances exert their antibacterial action by altering cell membrane structure, lowering lipid content and ultimately impede microbial development.[53] On the basis of the findings from the present study, the Moroccan *F. communis* fruits could be said to have moderate antibacterial activity. These findings corroborated the work of Maggi *et al*. (2009) on *F. communis* fruit oils who reported a very strong antibacterial activity against *S. aureus*, *E. coli* and *B. subtilis.*[54]

Acute Toxicity

The effect of a single oral administration of the hydroethanol extract of the edible fruits of *F. communis* (HEFE) on the overall behaviour, body weight, and organ weight of mice was investigated.

Effects of HEFE on the general behaviour of mice

A single oral administration of HEFE at doses of 200, 300, and 400 mg/kg to mice showed no sign of toxicity. Furthermore, the administration of HEFE did not cause any death in the treated mice at the doses tested. One can therefore infer that the median lethal dose (LD_{50}) of HEFE is higher than 400 mg/kg (Table 7).

Effects of acute administration of HEFE on the body weight of mice

The body weight of mice in the control group 1, and those in group 2 which were administered 200 mg/kg of HEFE remained stable, whereas the mice administered the 300 mg/kg dose of HEFE (Group 3) gradually gained weight over four days following the extract administration. In contrast, the mice in group 4 which were administered 400 mg/kg dose of HEFE demonstrated a decrease in body weight (Figure 4).

Sub-acute toxicity

The sub-acute toxicity of the HEFE was carried out by monitoring the effect of the extract on the general behaviour, the body weight and the organ weight of mice following a 28 days oral administration.

Effect of HEFE on the general behaviour of mice

The general behavioural effect of the daily oral administration of HEFE at doses of 200, 300, and 400 mg/kg for 28 days in mice is shown in Table 7. Observation over 28 days period shows no sign of toxicity (vomiting, drowsiness, aggression, diarrhoea, and mobility) for the group that received 200 mg/kg of the extract and the control group. Whereas there was a decrease in the mobility of the mice administered the 300 mg/kg and 400 mg/kg doses of the extract from the 27th day of administration.

Table 6: Antimicrobial activity of *F. communis* extracts against microbial strain tested

Microbial strain	Inhibition zone diameter (mm)		
	MEFE	**AEFE**	**Ampicillin**
E. coli K12	7.20 ± 0.20 [a]	8.00 ± 0.40 [a]	20.00 ± 0.00 [b]
S. aureus ATCC6633	6.07 ± 3.52 [a]	0.00 ± 0.00 [b]	16.00 ± 0.00 [c]
B. subtilis DSM6333	10.80 ± 0.20 [a]	9.00 ± 0.20 [a]	18.00 ± 0.00 [b]
C. albicans ATCC10231	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]

Values are mean ± SD, (n = 3). Values with different lower case letters in the same column are significantly different (P < 0.05).
AEFE = Aqueous extract of edible fruits, MEFE = Methanol extract of edible fruits

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

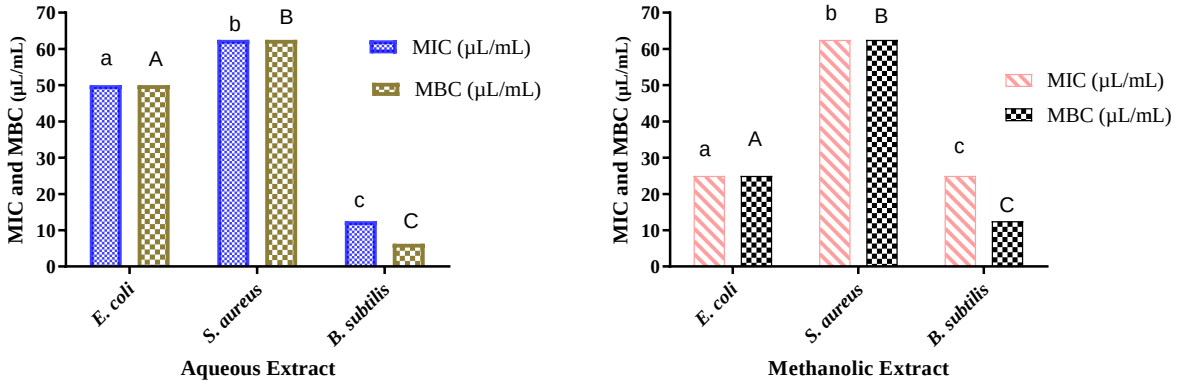

Figure 3: Antimicrobial activity (MIC and MBC) of methanol and aqueous extract of *F. communis*. Bars represent mean ± SD, (n=3). Bars with the same lower-case letter (for MIC) or the same upper case letter (for MBC) are not significantly different (P > 0.05).

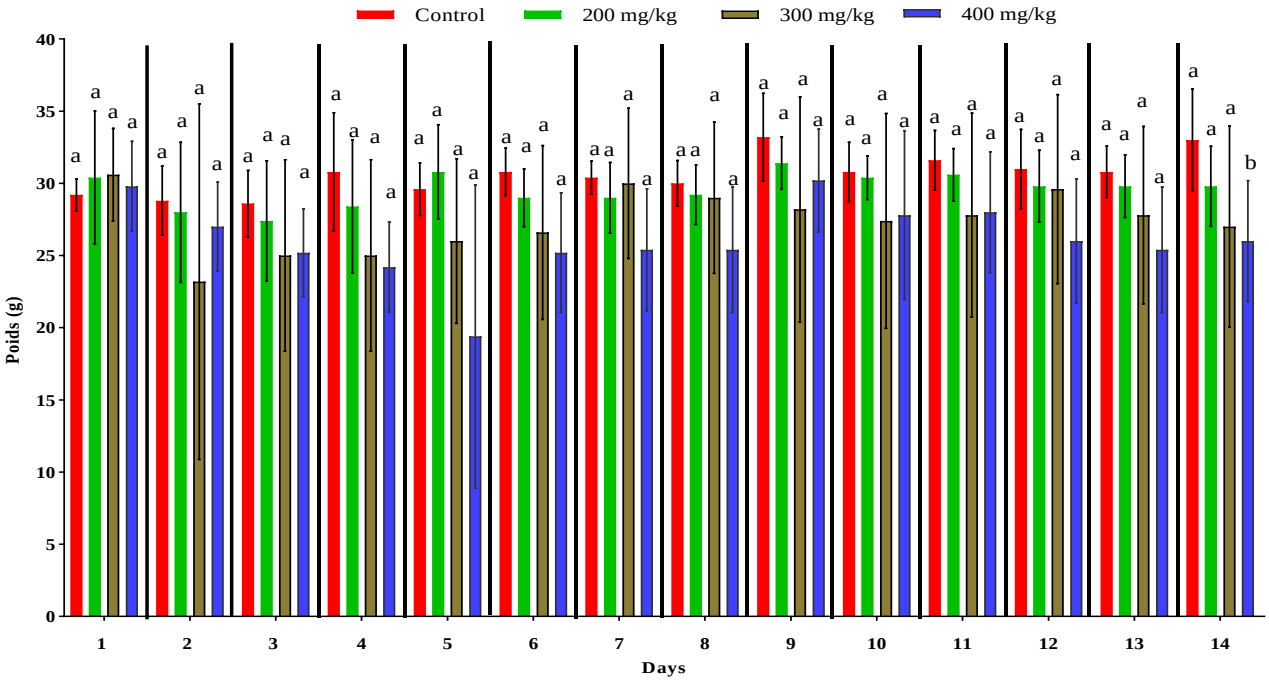

Figure 4: Effect of HEFE on body weight of mice after a single oral administration. Bars represent mean ± SD, (n = 3). Bars with the same lower case letter are not significantly different compared with the control (P > 0.05).

Effect of the HEFE on the body weight of mice
All the mice administered HEFE at the different doses (200, 300, and 400 mg/kg) experienced relative weight loss. However, the weight of the control mice remained stable with a slight increase after 15 days (Figure 5).

Effects of HEFE on organ weight
The effect of a 28-day administration of HEFE on the weight of some vital organs (liver, spleen, and kidneys) is presented in Table 8. It is clear from the results that the administration of HEFE at doses of 200, 300, and 400 mg/kg did not significantly affect the relative weights of the vital organs.

Histopathological effect of HEFE
The examination of the kidney and liver histology sections after 28-day administration of HEFE at doses of 200, 300, and 400 mg/kg showed that the extract at all the doses tested did not result in any abnormality in the tissue architecture of the kidneys and liver (Figure 6).

Effect of HEFE on some biochemical parameters
The effect of *HEFE* on hepatic and renal functions was assessed by analysing liver function enzymes (AST, ALT, and ALP), and renal function parameters (urea and creatinine). There were no significant alterations in the serum levels of these parameters after a 28-day oral administration of the extract at doses of 200, 300, and 400 mg/kg. This observation suggests that HEFE has no hepatotoxic and renotoxic effects at the doses tested (Table 9).
The use of plants in traditional medicine needs to be substantiated by scientific evidence regarding the efficacy, quality standards, and safety of these plants so that traditional medicines are used in accordance with established quality and safety standards.[55] Toxic substances of natural or synthetic origin can affect and damage vital organs in the body, especially the liver, which is susceptible to chemical

attacks due to its involvement in the biotransformation of chemicals.[56] Currently, it is known that the biological effects of *F. communis* on human health depend on the chemotype used: the poisonous chemotype, containing mainly prenyl coumarins such as ferulenol, causes intoxications, ferulosis and death,[57,58] while the non-poisonous chemotype, which contains daucane esters whose main component is ferutinin, is traditionally used for its hormonal effects and has been classified as a phytoestrogen.[59,60]

From the analysis of the results, it appears that *F. communis* do not have toxic effects at the doses tested (200, 300, and 400 mg/kg), either on the general behaviour of the mice, on their weight or on their organs. The study carried out by Derbane *et al.* (2008)[35] on the ethanol extract of the roots of *F. communis* shows that the extract caused toxic effect manifested as modifications of the behaviour of the animals during the 24 hours following the oral administration of a dose of 10 mg/kg. Toxic effect on the digestive tract manifested as an increase in stool secretion and abdominal swelling was also observed within 48 hours after the administration of *F. communis* extract. In addition, the study of Derbane *et al*. (2008) also observed that at a dose of 1000 mg/kg, *F. communis*. extract caused mortalities in mice,[35] suggesting that the plant is toxic. Contact toxicity testing of *F. communis* shows no severe skin symptomatology in humans, this may either be due to the dose used or that the skin of the animals provided better protection against the toxic effect of the plant extract.[35] Many environmental toxicants and clinically useful drugs can cause severe damage to various organs by eliciting the formation of free radicals.[61] In the present study, there were no histological abnormality and no signs of organ toxicity after HEFE administration. However, more studies are needed on the fruits and other parts of the plant to better elucidate its pharmacological and/or toxic effects.

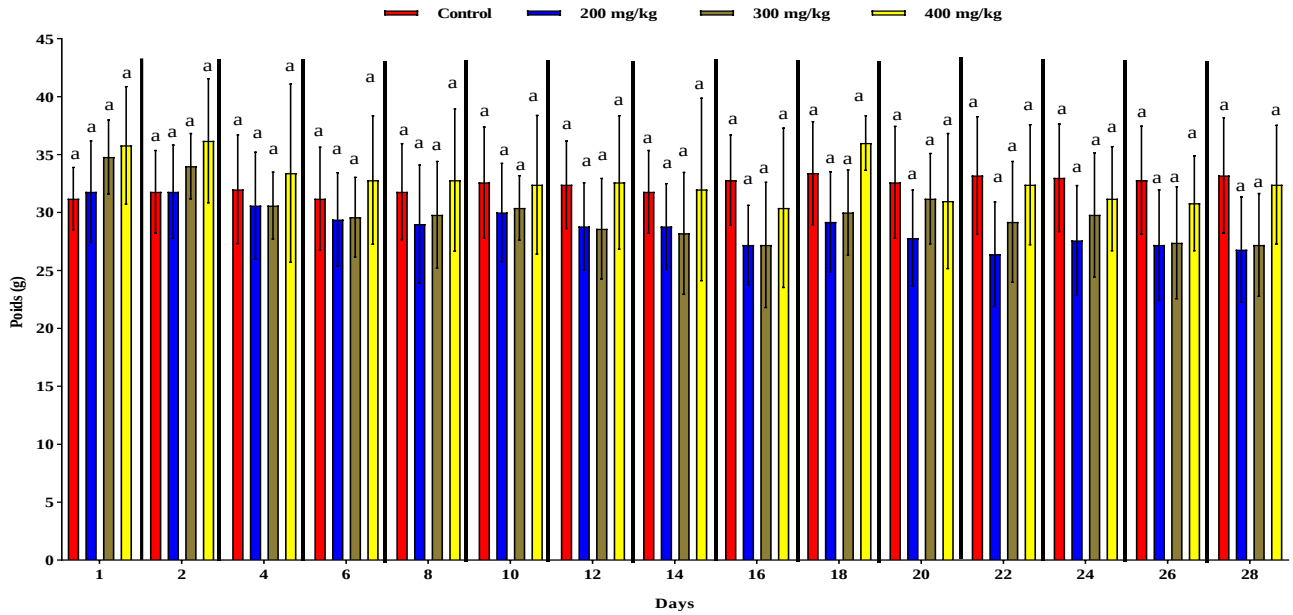

Figure 5: Effect of HEFE on body weight of mice following a 28-day oral administration. Bars represent mean ± SD, (n = 3). Bars with the same lower case letter are not significantly different compared with the control (P > 0.05).

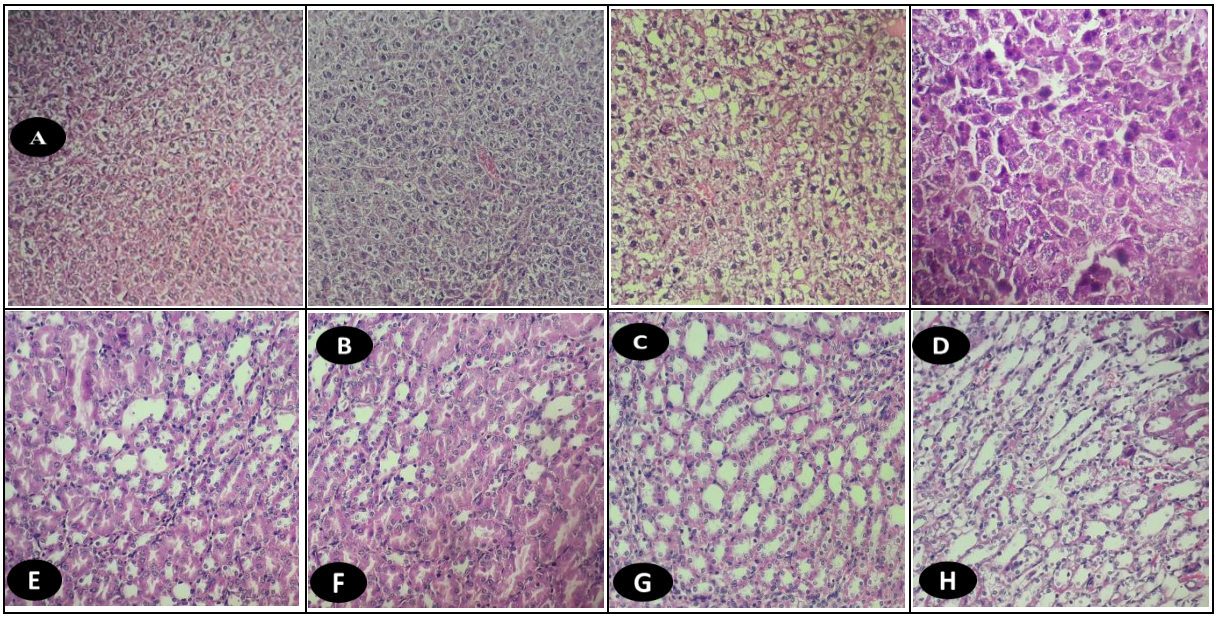

Figure 6: Histological sections of the liver and kidney of HEFE-treated mice. **A:** Liver 400 mg/kg **B:** Liver 300 mg/kg **C:** Liver 200 mg/kg **D:** Liver control **E:** Kidney 400 mg/kg **F:** Kidney 300 mg/kg **G:** Kidney 200 mg/kg **H:** Kidney control.

Table 7: Effect of HEFE on general behaviour in mice

Sign	Group	Days													
		2	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**
Mobility	Control	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	200 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	300 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	De
	400 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	De
Aggressiveness	Controls	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	200 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	300 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	400 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tremor	Controls	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	200 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	300 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	400 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	Controls	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	200 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	300 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	400 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Vomiting	Controls	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	200 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	300 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	400 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Vigilance	Controls	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	200 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	300 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	400 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N

De: Decrease; N: Normal in comparison with the control.

Table 8: Variation in relative organ weight of HEFE treated mice compared to control

Organs	Control	Doses		
		200 mg/kg	300 mg/kg	400 mg/kg
Liver	1.78 ± 0.278[a]	1.12 ± 0.197[a]	1.284 ± 0.612[a]	1.48 ± 0.397[a]
Spleen	0.218 ± 0.097[a]	0.14 ± 0.061[a]	0.068 ± 0.016[b]	0.214 ± 0.057[a]
Right kidney	0.274 ± 0.051[a]	0.144 ± 0.029[a]	0.142 ± 0.032[a]	0.234 ± 0.057[a]
Left kidney	0.308 ± 0.044[a]	0.154 ± 0.036[a]	0.146 ± 0.030[a]	0.228 ± 0.052[a]

Values are mean ± SD, (n = 3). Values with different lower case letters in the same row are significantly different (P < 0.05).

Table 9: Effect of HEFE on hepatic and renal function parameters in mice

		ASAT(U/L)	ALAT(U/L)	ALP (U/L)	UREA (mg/dL)	CREA (mg/dL)
Control (NaCl 0.9%)		224.8 ± 36.33[a]	25.70 ± 2.58[a]	145.0 ± 2.88[a]	15.70 ± 0.86[a]	0.18 ± 0.04[a]
Dose (mg/kg)	200	250.3 ± 10.02[a]	28.03 ± 4.27[a]	186.7 ± 26.03[a]	18.07 ± 4.08[a]	0.30 ± 0.04[a]
	300	268.9 ± 19.91[a]	36.07 ± 0.43[a]	126.0 ± 14.0[a]	22.33 ± 2.02[a]	0.26 ± 0.02[a]
	400	182.7 ± 7.87[b]	37.47 ± 1.68[a]	167.7 ± 2.33[a]	13.50 ± 1.79[a]	0.30 ± 0.02[a]

Data represent mean ± SD, (n = 5). Values with different lower case letters in the same row are significantly different (P < 0.05).
AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, CREA = Creatinine.

Conclusion

The results from the present study have shown that the extracts of *Ferula communis* fruits have remarkable antioxidant and antimicrobial activities. Acute and sub-acute oral administration of the hydroethanol extract of the fruits at doses of 200, 300, and 400 mg/kg showed no sign of toxicity in mice, which suggest that the fruit extract of *Ferula communis* may be safe when used at lower doses. However, further studies are needed to substantiate these claims

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Uttara B, Singh AV, Zamboni P, Mahajan R. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 2009; 7(1):65–74.
2. Moon JK, Shibamoto T. Antioxidant assays for plant and food components. J Agric Food Chem. 2009; 57(5):1655–1666.
3. Edziri H, Mastouri M, Aouni M, Verschaeve L. Polyphenols content, antioxidant and antiviral activities of leaf extracts of *Marrubium deserti* growing in Tunisia. South Afr J Bot. 2012; 80:104–109.
4. Habchaoui J, Saad I, Khomsi ME, Fadli M, Bourkhiss B. Ethnobotanical Study of Medicinal Herbs in the Saïss Urban Commune (Region of Fez/Morocco). Trop J Nat Prod Res. 2023; 7(11):5034–5039.
5. Okolie NP, Falodun A, Davids O. Evaluation of the Antioxidant Activity of Root Extract of Pepper Fruit (*Dennetia tripetala*), and It's Potential for the Inhibition of Lipid Peroxidation. Afr J Trad Compl Altern Med. 2014 Apr 3; 11(3):221–227.
6. Kara M, Assouguem A, Abdou RZ, Bahhou J. Phytochemical Content and Antioxidant Activity of Vinegar Prepared from Four Apple Varieties by Different Methods. Trop J Nat Prod Res. 2021; 5(9):1578–1585.
7. Singh J, Prasad R, Kaur HP, Jajoria K, Chahal AS, Verma A, . Bioactive Compounds, Pharmacological Properties, and Utilization of Pomegranate (*Punica granatum L.*): A Comprehensive Review. Trop J Nat Prod Res. 2023; 7(9):3856–3873.
8. Singh J, Kaur H, Kaur R, Garg R, Prasad R, Assouguem A, . Tropical Journal of Natural Product Research A Review on the Nutritional Value and Health Benefits of Different Parts of Grape (*Vitis vinifera* L.). Trop J Nat Prod Res. 2023; 3874–3880.
9. Atki YE, Aouam I, Kamari FE, Taroq A, Zejli H, Taleb M, . Antioxidant activities, total phenol an flavonoid contents of two *Teucrium polium* subspecies extracts. Moroc J Chem. 2020; 8(2):8–455.
10. Wenzel RP and Edmond MB. The impact of hospital-acquired bloodstream infections. Emerg Infect Dis. 2001; 7(2):174–177.
11. Brown ED and Wright GD. New Targets and Screening Approaches in Antimicrobial Drug Discovery. Chem Rev. 2005; 105(2):759–774.
12. Watson MF. The Genera of the Umbelliferae: A nomenclator. M. G. Pimenov and M. V. Leonov. London: Royal Botanic Gardens, Kew and Botanical Garden of Moscow University, Russia. 1993. 156 pp. ISBN 0 947643 58 3. £12.00 (paperback). Edinb J Bot. 1995; 52(1):92–93.
13. El Alaoui-Faris, FE ,. - Contribution à l'étude biosystématique du genre Ferula L. au Maroc. Fac. Sc. Rabat. Maroc; 1993. 264 pp.
14. El Alaoui-Faris FE, Cauwet-Marc AM, Cauwet-Marc AM. Nombre chromosomique de quelques espèces de férules marocaines (Ferula, Apiaceae). Fl Medit. 2006; 16:341–354.
15. Zargari A. Medicinal plants.(ed. 3) Tehran. Tehran University Publications; 1996.
16. Eigner D and Scholz D. [The magic book of Gyani Dolma]. Pharm Unserer Zeit. 1990;19(4):141–152.
17. Afifi FU and Abu-Irmaileh B. Herbal medicine in Jordan with special emphasis on less commonly used medicinal herbs. J Ethnopharmacol. 2000; 72(1):101–110.
18. Rubiolo P, Matteodo M, Riccio G, Ballero M, Christen P, Fleury-Souverain S, . Analytical discrimination of poisonous and nonpoisonous chemotypes of giant fennel (*Ferula communis* L.) through their biologically active and volatile fractions. J Agric Food Chem. 2006; 54(20):7556–7563.
19. Carta A. Ferulosis; isolation of the substance with hypoprothrombinemizing action from the galbanum of *Ferula communis*. Boll Della Soc Ital Biol Sper. 1951; 27(5):690–693.
20. Lamnaouer D. Anticoagulant activity of coumarins from *Ferula communis* L. Ther. 1999; 54(6):747–751.
21. Appendino G, Tagliapietra S, Gariboldi P, Nano GM, Picci V. Omega-Oxygenated prenylated coumarins from *Ferula communis*. Phytochem. 1988; 27(11):3619–3624.
22. Slinkard K and Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am J Enol Vitic. 1977; 28(1):49–55.
23. Kara M, Assouguem A, Fadili ME, Benmessaoud S, Alshawwa SZ, Kamaly OA, . Contribution to the Evaluation of Physicochemical Properties, Total Phenolic Content, Antioxidant Potential, and Antimicrobial Activity of Vinegar Commercialized in Morocco. Molecules 2022; 27(3):770.
24. Wu HC, Chen HM, Shiau CY. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res Int. 2003; 36(9–10):949–957.
25. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med. 1999; 26(9–10):1231–1237.
26. Lfitat A, Zejli H, Bousraf FZ, Bousselham A, El Atki Y, Gourch A, . Comparative assessment of total phenolics content and in vitro antioxidant capacity variations of macerated leaf extracts of *Olea europaea* L. and *Argania spinosa* (L.) Skeels. Mater Today Proc. 2021; 45:7271–7277.
27. Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Jap J Nutr Diet. 1986; 44(6):307–315.
28. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999; 269(2):337–341.
29. El Barnossi A, Moussaid F, Iraqi Housseini A. Antifungal activity of Bacillus sp. Gn-A11-18isolated from decomposing solid green household waste in water and soil against *Candida albicans* and *Aspergillus niger*. Akhssas A, Baba K, Bahi L, Benradi F, Cherkaoui E, Khamar M, ., editors. E3S Web Conf. 2020;150:02003.
30. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 2007; 42(4):321–324.
31. Kara M, Assouguem A, Kamaly OMA, Benmessaoud S, Imtara H, Mechchate H, . The Impact of Apple Variety and

the Production Methods on the Antibacterial Activity of Vinegar Samples. Molecules. 2021; 26(18):5437.
32. Agour A, Mssillou I, Mechchate H, Es-safi I, Allali A, Barnossi AE, . *Brocchia cinerea* (Delile) Vis. Essential Oil Antimicrobial Activity and Crop Protection against Cowpea Weevil *Callosobruchus maculatus* (Fab.). Plants. 2022; 11(5):583.
33. Costa-Silva JH, Lima CR, Silva EJR, Araújo AV, Fraga M, e Ribeiro AR, . Acute and subacute toxicity of the *Carapa guianensis* Aublet (Meliaceae) seed oil. J Ethnopharmacol. 2008; 116(3):495–500.
34. Darbar S, Saha S, Pramanik K, Chattopadhyay A. Preliminary Assessment of Acute and 28-Day Repeated Dose Oral Toxicity of a Newly Developed Herbal Mixture on Experimental Animal. Indian J Pharm Edu Res. 2019; 54(1):135–142.
35. Derbane R, Messaadi F, Teffaha M, Lahouel ME, Sebti ME. Etude de la toxicité de deux plantes: *Ferula communis* et *Lonicera implexa* [PhD Thesis]. Université de Jijel; 2008.
36. Salleh NAM, Pa'ee F, Manan NA, Sabran SF, Bakar FIA, Muhammad N, . Evaluation of *Salvinia molesta* D.S.Mitch (Salviniaceae) for Antioxidant and Antibacterial Properties. Trop J Nat Prod Res. 2023; 7(11):5106–5114.
37. Rahali FZ, Kefi S, Bettaieb Rebey I, Hamdaoui G, Tabart J, Kevers C, . Phytochemical composition and antioxidant activities of different aerial parts extracts of *Ferula communis* L. Plant Biosyst- Int J Deal Asp Plant Biol. 2019; 153(2):213–221.
38. Hmamou A, Eloutassi N, Alshawwa SZ, Al kamaly O, Kara M, Bendaoud A, . Total Phenolic Content and Antioxidant and Antimicrobial Activities of *Papaver rhoeas* L. Organ Extracts Growing in Taounate Region, Morocco. Molecules. 2022; 27(3):854.
39. Egharevba E, Chukwuemeke-Nwani P, Eboh U, Okoye E, Bolanle O, Oseghale I, . Evaluation of the Antioxidant and Hypoglycaemic Potentials of the Leaf Extracts of *Stachytarphyta jamaicensis* (Verbenaceae). Trop J Nat Prod Res. 2019; 3:170–174.
40. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004; 85(2):231–237.
41. Hybertson BM, Gao B, Bose SK, McCord JM. Oxidative stress in health and disease: The therapeutic potential of Nrf2 activation. Mol Aspects Med. 2011; 32(4–6):234–246.
42. Liang D, Zhou Q, Gong W, Wang Y, Nie Z, He H, . Studies on the antioxidant and hepatoprotective activities of polysaccharides from *Talinum triangulare*. J Ethnopharmacol. 2011; 136(2):316–321.
43. Rains JL and Jain SK. Oxidative stress, insulin signaling, and diabetes. Free Rad Biol Med. 2011; 50(5):567–575.
44. Dai J and Mumper RJ. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules. 2010; 15(10):7313–7352.
45. Masella R, Di Benedetto R, Varì R, Filesi C, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem. 2005; 16(10):577–586.
46. Dennis-Eboh U, Achuba FI, George BO. Evaluation of the Phytochemical and Antioxidant Properties of Cold, Hot Water and Wine Extracts Produced from *Ficus capensis* Leaf: Trop J Phytochem Pharm Sci. 2023; 2(2):47–54.
47. Gouveia S and Castilho PC. *Helichrysum monizii* Lowe: Phenolic Composition and Antioxidant Potential: *H. monizii* Phenolic Composition and Antioxidant Potential. Phytochem Anal. 2012; 23(1):72–83.
48. Que F, Mao L, Pan X. Antioxidant activities of five Chinese rice wines and the involvement of phenolic compounds. Food Res Int. 2006; 39(5):581–587.
49. El Atki Y, Aouam I, Taroq A, el Kamari F, Timinouni M, Badiaa L, . Antibacterial effect of combination of cinnamon essential oil and thymol, carvacrol, eugenol, or geraniol. J Rep Pharm Sci. 2020; 9:104.
50. Bentabet N, Boucherit-Otmani Z, Boucherit K. Composition chimique et activité antioxydante d'extraits organiques des racines de *Fredolia aretioides* de la région de Béchar en Algérie. Phytothér. 2014; 12(6):364–371.
51. Kang DG, keun Yun C, Lee HS. Screening and comparison of antioxidant activity of solvent extracts of herbal medicines used in Korea. J Ethnopharmacol. 2003; 87(2–3):231–236.
52. Rota MC, Herrera A, Martínez RM, Sotomayor JA, Jordán MJ. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Contr. 2008; 19(7):681–687.
53. Helal GA, Sarhan MM, Abu Shahla ANK, Abou El-Khair EK. Effects ofCymbopogon citratus L. essential oil on the growth, lipid content and morphogenesis of *Aspergillus niger* ML2-strain. J Basic Microbiol. 2006; 46(6):456–469.
54. Maggi F, Cecchini C, Cresci A, Coman MM, Tirillini B, Sagratini G, . Chemical composition and antimicrobial activity of the essential oil from *Ferula glauca* L. (*F. communis* L. subsp. glauca) growing in Marche (central Italy). Fitoter. 2009; 80(1):68–72.
55. Muharni M, Choirunnisa N, Fitrya F. A Subchronic Toxicity Test of *Dillenia ochreata* Leaves Extract on Wistar rats. Trop J Nat Prod Res. 2023; 7(11):5244–5249.
56. Husni E, Dillasamola D, Jannah M. Subacute Toxicity Test of Ethanol Extract of Sungkai Leaf (*Peronema Canescens* Jack.) on Sgot and Sgpt Levels. Trop J Nat Prod Res. 2023; 7(11):5046–5049.
57. Appendino G, Cravotto G, Sterner O, Ballero M. Oxygenated Sesquiterpenoids from a Nonpoisonous Sardinian Chemotype of Giant Fennel (*Ferula c ommunis*). J Nat Prod. 2001; 64(3):393–395.
58. Kasaian J, Mosaffa F, Behravan J, Masullo M, Piacente S, Ghandadi M, . Reversal of P-glycoprotein-mediated multidrug resistance in MCF-7/Adr cancer cells by sesquiterpene coumarins. Fitoter. 2015; 103:149–154.
59. Hanafi-Bojd M, Iranshahi M, Mosaffa F, Tehrani S, Kalalinia F, Behravan J. Farnesiferol A from *Ferula persica* and Galbanic Acid from *Ferula szowitsiana* Inhibit P-Glycoprotein-Mediated Rhodamine Efflux in Breast Cancer Cell Lines. Planta Med. 2011; 77(14):1590–1593.
60. Iranshahi M, Amin G, Shafiee A. A New Coumarin from *Ferula persica*. Pharm Biol. 2004; 42(6):440–442.
61. Noguchi T, Kuo-Lan Fong, Lai EK, Alexander SS, King MM, Olson L, . Specificity of a phenobarbital-induced cytochrome P-450 for metabolism of carbon tetrachloride to the trichloromethyl radical. Biochem Pharmacol. 1982; 31(5):615–624.