

PROXIMATE AND MINERAL COMPOSITION, BIOLOGICAL PROPERTIES, AND ACUTE TOXICITY OF *ARISARUM VULGARE* O. TARG. TOZZ., A TRADITIONAL WILD FAMINE FOOD PLANT IN MOROCCO

Abdelghani Aboukhalaf^{1,2}, Houda El Yacoubi¹, Atmane Rochdi¹

¹Laboratory of Natural Resources and Sustainable Development, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kénitra, Morocco

²Research Unit on Nutrition & Food Sciences, Laboratory of Anthropogenetic, Biotechnologies and Health, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida, Morocco

ABSTRACT

Background. During famine periods in Morocco, *Arisarum vulgare* O. Targ. Tozz was traditionally consumed in rural areas and contributed to population survival. Despite its historical importance, limited research exists on its nutritional composition, bioactive properties and safety.

Objective. The current study aimed to evaluate the proximate and mineral compositions, phytochemical profile and acute toxicity of *A. vulgare* tubers.

Material and Methods. Nutritional composition was determined using standard food analysis methods, whereas polyphenol and flavonoid contents and antioxidant activity were assessed using colorimetric assays. Antimicrobial activity was evaluated by disc diffusion and broth microdilution methods, and safety was assessed by performing acute toxicity.

Results. The finding revealed that this plant is rich in carbohydrates but low in protein and fat, while mineral and phenolic contents were at moderate levels. Biological evaluation of the extracts showed remarkable antioxidant activity and wide antibacterial and antifungal effects. The aqueous extract obtained by traditional decoction showed no acute toxicity with LD50 higher than 5000 mg/kg, which suggests that the traditional preparation method plays an important role in reducing the toxicity of *A. vulgare*.

Conclusions. The results highlight the nutritional value of *A. vulgare* tubers and their content in bioactive compounds showing antioxidant and antimicrobial activities.

Keywords: *Arisarum vulgare* O. Targ. Tozz, famine food, nutritional profile, antioxidant activity, antimicrobial activity, toxicity

INTRODUCTION

Morocco has experienced several historical periods of famine and food scarcity, particularly during the second half of the twentieth century, which profoundly affected rural populations [1]. During these crises, limited access to staple foods such as cereals and potatoes forced communities to rely on alternative food resources as emergency food sources, making traditional knowledge of local flora essential for survival [2, 3]. Wild edible plants (WEPs) have long served as a safety net for poor rural communities, especially in remote areas, where they were mainly used as subsistence or famine foods [3]. Ethnobotanical surveys conducted in different regions

of Morocco have documented a rich diversity of WEPs traditionally used during periods of food shortage, highlighting their importance in local food systems and cultural heritage [4-7].

Among these species *Arisarum vulgare* O. Targ. Tozz (Araceae) is a tuberous wild plant that has been traditionally consumed during famine periods [8]. In Morocco, this species was locally known by the vernacular name “*Irni*”, a term closely associated with years of hardship and food deprivation. The tubers were used as a famine food and incorporated into various traditional dishes like vegetable preparations and staple foods such as couscous and bread, often after specific processing methods intended to reduce their inherent toxicity, notably

Corresponding author: Abdelghani Aboukhalaf, Laboratory of Natural Resources and Sustainable Development, Department of Biology, Faculty of Sciences, Ibn Tofail University, 14000 Kénitra, Morocco; email: aboukhalaf.a@ucd.ac.ma

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Publisher: National Institute of Public Health NIH - National Research Institute

prolonged cooking (up to 24 h) [2, 5]. Despite its reputation as a potentially toxic plant, its use during food shortage underscores its historical role as an emergency resource when few alternatives were available. *A. vulgare* contains alkaloids such as irniine and bbugaine, which are associated with significant toxicity. These compounds have been shown to induce hepatotoxicity, and DNA damage in liver cell models, which may explain the toxic effects observed after consuming the tubers [8, 9]. The accumulation of toxic secondary metabolites in plants is widely recognized as an adaptive defense strategy against both biotic and abiotic stress factors, and such compounds are often produced as part of complex chemical defense systems that enhance plant survival under adverse environmental conditions [10]. Beyond its use as a subsistence food, *A. vulgare* has also been traditionally used in folk medicine for the management of various ailments, including infections, pain, headaches, digestive disorders, skin conditions, respiratory complaints, wounds, and burns, as well as for the treatment of cancer in traditional practices [8, 11, 12].

To date, comprehensive scientific data addressing its nutritional value biochemical composition, and toxicological profile are poorly documented. The objective of the present study was therefore to provide an integrated evaluation of *A. vulgare* tubers by investigating their nutritional composition, mineral content, phytochemical profile, antioxidant and antimicrobial activities, as well as their acute toxicity. This study aims not only to assess its traditional use as a famine food but also to explore its potential as a supplementary food resource in local diet, provided that its safety is ensured.

MATERIAL AND METHODS

Sample collection and preparation

Fresh tubers of *A. vulgare* were collected in March 2025 from Sidi Bennour, located in central Morocco. About 20 tubers from different plants were collected, randomly selected, and combined to obtain a representative sample for analysis. After collection, the samples were rinsed with distilled water and air dried in a shaded area. The dried plant material was then milled into a fine powder using a laboratory mill and sieved to obtain a uniform particle size. The resulting powder was extracted by maceration in methanol for 24 h, as this solvent is effective in extracting a wide range of bioactive compounds related to antioxidant and antimicrobial activities [13]. In parallel, an aqueous decoction was prepared for the acute toxicity test to reflect the traditional way the plant is used and to better represent how it is consumed. The extracts were subsequently filtered through Whatman

filter paper (No. 1), and the filtrates were concentrated under reduced pressure using a rotary evaporator at 55°C. The dried extracts were stored at 4°C until further use.

Animals

Acute toxicity was evaluated using healthy, non-pregnant young female Wistar rats aged 8-12 weeks (170-180 g). A total of 15 rats were used and divided into three groups (n = 5 per group): a control group and two treated groups receiving doses of 2000 and 5000 mg/kg of aqueous extract, respectively. The animals were housed in standard cages and acclimatized for one week under controlled environmental conditions (23 ± 2°C; 12 hours light/dark cycle) prior to the initiation of treatment. Food and water were provided *ad libitum* throughout the acclimatization period. All animal experiments were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and were approved by local institutional animal ethics committee (Approval No: 07/2025/NRSDL, approved in July 2025).

Determination of nutritional composition

The proximate composition of *A. vulgare*, including moisture, ash, crude protein, fat and carbohydrate contents, was determined following previously described standard methods. Moisture and ash contents were determined by oven-drying and muffle furnace methods [15], respectively, while crude protein was quantified using the Kjeldahl method [16]. Total fat content was assessed by Soxhlet extraction technique using hexane as solvent [15]. Carbohydrate content was calculated by difference according to the equation: Carbohydrates (%) = 100% - (moisture + ash + fat + crude protein).

Mineral element composition was determined using inductively coupled plasma-atomic emission spectrometry (ISP-AES; Jobin Yvon, Ultima 2) equipped with an axial viewing plasma, following the method described by [16]. Major elements (K, Ca, Mg, Na, and S) and trace elements (Fe, Zn, Cu, and Mn) were quantified.

Total phenolic content (TPC)

TPC was determined using Folin-Ciocalteu method as described by Kim et al. [17]. Briefly, 100 µL of methanolic extract was mixed with 1 mL of diluted Folin-Ciocalteu reagent (1:10, v/v). After 5 min, 1 mL of Na₂CO₃ solution (7%) was added, followed by immediate dilution with 0.4 mL of distilled water. The mixture was incubated for 1.5 h in the dark, and absorbance was measured at 760 nm using a spectrophotometer. TPC was calculated from a gallic acid calibration curve and expressed as gallic

acid equivalents per gram of extracts (mg GAE/g extract).

Total flavonoid content (TFC)

TFC of the tuber methanolic extract of *A. vulgare* was determined using aluminum chloride colorimetric method [18]. Briefly, 400 μ L of extract was mixed with 120 μ L of 5% NaNO₂. After 5 min, 120 μ L of AlCl₃ (10%) was added, followed by the addition of 800 μ L of 1M NaOH after a further 6 min. Absorbance was measured at 510 nm using a spectrophotometer. All analyses were performed in triplicate, and TFC was expressed as milligrams of quercetin equivalents per gram of extract (mg QE·g⁻¹ extract).

In vitro antioxidant evaluation

Antioxidant activity was evaluated using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay as described by Ksouri et al. [19]. Methanolic extract at various concentrations (1-1000 μ g/mL) were combined with an equal volume methanolic DPPH solution (0.004%, w/v). The mixtures were kept incubated for 30 min at room temperature in the dark, and absorbance recorded at 517 nm. All measurements were carried out in triplicate, and results were expressed as IC₅₀ values (μ g/mL), defined as the concentration required to scavenge 50% of the initial DPPH radicals. Ascorbic acid was used as a reference standard.

Antimicrobial activity

Microorganism strains

A total of six bacterial strains, comprising three Gram-positive bacteria and three Gram-negative bacteria, as well as two fungal strains, were used in this study. The strains were obtained from the Institute Pasteur Collection (CIP) and the American Type Culture Collection (ATCC). The tested microorganisms included *Bacillus subtilis* (ATCC66331), *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC19433), *Citrobacter freundii* (ATCC8090), *Escherichia coli* (CIP54127), *Pseudomonas* sp., *Cryptococcus neoformans* (CIP 960) and *Candida albicans* (48.72).

Disk diffusion assay

The antimicrobial potential of the extract was evaluated by the disc diffusion assay, following the procedure reported by Aboukhalaf et al. [20].

Minimum inhibitory concentration (MIC) determination

The MIC of the *A. vulgare* extract was determined using a resazurin-based microdilution method, according to Lahlou et al. [21].

Minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) determinations

Wells showing no visible growth after MIC determination were subcultured to recovery media for MBC and MFC assessment and incubated under appropriate conditions. Absence of growth indicated bactericidal or fungicidal activity. MBC/MIC and MFC/MIC ratios were calculated with values ≤ 4 considered bactericidal/ fungicidal and values > 4 considered bacteriostatic/fungistatic [21].

Acute toxicity study

Acute oral toxicity study of the aqueous decoction extract of *A. vulgare* was evaluated in accordance with Organization for Economic Co-operation and Development (OECD) guideline 425 at limit doses of 2000 and 5000 mg/kg body weight [22].

Statistical analysis

All data are expressed as mean \pm standard deviation (SD). Differences among samples were analysed using one-way analysis of variance (ANOVA) at a 95% confidence level. Data normality was checked prior to analysis. Values were considered statistically significant at $p < 0.05$.

RESULTS

Nutritional composition

The proximate composition of *A. vulgare* tubers is presented in Table 1. The moisture content was

Table 1. Nutritional composition of *A. vulgare*

Proximate (%)	DW	FW
Moisture	8.35 \pm 0.55	78.13 \pm 0.17
Ash	6.98 \pm 0.76	1.53 \pm 0.16
Proteins	5.54 \pm 0.32	1.21 \pm 0.07
Fat	1.07 \pm 0.30	0.23 \pm 0.07
Carbohydrates	78.06 \pm 1.89	17.07 \pm 0.41
Mineral composition (mg/100 g)	DW	FW
Mg	16.62 \pm 0.31	3.63 \pm 0.07
Ca	95.18 \pm 0.85	20.82 \pm 0.19
P	19.97 \pm 0.14	4.37 \pm 0.03
K	53.12 \pm 0.32	11.62 \pm 0.07
Na	43.97 \pm 0.27	9.61 \pm 0.06
Zn	0.235 \pm 0.001	0.051 \pm 0.0002
Cu	0.043 \pm 0.0002	0.009 \pm 0.00004
Fe	4.040 \pm 0.014	0.88 \pm 0.003
Mn	0.161 \pm 0.003	0.035 \pm 0.001

Data are expressed as mean \pm standard deviation (SD); (n = 3); on dry weight (DW) and fresh weight (FW) basis.

relatively low (8.35%). Carbohydrates were the predominant component of *A. vulgare* (78.06%), followed by ash (6.98%), protein (5.54%), and fat (1.07%). Mineral analysis revealed appreciable levels of macroelements (Table 1), including calcium (20.82 mg/100 g fresh weight (FW)), potassium (11.62 mg/100 g FW), sodium (9.61 mg/100 g FW), phosphorus (4.37 mg/100 g FW), and magnesium (3.63 mg/100 g FW). Among microelements, iron content reached 40.88 mg/100 g FW, while zinc and manganese were present in moderate amounts and copper in lower concentrations.

Total phenolic and flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) of the methanolic extract are presented in Table 2. The extract showed a TPC of 29.76 mg GAE/g extract and a TFC of 0.69 mg GAE/g extract.

Antioxidant capacity

The antioxidant capacity of the *A. vulgare* tubers extract, along with standard antioxidant (ascorbic acid), was evaluated using the DPPH radical scavenging assay through the determination of IC₅₀ values. Lower IC₅₀ values indicate stronger antioxidant activity. Table 2 presents the obtained results. As shown, the studied extract exhibited strong free radical scavenging activity, with a low value (230.19 µg/mL). The IC₅₀ value is further lower for ascorbic acid (8.15 µg/mL) used as positive control.

Antimicrobial activity

The antimicrobial activity of *A. vulgare* was evaluated against a panel of pathogenic microorganisms using inhibition zone measurements, MIC and MBC/MFC assays (Table 3 and 4). The extract exhibited strong inhibitory effects against all tested Gram-

Table 2. Total phenol and flavonoid contents and antioxidant activity of methanolic extract of *A. vulgare*

	Total phenolic Content mg GAE/g	Total flavonoids Content mg QE/g	Antioxidant activity (IC ₅₀ µg/mL)	
			Extract	Ascorbic acid
Extract	29.76 ± 1.93	0.69 ± 0.55	230.19 ± 0.73	8.15 ± 0.01

Data are expressed as mean ± standard deviation (SD); (n = 3).

Table 3. Inhibition zone diameter (mm) of *A. vulgare* methanolic extract in the disk diffusion method

Microorganisms	Disk inhibitory zone (mm)		
	<i>A. vulgare</i> extract	Ampicillin	Econazole
<i>S. aureus</i>	22 ± 0.32	28 ± 0.06	-
<i>E. faecalis</i>	22 ± 0.97	29 ± 0.04	-
<i>Bacillus</i> sp.	26 ± 0.76	28 ± 0.06	-
<i>C. freundii</i>	20 ± 0.44	26 ± 0.08	-
<i>E. coli</i>	24 ± 0.22	28 ± 0.13	-
<i>Pseudomonas</i> sp.	17 ± 0.64	23 ± 0.25	-
<i>C. albicans</i>	13 ± 1.33	-	22 ± 0.2
<i>C. neoformans</i>	-	19 ± 0.2	24 ± 0.2

Data values are presented as mean ± standard deviation (SD); (n = 3); - = no activity or not determined.

Table 4. Antimicrobial parameters (MIC, MBC, MFC) of the tested extract

Strains	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC ratio	MFC (mg/mL)	MFC/MIC ratio	Type of activity
<i>S. aureus</i>	0.097	0.097	1	-	-	Bactericidal
<i>E. faecalis</i>	0.097	3.125	32	-	-	Bacteriostatic
<i>Bacillus</i> sp.	0.78	3.125	4	-	-	Bactericidal
<i>C. freundii</i>	0.194	3.125	16	-	-	Bacteriostatic
<i>E. coli</i>	1.56	6.25	4	-	-	Bactericidal
<i>Pseudomonas</i> sp.	0.194	3.125	16	-	-	Bacteriostatic
<i>C. albicans</i>	-	-	-	-	-	-
<i>C. neoformans</i>	6.25	-	-	12.5	2	Fungicidal

- = not determined.

positive and Gram-negative bacteria and yeasts tested, with inhibition zone diameters ranging from 13 to 26 mm. In contrast, no activity was detected against the yeast *C. albicans*. The tested extract exhibited a maximum zone of inhibition against *Bacillus* sp., while a minimum zone of inhibition was observed against *C. neoformans*.

The antimicrobial efficacy of the extract was further confirmed by MIC determination (Table 4), with MIC values ranging from 0.097 to 6.25 mg/mL. The highest MIC value was observed for *S. aureus* and *E. faecalis* (0.097 mg/ml).

Furthermore, the extract exhibited bactericidal activity against *S. aureus*, *E. coli* and *Bacillus* sp., as well as fungicidal activity against *C. neoformans*, as indicated by MBC/MIC and MFC/MIC ratios ≤ 4 . In contrast, bacteriostatic effects were observed against *E. faecalis*, *C. freundii* and *Pseudomonas* sp., with MBC/MIC ratios > 4 .

Acute toxicity

The acute toxicity of *A. vulgare* water decoction was assessed at doses of 2000 and 5000 mg/kg body weight. Throughout the 15 day observation period, no mortality or treatment related clinical signs of toxicity were recorded, in particular, symptoms commonly

associated with acute toxicity, including drowsiness, edema, loss of reflexes, diarrhea, tremors, excessive urination, or salivation, were not observed in treated animals.

Furthermore, no statistically significant differences ($p > 0.05$) were noted between the control and treated groups in terms of body weight evolution (Figure 1), food intake, or water consumption (Table 5) during the experimental period.

DISCUSSION

Nutritional composition

To the best of our knowledge, this is the first study reported the nutritional composition of *A. vulgare*. The low moisture content observed is favorable for storage stability and limits microbial spoilage. Carbohydrates are among the main nutrients in the human diet and their levels in plants are an important indicator of nutritional and caloric value [23]. In the present study, the high carbohydrate content indicates that this species can serve as a source of energy. Although its nutritional contribution depends on its inclusion in a diversified diet.

The ash content was higher than that reported for several wild edible tuberous plants traditionally

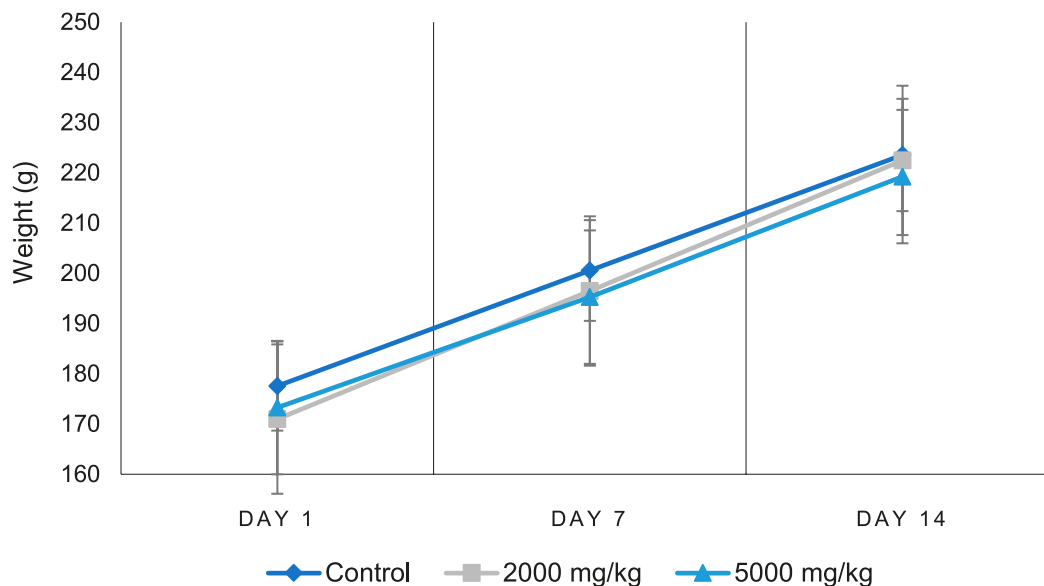


Figure 1. Body changes in rats treated with *A. vulgare* aqueous extract during the acute toxicity study (mean \pm SD, $n = 5$). No significant differences versus control ($p > 0.05$).

Table 5. Effect of *A. vulgare* extract on food intake (g) and water intake (mL) in experimental rats during acute toxicity study

	Groups							
	Food intake (g)				Water intake (mL)			
	Control	2000 mg/kg	5000 mg/kg	p-value	Control	2000 mg/kg	5000 mg/kg	p-value
<i>A. vulgare</i>	47.15 \pm 2.06 ^a	46.96 \pm 1.96 ^a	46.72 \pm 2.18 ^a	> 0.05	47.22 \pm 2.43 ^a	47.76 \pm 1.36 ^a	46.54 \pm 2.56 ^a	> 0.05

Data are presented as mean \pm standard deviation (SD); ($n = 5$ per group); ^a = no significant differences versus control.

consumed in Lokop forest, (East Aceh, Indonesia) [24], suggesting a higher mineral contribution. The protein content (5.54%) exceeded previously reported values for similar wild tubers (1.72-4.27%) [24], although still lower than that of protein-rich leafy vegetables, this protein level remains nutritionally relevant. The low fat content of this species (1.07%) suggests that *A. vulgare* can be classified as a low-fat food. However, its potential health effects depend on dietary patterns and nutritional balance.

Calcium is an essential mineral vital for bone and tooth development, muscle function, nerve transmission, and detoxification processes [25]. The calcium content of *A. vulgare* (20.82 mg/100 g FW) is comparable to that of commonly consumed tuber vegetables in Morocco such as sweet potato (22 mg/100 g FW), carrot (30 mg/100 g FW) and, turnip (33 mg/100 g FW), although higher than potato (8 mg/100 g FW) [26]. Potassium content was higher than sodium, which results in a Na/K ratio below 1. As an indicator of cardiovascular health, a low Na/K ratio is generally associated with a reduced risk of hypertension and related metabolic disorders [27]. Therefore, the low Na/K ratio observed enhances the dietary value of *A. vulgare* tubers and supports their inclusion in mineral balanced diets.

Iron is required for haemoglobin formation and oxygen transport [28]. The high Fe content compared to conventional tuberous and root vegetables such as sweet potato (0.57-0.73 mg/100 g FW) [29], carrot (0.30 mg/100 g FW), beetroot (0.8 mg/100 g FW) and radish (0.34 mg/100 g FW) [30] highlights the potential of *A. vulgare* as a source of this essential micronutrient. Zinc and manganese, which are involved in immune function, antioxidant defense, and enzymatic activity [28], were present in moderate amounts, while copper, essential for iron metabolism and redox processes, was found at lower levels. These levels suggest a limited to moderate contribution to daily micronutrient requirements. Overall, these results suggest that *A. vulgare* can contribute to mineral intake. Nevertheless, its contribution varies among minerals and may also be affected by traditional processing methods such as prolonged cooking.

Total phenolic and flavonoid contents

Polyphenols are considered essential functional components of the human diet. They are classified into several groups, among which flavonoids, represent the largest and most widespread family [31]. In the current study, the moderate total phenolic content and low flavonoid content suggest that phenolic acids and other non-flavonoid phenolics may constitute the major contributors of total phenolic of the extract. The obtained TPC and TFC values were found to be lower than the values reported for the tubers of the

same species by Bouafia et al. [11], who recorded 176.00 ± 1.08 mg GAE/g of dry extract for TPC and 81.21 ± 1.24 mg QE/g of dry extract for TFC. In contrast, Kadri et al. [14] reported lower total phenolic contents in the hydromethanolic extract of *A. vulgare* seeds, with value of 12 mg GAE/g of extract, which is lower than the value obtained in the present study. However, the same study reported a higher TFC (3.4 mg QE/g extract) than the value recorded in the present finding. Such variations in TPC and TFC contents may be attributed to differences in climatic conditions, seasonal factors, geographical location, plant part used, harvest time, and extraction methods.

Antioxidant activity

The antioxidant activity observed in this study indicates that the extract possesses a noticeable free radical scavenging capacity. This activity was higher than that reported by Messaoudi et al. [32], but lower than the activity observed by Bouafia et al. [11] for the ethanolic and aqueous extracts of the same plant parts. Such variability in antioxidant activity may be attributed to differences in climatic and environmental conditions. The antioxidant capacity of the extracts of the examined species in DPPH assay is likely related to the presence of specific phenolic compounds, such as rutin, hesperidin, isoquercitrin, catechin, chlorogenic acid, syringic acid, salicylic acid, kaempferol, and luteolin, which are well known for their strong antioxidant properties [11].

Antimicrobial activity

The results of the present study indicate that *A. vulgare* exhibited a broad-spectrum antimicrobial activity associated with stronger effects against Gram-positive bacteria than Gram-negative bacteria. This difference may be explained by structural differences in bacterial cell walls. These findings are consistent with previous findings by Bouafia et al. [11], who demonstrated that the ethanolic extract of *A. vulgare* was effective against *S. aureus*, *E. coli*, *Enterococcus faecium*, and *Salmonella typhimurium*, with the highest inhibition zone observed against *E. faecium*. Similarly, Messaoudi et al. [32] reported that the methanolic extract exhibited antimicrobial activity against *Klebsiella pneumoniae*, *S. aureus* and *C. albicans*.

The low MIC values observed in the present study, particularly for *S. aureus* and *E. faecalis*, further support the strong antimicrobial potential of the extract. These results are consistent with the findings presented by Aydin et al. [12], who observed inhibitory effects of *A. vulgare* extracts against *E. coli* and *S. aureus*.

Additionally, the bactericidal and fungicidal effects observed against several strains, as indicated by MBC/

MIC and MFC/MIC ≤ 4 , highlight the effectiveness of the extract in inhibiting microbial growth. However, the bacteriostatic effects observed for some strains suggest a variable response depending on the microorganism.

The antimicrobial properties of *A. vulgare* may be attributed to its content of bioactive compounds, particularly alkaloids such as bbugaine and irniine, which have been previously reported to play a role in pathogen control [33].

Acute toxicity

The absence of mortality and clinical signs of toxicity suggest that the aqueous decoction of *A. vulgare* did not induce acute toxicity at the tested doses. No significant differences in body weight, food intake, or water consumption were observed, supporting its safety under the experimental conditions. Although *A. vulgare* is traditionally considered toxic, its consumption has been reported during periods of famine, which suggests that traditional preparation methods such as aqueous decoction may reduce its toxicity.

CONCLUSION

This study provides insight into the nutritional and biological characteristics and acute toxicity of *A. vulgare* tubers traditionally used as a subsistence food during periods of food scarcity. Proximate analysis indicated that the tubers are predominantly composed of carbohydrates, which highlights their role as a source of energy rather than a complete nutritional food. Protein and fat were relatively low, while mineral analysis revealed moderate levels of essential elements, particularly calcium and iron. Phytochemical analysis showed moderate amounts of phenolic compounds and flavonoids, and the methanolic extract exhibited antioxidant and antimicrobial activities. Acute toxicity assessment did not reveal adverse effects at the tested doses, suggesting a favorable short-term safety profile.

Therefore, *A. vulgare* should be regarded mainly as an emergency or subsistence food providing energy and certain micronutrients rather than a rich source of nutrients. Their traditional use during famine periods may be explained by their availability and caloric contribution. Further studies including chronic toxicity and biochemical and histopathological evaluations, are required to ensure their long term safety for human consumption.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Received: 23.01.2026

Revised: 18.04.2026

Accepted: 21.04.2026

Published online first: 05.05.2026