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Research Article

Phytochemical screening, nutritional value, antioxidant and antimicrobial activities and acute toxicity of *Scolymus hispanicus*: A wild edible plant in Morocco

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Abstract

Scolymus hispanicus L. (Golden thistle) is a well-known wild edible plant (WEP) in Morocco. However, the species value is not well investigated and its economic value is considerably low. Therefore, the aim of this work was to evaluate the phytochemical composition, the biological activities, and the acute toxicity of crude extract of *S. hispanicus* aerial parts. The mineral composition was assessed using an ICP-AES spectrometer and, the contents in polyphenol, flavonoids and tannins using colorimetric methods. The antioxidant activity was tested by DPPH assay. Disc diffusion and broth micro dilution methods were used to evaluate the antimicrobial activity. Moreover, the safety of the plant extract was validated by performing acute toxicity. The findings revealed that this plant is a rich source of protein, carbohydrates and minerals especially iron and, have high contents of polyphenols, flavonoids and tannins. The biological evaluation of the plant extracts exhibited a remarkable antioxidant content and, a wide antibacterial activity and yeast inhibition. The results indicated also that the tested extract is safe with an LD_{50} higher than 5000 $mg.kg^{-1}$. The study data suggest that *S. hispanicus* could be a promising functional and nutraceutical food with antioxidant and antimicrobial potential and can contribute to a balanced diet.

Keywords
Scolymus hispanicus, nutritional composition, antimicrobial effect, antioxidant effect, phenolic content, toxicity, functional food

Abbreviations
ATCC – American Type Culture Collection; BHT – butylated hydroxytoluene; CE – catechin equivalent; CFU – colony forming unit; CIP – institute Pasteur Paris Collection; DM – dry matter; DPPH – 2,2-diphenylpicrylhydrazyl; GAE – gallic acid equivalent; IC_{50} – Median Inhibition Concentration; ICP-AES – inductively coupled plasma atomic emission spectroscopy; LD_{50} – median lethal dose; MBC – minimal bactericidal concentration; MFC – minimal fungicidal concentration; MIC – minimum inhibitory concentration; OECD – Organization for Economic Co-operation and Development; QE – quercetin equivalents; TCC – total condensed tannins compounds; TFC – total flavonoids compounds; TPC – total phenolic compounds; WEPs – wild edible plants

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Introduction

Morocco is considered among the countries of the Mediterranean basin with the richest plant biodiversity and a wide variety of wild edible plants (WEPs). The latter are defined as "plant species that are neither cultivated nor managed, but accessed from various types of natural vegetation and used as food" (Aboukhalaf et al. 2022). In addition to complying with the pillars of sustainability (Pieroni et al. 2021), these plants have proven to be a potential source of essential nutrients and microelements such as proteins, fibers, minerals and vitamins, and are a good source of bioactive compounds including polyphenols and flavonoids that are associated with potential health benefits (Rana et al. 2019; Sánchez-Mata et al. 2012). Additionally, they are used in the prevention and management of a wide range of chronic diseases, such as diabetes, cancer, obesity, cardiovascular disease, immune dysfunction, etc. (Marrelli et al. 2020a; Marrelli et al. 2020b; Mzoughi et al. 2019). Interest in research on nutritional and phytochemical enhancement of (WEPs) is currently increasing worldwide in order to reduce malnutrition. Malnutrition, particularly due to iron deficiency, is a widespread health problem, affecting 1.62 billion people (about 25%) of the world's population (McLean et al. 2009). The low availability of iron in the daily diet is the main obstacle to meeting the needs for this nutrient (Mzoughi et al. 2019). Valuing wild edible plants could contribute to a more sustainable and cost-effective strategy to address micronutrient deficiencies. Golden thistle (*Scolymus hispanicus*) commonly known as *El Guernina*, *Taghediwt* or *Agheddou* is a well-known edible plant widespread in Morocco and other Mediterranean countries (Marmouzi et al. 2017). In Morocco, the tender stems and leaves of this plant can be eaten raw as a snack and salad or prepared as vegetables in several traditional dishes like broth, couscous and *Beqoula* (Aboukhalaf et al. 2022; Nassif el al. 2013). The plant also has many traditional medicinal properties. It is used to treat coughs, colds, Malta fever, and eye infections (Aboukhalaf et al. 2022; Berdja et al. 2021). It has important applications in herbal medicine as an appetite stimulant, digestive, diuretic, depurative, choleretic, lithiuretic, diaphoretic and antipyretic (Altiner et al. 2016; Berdja et al. 2021; Marmouzi et al. 2017).

Materials and Methods

Collection of plant material. The samples of the wild edible plant *S. hispanicus* were collected in February 2022, in the province of Sidi Bennour (32° 39' 8.50" N, 8° 25' 39.68" W) in central Morocco. The scientific identification was undertaken by a taxonomist, at the Department of Biology at Chouaïb Doukkali University (El Jadida, Morocco) and reference specimens (KW37) were deposited in the herbarium of the same department. Aerial parts of the WEP were used for the determinations.

Preparation of crude extracts. The aerial parts of *S. hispanicus* collected samples were washed with distilled water and dried in an oven at 37°C for one week. The dried material was milled into powder (<1 mm) using an electric blender (Moulinex type LM 207, France), and then extracted by maceration method with methanol for 48 h. The obtained extract was subsequently filtered with a filter paper (Whatman. No. 1) and concentrated through Rota evaporator. Dried extract was stored in refrigerator at 4°C till further study.

Animals. An acute toxicity test was carried out on female Wistar Albino rats (healthy and non-pregnant) aged 8 to 12 weeks, weighing 160-190 g, obtained from the animal facility of the Faculty of Sciences of Chouaïb University Doukkali in Morocco. For acclimatization to the laboratory conditions all animals were kept in their cages 7-9 d before the start of dosing, at temperatures of 23±3°C and in 12 h light-dark cycles and with *ad libitum* access to food and in water.

Determination of nutritional composition. The proximate composition (moisture, ash content, crude protein, carbohydrates and fat) of *S. hispanicus* was analyzed according to the methods described previously. Moisture was measured using the oven-drying method (Gharby et al. 2017). The ash content was determined by using a muffle furnace (Gharby et al. 2017). Crude proteins were quantified using the Kjeldahl procedure (Gharby et al. 2017). The fat content is estimated by the Soxhlet gravimetric technique using hexane as the solvent for the extraction (Lahmar et al. 2017). Carbohydrate content was quantified using the equation (1):

Carbohydrates, % = 100% - (water content + total ash + total fat + crude protein) (Jadid et al. 2018) *(1)*

The micronutrients (K, Ca, Mg, B, Fe, Cu, S, Zn, Mn, Mg and Na) were determined using an ICP-AES spectrometer (Jobin Yvon, Ultima 2) equipped with an axial viewing plasma as previously described by Mohammad et al. (2013).

Quantification of phenolic content. Quantification of TPC was carried out according to the method of Folin-Ciocalteu written by Kim et al. (2003) with a slight modification. Briefly, 100 µl of the extract solution (1 mg/ml) was mixed with 1 ml of freshly diluted Folin-Ciocalteu reagent (1:10 v/v) and within 5 min, a volume of 1 ml of 7% Na_2CO_3 solution was added. The solution is immediately diluted with 400 µl of distilled water, and the mixture is left to stand for 90 min in the dark. Absorbance was then measured at 760 nm using a spectrophotometer. The total phenolic compounds of the extract are calculated by the equation obtained from the standard calibration curve of gallic acid (GA) and expressed in terms of gallic acid equivalent (mg GAE.g^{-1} extract).

Quantification of flavonoid content. Quantification of TFC of the extract of aerial parts of *S. hispanicus* was assessed by aluminum chloride colorimetric method as stated by Kim et al. (2003). A volume of 400 µl of the extract is placed in a tube with 120 µl of 5% $NaNO_2$, added after 5 min to 120 µl of $AlCl_3$ (10%), then with 800 µl of NaOH (1M) after another 6 min. The absorbance of the reaction mixture was measured by spectrophotometry at 510 nm. The test was performed three times and the flavonoid content is expressed as milligrams of quercetin equivalents per gram of sample extract (mg QE.g^{-1} extract).

Condensed tannin content. Condensed tannin contents were determined according to the method of Broadhurst et al. (1978) with a slight modification using catechin as the reference compound. A volume of 400 µl of extract was added to 3 ml of a 4% solution of vanillin in methanol and 1.5 ml of hydrochloric acid. After 15 min the absorbance was registered at 500 nm against methanol as a blank. The condensed tannin content was expressed in mg catechin equivalent. g^{-1} extract

In vitro antioxidant evaluation. The antioxidant activity was determined using the free radical scavenging assay of the 2,2-diphenyl-2 picrylhydrazyl ($DPPH^{\bullet}$) method described by Ksouri et al. (2015). Different concentrations of methanolic extracts (500 µg.ml^{-1}) were mixed with an equal volume of the methanol/DPPH solution (0.004% w/v), The mixture was kept at room temperature in the dark for 30 min then the absorbance is read at 517 nm. The test was performed in triplicate and expressed as mg Trolox equivalent. g^{-1} extract as it is a more descriptive and meaningful expression than tests that express antioxidant potential as the percentage decrease in absorbance.

Antimicrobial activity

Microorganism strains. Six strains of bacteria (three strains of Gram-positive bacteria and three strains of Gram-negative bacteria) and two strains of fungi were provided from the Institute Pasteur Paris Collection (CIP) and the American Type Culture Collection (ATCC), and were used in this study: *Enterococcus faecalis* (ATCC19433), *Staphylococcus aureus* (ATCC25923), *bacillus subtilis* (ATCC66331), *Escherichia coli* (CIP54127), *Citrobacter freundii* (ATCC8090), *Pseudomonas* spp., *Candida albicans* (48.72), and *Cryptococcus neoformans* (CIP 960). All the strains were maintained at freeze temperature until use.

Disk diffusion assay. Screenings of extract for antimicrobial activity was done by the disc diffusion method (Jorgensen et al. 2015).

Minimum inhibitory concentration Determination (MIC). The MIC of *S. hispanicus* extract was determined by the quantitative method of microdilution using resazurin as viability indicator (Btissam et al. 2018).

Minimal bactericidal concentration and minimal fungicidal concentration determinations. All the well plate showing no growth after MIC tests were reinoculated for the determination of the MBC and MFC. The broths were incubated according to growth requirement of each microorganism. The absence of growth in the recovery medium was evidence of bactericidal and fungicidal activities. Moreover, the ratio MBC/MCI and MFC/MCI of each sample was calculated to assess the antimicrobial power. If the ration ≤ 4, the effect is bactericidal/ fungicidal and when the ratio > 4, it's bacteriostatic/fungistatic (Harchaoui et al. 2022).

Acute toxicity study. The acute oral toxicity study of the methanolic extract of *S. hispanicus* was evaluated based on the guideline 425 of the Organization for Economic Co-operation and Development at limit doses of 2000 and 5000 mg.kg^{-1} body weight (OECD 2008).

Statistical analysis. All studied data are expressed as mean ± standard error means (SEM). ANOVA-one way with a confidence level of 95% was used to look for differences among the study results. Means were considered to be significant at $p<0.05$.

Results and Discussion

Proximate analysis. The findings for proximate composition of *S. hispanicus* aerial parts are shown in Table 1. The species contains high values in protein and ash (27.17%, 26.66% respectively). These results make the aerial parts of *S. hispanicus* a good potential source of additional protein and mineral elements for the human diet. The values of these contents were found to be higher than those reported by Altiner et al. (2016) and by García-Herrera et al. (2014). Similarly, the total fat content of this species, which is higher than the levels reported by Altiner et al. (2016) and García-Herrera et al. (2014), was 1.57% in this study. On the other hand, carbohydrates are biomolecules known for several roles in many life processes including metabolism, energy storage, structural support, immunological recognition, antibiosis etc. (Guillén et al. 2010). In this study, the carbohydrates (40.07%) represented the major component in the proximate analysis of this species. Other authors have reported higher carbohydrate levels in *S. hispanicus* (García-Herrera et al. 2014). Based on the findings of this study, *S. hispanicus* can be suggested as an alternative source of a healthy food for human which has high-protein diet but low in fat. In addition, this species could be used to supplement and fortify bakery products such as bread, crackers, cakes and biscuits in order to improve their nutritional value and secure a higher food intake. The use in bread and bakery products is an effective strategy due to the high consumption of these foods.

Mineral analysis. The minerals contained in *S. hispanicus* are divided into Macroelements and microelements. As shown in Table 1, the most abundant macronutrients in this species were sodium (411.63±1.01mg.100g^{-1}) and potassium (253.81±1.82 mg.100g^{-1}). The other amounts of Macroelements, in descending order, were calcium (151.07±1.38 mg.100g^{-1}), sulfur (150.58±1.23 mg. 100g^{-1}), magnesium (55.507±0.58 mg. 100g^{-1}) and phosphorus (38.351±0.34 mg.100g^{-1}).

Ca and Mg were within the range of values reported for this species by the study by García-Herrera et al. (2014). However, the Na contents found in this study are higher than those reported by the studies of these same authors on *S. hispanicus* collected in different sites (11.2-65.3 mg.100g^{-1} w/w). Among the microelements, the highest values found were that of iron (6.855±0.024 mg.100g^{-1}) followed by manganese (0.726±0.004 mg.100g^{-1}), boron (0.482±0.0009 mg.100g^{-1}) and zinc (0.246±0.001 mg.100g^{-1}) while the lowest content value was found for copper of 0.133±0.0003 mg.100g^{-1}).

Fe and Mn were higher in this study compared to the contents reported by the study carried out in central Spain. On the contrary, the same study carried out in central Spain found a higher Zn content compared to the present study (García-Herrera et al. 2014). It turns out that the studied WEP is an excellent source of minerals, especially Fe, compared to other conventional vegetables such as beans (Fe = 4.93 mg.100g^{-1}), spinach (Fe = 1.05 mg.100g^{-1}), lettuce (Fe = 0.95 mg.100g^{-1}), broccoli (Fe = 0.69 mg.100g^{-1}), and other cultivated vegetables considered to be high in Fe (USDA 2018). Iron is one of the most important micronutrients needed by the human body (Godswill et al. 2020). It has several vital biological roles, including DNA and RNA synthesis, oxygen transport, cellular respiration, energy generation and regulation of gene expression (Winter et al. 2013). Moreover, iron deficiency remains the most common nutritional deficiency and the main cause of anemia, which affects more than one billion people in the world (Stelle et al. 2019).

Table 1. Proximate composition and mineral composition of *S. hispanicus*

Proximate composition, %	
Moisture	13.63±0.32
Proteins	18.08±0.19
Fat	1.57±1.85
Carbohydrates	40.07±0.00
Ash	26.66±0.56

Mineral composition mg.100 g^{-1} dry mater	
Macroelements	
Mg	55.51±0.58
Ca	151.07±1.38
P	38.35±0.34
K	253.81±1.82
Na	411.63±1.01
S	150.58±1.23
Microelements	
Zn	0.246±0.001
Cu	0.133±0.0003
Fe	6.855±0.024
Mn	0.726±0.004
B	0.482±0.0009

By consuming them as a main or as side dishes, edible wild plants such as *S. hispanicus* containing sufficient iron, content, cases of iron deficiency can be avoided. Indeed, consumption of 100 g of this plant could cover the Recommended Dietary Intakes in Fe, at around 75% for men and around 33% for women. These results suggest also that the aerial parts powder of *S. hispanicus* can be recommended as a natural functional (iron-rich) ingredient for developing iron-fortified foods and avoiding the use of oral iron supplements, which are associated with undesirable side effects.

Total phenolic content, flavonoids and tannins. In this study, the contents of total phenolic compounds, flavonoids and condensed tannins were also determined in the methanol extract of *S. hispanicus* and presented in Table 2. As indicated, the methanol extract has a remarkably high content of TPC (89.6 mg GAE.g^{-1} extract) and TFC (71.89 mg QE.g^{-1} extract). These values were found to be higher than those obtained in basal leaves of the same species by the study of Morales et al. (2014) reporting a value of 21.51 mg GAE.g^{-1} extract for TPC and 8.39 mg CE.g^{-1} extract for TFC. However, the results reported by the Berdja et al. (2021) study on the aqueous extract of *S. hispanicus* indicated that this species had 270.321±25.44 µg GAE.mg^{-1} extract for TPC and 164.94±9.45 µg QE.mg^{-1} extract which is higher than the values obtained in the present study. These different amounts of TPC and TFC obtained in these two studies may be due to the different conditions related to the climate, the season, the geographical location, the time of the harvest of the plant, the used part of the plant, as well as the solvents chosen and the extraction procedure used (Aboukhalaf et al. 2020; Miliauskas et al. 2004). Polyphenols are considered among the most commonly found compounds in plants with various virtues. Indeed, these compounds are known to have several therapeutic and protective effects such as antioxidant, anti-aging, anti-inflammatory and anti-proliferative activities (Lin et al. 2016; Taghouti et al. 2018). Thus, the consumption of such plants rich in phenolic compounds could reduce the incidence of chronic diseases such as diabetes, cardiovascular and cancer diseases, Alzheimer's disease, Parkinson's disease and inflammation (Ambriz-Pérez el al. 2016; Lin et al. 2016). The TCC of the methanolic extract of *S. hispanicus* was 58.14 mg of CE.g^{-1} of extract. Tannins are water-soluble polyphenols that are distributed in many plant foods (Chung el al. 1998). It is known that many tannins have inhibitory effects on mutagenesis and carcinogenesis due to their antioxidative property (Chung et al. 1998). *S. hispanicus* by its richness in these bioactive compounds could therefore promote their use as additive in the food industry to maintain the quality and shelf life of food product and improve their sensory characteristics.

DPPH radical scavenging activity assay. The antioxidant activity was tested for the *S. hispanicus* aerial parts extract by DPPH radical scavenging capacity evaluated by determination of IC_{50} values. The Table 2 presents the obtained results. As can be seen, the studied extract exhibited a strong anti-free radical activity, with a value (IC_{50} = 2.09±1.43 mg TEAC.g^{-1} extract). There are a few reports about antioxidant activity of *S. hispanicus* in the available literature which makes difficult the comparison of our results. In a previous study (Altiner et al. 2016), we have found the following antioxidant activity values: 34.29 and 13.53 µmol Trolox.g^{-1}fw for hydrolysable and extractable phenolic extracts respectively. Other studies have also shown an important antioxidant capacity of this plant (Morales et al. 2014; Berdja et al. 2021).

Table 2. Total phenol, flavonoids, condensed tannins contents and antioxidant activity of methanolic extract of *S. hispanicus*

	Total phenolic content, mg GAE.g^{-1}	Total flavonoids content, mg QE.g^{-1}	Tannins content, mg CE.g^{-1}	Antioxidant activity, DPPH$^{\bullet}$ mg TEAC.g^{-1}
Extract	89.6±1.21	71.89±0.55	58.14±1.97	2.09±1.43

Data are expressed as mean ± standard error mean (SEM) (n = 3)

The antioxidant potential of the methanolic extracts of the examined species in DPPH$^{\bullet}$ test, can be explained by its high phenolic and flavonoids contents such as caffeic acid, quercetin, luteolin, kaempferol, apigenin and isorhamnetin (Petropoulos et al. 2019). Indeed, several studies have revealed a positive correlation between the antioxidant activity and phenolic content (Lante et al. 2022; Bakour et al. 2020; Lahmar et al. 2017; Miliauskas, et al. 2004). In Morocco *S. hispanicus* is traditionally cooked with meat (Aboukhalaf et al. 2022; Nassif el al. 2013). Therefore, its extract due to its high antioxidant activity can be used as an effective natural antioxidant that can replace synthetic antioxidants in the production of healthier meat and meat products. Many studies have proposed the strategy of the use of plant extracts to improve meat quality and prolong their shelf life (Kolev et al. 2022; Kim et al. 2013).

Antimicrobial activity. The antimicrobial activity of the methanolic extract of *S. hispanicus* against the different pathogenic microorganisms examined in the present study was assessed by the presence or absence of zones of inhibition, MIC and MBC/MFC values (Table 3 and Table 4).

The results reveal antimicrobial properties of the plant extract against all gram-negative and gram-positive bacteria and yeasts tested to varying degrees. However, no activity was observed against the yeast *C. neoformans*. The tested extract showed a maximum zone of inhibition against *S. aureus* (19.0±0.2 mm) and against *E. faecalis* (18.0±0.15 mm) while a minimum zone of inhibition was shown by *E. coli* and *C. albicans* (14.0±0.6 mm and 13.0±1.33 mm respectively). It appears that Gram positive bacteria are more sensitive to the extract examined in this study than Gram negative bacteria. This difference in sensitivity could be due to the difference in cell wall composition of Gram-negative and Gram-positive bacteria (Deng et al. 2014). The results of the present study are consistent with those previously reported by Aboukhalaf et al. (2020). However, contrary to our result, they also reported antifungal activity against *C. neoformans* (Aboukhalaf et al. 2020). The tests of the studied extracts efficacy on the microbial strains used, was determined by measuring the minimum inhibitory concentration (Table 4). The obtained result is consistent with that recorded in the preliminary test using the disc diffusion method, for which the greater is the diameter around; the more interesting is the CMI.

Table 3. Disk inhibitory zone of *S. hispanicus* extract against microorganisms

		Disk inhibitory zone, mm		
	Microorganisms	***S. hispanicus* extract**	**Amplicillin, 30 µg**	**Fluconazole**
Gram positive Bacteria	*S. aureus*	19±0.20	26±0.06	ND
	E. faecalis	18±0.15	27±0.13	ND
	Bacillus.sp.	17±0.55	27±0.20	ND
Gram negative Bacteria	*C. freundii*	17±0.33	25±0.06	ND
	E.coli	14±0.60	28±0.13	ND
	Pseudomonas sp.	16±0.60	24±0.20	ND
	C.albicans	13±1.33	ND	24±0.06
Yeasts	*C. neoformans*	ND	ND	26±0.20

ND - not determined

The tests of the studied extracts efficacy on the microbial strains used, was determined by measuring the minimum inhibitory concentration (Table 4). The obtained result is consistent with that recorded in the preliminary test using the disc diffusion method, for which the greater is the diameter around; the more interesting is the CMI. It is the case for *S. aureus*, *C. freundii* and *E. feacalis* for which a high sensitivity was shown for the *S. hispanicus* plant extract, with a MIC value of 3.125 mg.ml^{-1}. These results are in agreement with those of Marmouzi et al. (2017) that reported an inhibitory effect of *S. hispanicus* extracts against *E. coli*, *S. aureus*, *B. subtilus*, *S. enterica* and *P. aeruginosa* with respective MICs values of 1.56, 3.12, 1.56, 1.56 and 1.56 mg.mL^{-1}. Petropoulos et al. (2019) have also reported that the wild plant *S. hispanicus* exhibited antimicrobial effect against a wide range of microbial strains, namely *S. aureus*, *E. coli*, *Bacillus aureus*, *Salmonella tymphimurium*, *Penicillium ochrochloron*, *Aspergillus fumigatus*, *Aspergillus ochraceus*, *Aspergillus niger* and *Penicillium funiculaire*.

Table 4. MIC, MBC, MFC, MBC/MCI and the MFC/MCI ratio on the antimicrobial activity of the tested extracts

	Antimicrobial activity parameters						
	Microorganisms	**MIC, mg.ml^{-1}**	**MBC, mg.mL^{-1}**	**MBC/MIC, ratio**	**MFC, mg.ml^{-1}**	**MFC/MIC, ratio**	**Decision**
Gram positive	*S. aureus*	3.125	6.25	2	ND	ND	Bactericidal
	E. faecalis	3.125	6.25	2	ND	ND	
	Bacillus. Sp.	6.25	12.5	2	ND	ND	
Gram negative	*C. freundii*	3.125	12.5	4	ND	ND	
	E. coli	6.25	12.5	2	ND	ND	
	Pseudomonas sp.	6.25	12.5	2	ND	ND	
Yeasts	*C. albicans*	12.5	ND	ND	100	8	Fungistatic
	C. neoformans	_	ND	ND	ND	ND	_

Data values are presented as (Mean ± SEM), (n = 3); ND - not determined; _ - no activity

More precise data on the antimicrobial properties were obtained by determining bacteriostatic/ fungistatic and bactericidal/fungicidal concentrations. Table 4 shows that the MBC and MFC values ranged from 6.25 to 100 mg.ml^{-1}. In agreement with the previous antimicrobial test, the *S. aureus* and *E. faecalis* strains were more sensitive to the tested extract while *C. albicans* was the most resistant strain with a MFC value of 100 mg.ml^{-1}. It is also observed that the extracts of *S. hispanicus* were bactericidal for all the bacterial tested strains at higher MBC values than the MIC values while it was fungistatic against the yeast *C. neoformans*. The antimicrobial properties of this species may be attributed to their high content of phenolic acids, flavonoids and tannins. Thay have been reported to be involved in control of pathogens through the inhibition of hydrolytic enzymes, the inhibition of oxidative phosphorylation and nucleic acid biosynthesis, the inactivation of microbial adhesions, the non-specific interactions with carbohydrates, the cell envelope transport proteins, etc. (Cushnie et al. 2005; Omojate et al. 2014). The current data reveal that *S. hispanicus* could be considered a promising source of preservatives in the food industry as well as good candidates for raw material antibacterial and antifungal phytopreparations.

Acute oral toxicity study. The acute toxicity of the methanolic extract of *S. hispanicus* was determined as described in OECD Guideline 425 (OECD 2008), where the limit test dose of 5000 mg.kg^{-1} was used.

No clinical signs or symptoms and no mortality were observed in any of the treated animals at 4, 24 h and up to 14 d after oral administration of the plant extract tested at the doses of 2000 or 5000 mg.kg^{-1} (Table 5).

According to the Globally Harmonized Classification System of Chemical Substances and Mixtures, *S. hispanicus* extract can be classified as category 5 (an extract with a median lethal dose (LD_{50}) greater than 5000 $mg.kg^{-1}$).

Table 5. Initial and daily observation of changes in appearance, behavior, and living habits at 4, 24 h and up to 14 d after oral administration of the tested plant extract at a dose of 2000 or 5000 $mg.kg^{-1}$

Clinical signs	First hour	Second hour	Third hour	Fourth hour	Day 2 to day 14
Loss of reflux	-	-	-	-	-
Drowsiness	-	-	-	-	-
Oedema	-	-	-	-	-
Urination	-	-	-	-	-
Salivation	-	-	-	-	-
Diarrhea	-	-	-	-	-
Increased respiration rate	-	-	-	-	-
Eye closure at touch	+	+	+	+	+
Tremor	-	-	-	-	-
Mortality	-	-	-	-	-

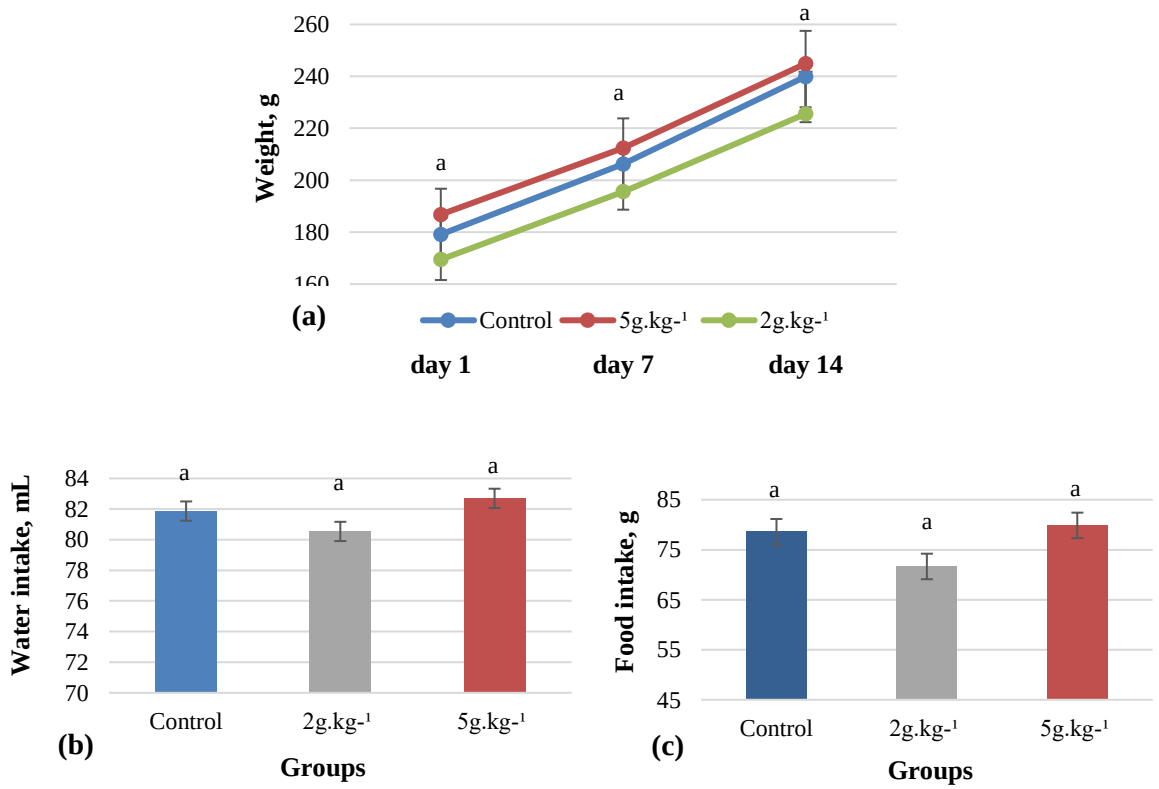


Figure 1. Effect of *S. hispanicus* extract on body weight (**a**), food (**b**) and water (**c**) intake of experiment rats in acute toxicity studies.

Values expressed as mean ± SEM, n= 5 animals /group. No significant differences ($p>0.05$) were found in comparison with controls.

Data regarding the evolvement of the animal's body weight during the study period are shown in Fig. 1. Statistical analysis revealed no significant changes in body weight gain throughout the study period ($p>0.05$) neither in animals having received a single dose of *S. hispanicus* extract nor in the control group. Furthermore, no significant differences ($p>0.05$) were observed in food and water intake (Fig. 1) between extract treated and control groups during the study period, which indicates that our extract has no toxic effects. The no significant change in general behaviors, body weight, and food and water intake indicate the safety of the wild plant extract. These obtained results are in agreement with that those of Marmouzi et al (2017) reporting that *S. hispanicus* extract has no toxic effect with oral administration of single doses (2 $g.kg^{-1}$) under the conditions of acute toxicity.

Conclusions

This study describes the nutritional value (proximate composition and mineral profile) of aerial parts of golden thistle, an edible wild plant demonstrating the potential to be used as an alternative green leafy vegetable for human consumption. The results revealed that golden thistle has high levels of minerals, particularly iron, providing scientific evidence of the potential of wild edible plants as sources of essential nutrients. Phytochemical analysis showed that Golden Thistle also had high levels of phenols, flavonoids and tannins. Moreover, its methanolic extract showed good antioxidant and antimicrobial activities. In addition, a toxicological evaluation confirmed the safety of this wild species. Therefore, this plant could be considered as a natural source of nutrients and bioactive antioxidant contributing to the fight against malnutrition and micronutrient deficiencies and as a prospective tool in the treatment of diseases related to oxidative stress and pathogenic infections. Moreover *S. hispanicus* might be also used as a new additive in the food industry to maintain the quality and shelf life of food product and improve their sensory characteristics.

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