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Antioxidant Activities and Laxative Effect of Bioactive Compounds from *Cynara cardunculus* var. *sylvestris*

(Aktiviti Antioksidan dan Kesan Laksatif Sebatian Bioaktif daripada *Cynara cardunculus* var. *sylvestris*)

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ABSTRACT

The present study aims to investigate the antioxidant and anti-constipation activities of the derived phenolic extracts in ribs wild cardoon (*Cynara sylvestris*). Analysed extracts (acetone, methanol and aqueous) exhibited high level of phenolic compounds and excellent antioxidant activities with significant IC_{50} values ($p \leq 0.05$), as assessed by the DPPH radical-scavenging activity (0.84±0.08 - 2.44±0.007 µg/mL), ABTS cationic radical test (0.96 ±0.01- 136.67±6.75 µg/mL) and β-carotene bleaching assay (2.09±0.02-51.12±1.32 µg/mL). Also, higher levels of insoluble dietary fibers were found (56.18 ± 0.91% DW of neutral detergent fiber). The *in vivo* investigation was performed on Wistar rats to explore the ability of *C. sylvestris* aqueous extract (CSAE) in the enhancement of the gastrointestinal transit and the treatment of induced constipation by Loperamide (commercialized as Idium). A significant increase of 15, 24.5 and 32.4% in gastrointestinal motility was recorded when doses of CSAE increased (75, 150 and 300 mg/kg b.w, respectively). Food intake, water consumption, number and weight of stools were also increased in a dose-dependent manner, compared to the positive control (untreated). Moreover, CSAE provided significantly ($p \leq 0.05$) and dose-dependently protection against oxidative stress by preserving normal antioxidant enzymes activities (SOD and GPx) in intestinal and colonic mucosa, and resorted hepatic enzymes (AST and ALT) and renal (urea and creatinine) levels to normal values. These results can be explained by the abundance of phenolic compounds and insoluble fibers in this plant. Therefore, its use can be encouraged in alimentary and pharmaceutical applications as antioxidant and laxative food supplements.

Keywords: Anti-constipation; *Cynara cardunculus* var. *sylvestris*; gastrointestinal motility; oxidative stress; phenolic compounds

ABSTRAK

Penyelidikan ini bertujuan untuk mengkaji aktiviti antioksidan dan anti-sembelit daripada ekstrak fenol yang diperoleh dalam kadun liar rusuk (*Cynara sylvestris*). Ekstrak yang dianalisis (aseton, metanol dan akueus) menunjukkan tahap sebatian fenol yang tinggi dan aktiviti antioksidan yang sangat baik dengan nilai IC_{50} yang ketara ($p \leq 0.05$), seperti yang dinilai oleh aktiviti penangkapan radikal DPPH (0.84±0.08 - 2.44±0.007 µg/mL), ujian radikal kationik ABTS (0.96 ±0.01- 136.67±6.75 µg/mL) dan ujian pelunturan β-karotena (2.09±0.02-51.12±1.32 µg/mL). Juga, paras serat makanan tidak larut yang lebih tinggi didapati (56.18 ± 0.91% DW serat detergen neutral). Penyelidikan *in vivo* telah dijalankan ke atas tikus Wistar untuk mengkaji keupayaan ekstrak akueus *C. sylvestris* (CSAE) dalam peningkatan transit gastro usus dan rawatan sembelit yang disebabkan oleh Loperamide (dikomersialkan sebagai Idium). Peningkatan ketara sebanyak 15, 24.5 dan 32.4% dalam motiliti gastro usus direkodkan apabila dos CSAE meningkat (masing-masing 75, 150 dan 300 mg/kg b.w). Pengambilan makanan, penggunaan air, bilangan dan berat najis juga dinaikkan secara kebergantungan dos, berbanding kawalan positif (tidak dirawat). Tambahan pula CSAE memberikan perlindungan yang ketara ($p \leq 0.05$) dan kebergantungan dos terhadap tekanan oksidatif dengan mengekalkan aktiviti enzim antioksidan normal (SOD dan GPx) dalam usus dan kolon mukosa dan menjadikan tahap enzim hepatik (AST dan ALT) dan buah pinggang (urea dan kreatinin) kepada nilai normal. Keputusan ini boleh dijelaskan oleh banyaknya sebatian fenol dan gentian tidak larut dalam tumbuhan ini. Oleh itu, penggunaannya boleh digalakkan dalam pengaplikasian makanan dan farmaseutikal sebagai makanan tambahan antioksidan dan laksatif.

Kata kunci: Anti-sembelit; *Cynara cardunculus* var. *sylvestris*; motiliti gastro usus; sebatian fenol; tekanan oksidatif

Introduction

Cynara cardunculus L. (Asteraceae) commonly known as 'cardoon' is a herbaceous plant, robust, widely localized in the Mediterranean basin, that is well adapted to difficult habitat conditions such as arid region with high temperatures, high salinity soils and drought (Gominho, Fernandez & Pereira 2000). This species comprises three botanical varieties named *C. cardunculus* var. *scolymus* (L.) Fiori (artichoke), *C. cardunculus* L. var. *altilis* (domestic cardoon) and *C. cardunculus* L. var. s*ylvestris* (Lamk) Fiori (wild cardoon) (Koubaa & Damak 2003). In Tunisia, it is widespread and is used in several traditional dishes and consumed mainly for its fleshy stems and leaf stalks. Whereas its flowers are traditionally used for the preparation of cheese (Ben Ammar et al. 2014). The leaves are known for their therapeutic potential, including diuretic, choleretic, cholagogue, antidiabetic, and antimicrobial agent (Falleh et al. 2008).

Cardoon is a vegetable that contains an important amount of phenolics compounds in its leaves and seeds (Falleh et al. 2008), minerals and vitamins (B, C) (Favier et al. 1995) that were well recognised to have great biological potentials such as antimutagenic, anti-inflammatory, anticancer, and antioxidant (Blanco et al. 2018). One of the disorders 'traditionally' treated with cardoon is constipation. Recently, the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) recognised preparations from *C. cardunclus* leaves as herbal remedies for the symptomatic relief of digestive disorders (EMA 2017). More recently, it has been demonstrated in preclinical studies, in clinical trials and *in vitro* models for artichoke leaf extract, alone or in combination with nutraceuticals the beneficial effects of wild artichoke in protection against oxidative stress conditions and fatty liver disease (Ferro et al. 2022; Rosaria et al. 2023).

Constipation is described as a decrease in the frequency of stools (1-3 defecations per week) associated with their difficulty passing. It is often associated with a feeling of discomfort, cramps and abdominal bloating. Constipation has many different causes, such as diet, physical activity, metabolic problems, fiber deficiency, anorectal problems, hormonal disorders such as hypothyroidism, medication side effects and rarely, heavy metal toxicity (Suares & Ford 2011). Furthermore, constipation affects about one in 10 people worldwide; it is more common in women and the elderly (Costil & Jouët 2017) and different types of laxatives used to combat constipation but have long-term side effects (VIDAL 2019).

Previous studies have reported that constipation is associated with oxidative stress. In fact, children with chronic constipation exhibit oxidative stress and reduced levels of vitamin C, vitamin E, SOD and catalase activities (Zhou et al. 2005). Furthermore, in animal models of Loperamide-induced constipation, the activities of antioxidant enzymes (SOD, GPx, and CAT) were significantly reduced, and MDA and H_2O_2 levels were increased (Jabri et al. 2017). Based on previous findings suggesting a link between oxidative stress and constipation, antioxidants compounds have been considered as new strategies to treat constipation by preventing oxidative reactions. Also, research for natural substances, derived from plants and with biological activity as the synthesized drugs but without side effects, constitutes an important scientific challenge, especially if plants can be obtained from marginal lands and grow under stressed climate conditions. *Cynara cardunculus* is one of the plants that has been widely described in the literature with therapeutic purpose. Its extracts have been used in traditional medicine against several gastrointestinal and digestive disorders (Ben Salem et al. 2022; Porro et al. 2024).

To the best of our knowledge, there is no study dealing with the anti-constipation activity attributed to biochemical compounds of wild cardoon (*C. sylvestris*). Thus, the aim of the present work was to assess the laxative and antioxidant effects of the bioactive compounds determined in ribs wild cardoon (*C. sylvestris*).

Materials and Methods

Plant material

Wild cardoon samples (*Cynara sylvestris*) were collected from the region of Le Kef (semi-arid bioclimate) in the Northwest of Tunisia, and identified by the taxonomy laboratory of the Faculty of Sciences of Tunis. The voucher specimen (ACC27) was deposited at the Herbarium of the Laboratory of Functional Physiology and Valorization of Bio-Resources at the Higher Institute of Biotechnology of Béja. Samples ribs were rinsed with distilled water, cut into small pieces and then blotted on filter paper. After drying at 40 °C in a ventilated oven (memmert UN30, Germany) for 3 days, the ribs were ground using a blender (GRINDOMIX GM200, Germany) and then stored in sterile bottles and protected from light.

DIETARY FIBER DETERMINATION

Insoluble fibers analysis has been evaluated by the determination of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents. These parameters were determined according to Van Soest, Robertson and Lewis (1991) method, using a semi-automatic analyzer (Fibertec 1023, Denmark). The NDF residue was obtained after hydrolysis of the sample with a neutral detergent solution: sodium dodecyl sulphate (SDS) and estimated as the following: %NDF = $((P_1-P_0)/m) \times 100$, where P_0 is the weight of empty crucible (g); P_1 is the weight of crucible with residue after drying (g); and m is the sample dry weight (g).

The ligno-cellulose residue (ADF) was obtained using cetyl-trimethyl-ammonium- bromide (CTAB) in a pH acidified medium using H_2SO_4 from the NDF residue. The ADF content was calculated according to the following relation: %ADF = $((P_2-P_0)/m) \times 100$, where P_0 is the empty crucible weight (g); P_2 is the weight of crucible with residue after the second drying (g); and m is the sample dry weight (g).

The lignin residue (ADL) was estimated after destruction of cellulose by sulfuric acid (72%) from the ADF residue and the content was calculated as the following: %ADL = $((P_3-P_0)/m) \times 100$ where P_0 is the empty crucible weight (g); P_3 is the weight of crucible with residue after the third drying (g) and m is the sample dry weight (g). At the end of the experiment, the same residue was calcined at 550 °C in a muffle furnace (F48000, France) for 5 h until obtaining a white ash; a simple correction was made by subtracting ash weight from each obtained fiber fraction.

PREPARATION AND YIELD OF THE PLANT EXTRACTS

Ribs of *C. sylvestris* were entirely extracted with distilled water, acetone or methanol (80%) at a ratio of 1:10 (w/v) for 48 h under dark conditions. After filtration through a Whatman No. 4 filter paper, the extracts were evaporated using rotary evaporator (DLAB RE100-Pro, Malaisie) and freeze-dried (LABCONCO, USA). In order to obtain maximum yield, the extraction was repeated thrice on the same pomace. Each extract was kept in glass tube and stored in chiller (Brandt, France) at 4 °C in the dark. The yield (%) of evaporated dried extracts was calculated as $DW_{extr}/DW_{samp} \times 100$, where DW_{extr} is the weight of extract after freeze-drying; and DW_{samp} is the dry weight of sample.

PHENOLIC COMPOUNDS ANALYSIS

Total phenolics determination

Polyphenols content was evaluated according to Wood et al. (2002) method, using the Folin-ciocalteu reagent. The absorbance was read at 760 nm using UV-Vis Spectrophotometer (Jenway 7205, UK) against a blank and gallic acid was used as a standard (DO = 0.004C-0.032 ; R^2 = 0.997, concentrations were in the ranges: 0-200 µg/mL). Assay was carried out in triplicate and total polyphenols content in ribs of *C. sylvestris* was determined as mg gallic acid equivalent/g dry weight of the plant material (mg GAE/g DW).

Flavonoids determination

Flavonoids content was determined using previously described method (Tajini, Boualy & Ouerghi 2020). Quercetin was applied as a positive control (DO = 0.004C+0.008; R^2 = 0.986; concentrations were in the ranges: 0-250 µg/mL) and the absorbance was measured at 510 nm using a UV-visible spectrophotometer (Jenway 7205, UK) against a blank. Assay was performed in triplicate and the total flavonoid content in samples was calculated as mg quercetin equivalent/g dry weight of the plant material (mg QE/g DW).

Condensed tannins determination

Condensed tannins content was measured according to Price, Van Scoyoc and Butler (1978) method as previously described by Tajini, Boualy and Ouerghi (2020) and catechin was used as a standard (DO = 0.0033C-0.001; R^2 = 0.998, concentrations were in the ranges: 0-3000 µg/mL), values were expressed as mg catechin equivalent/g dry weight of the plant material (mg CE/g DW).

ANTIOXIDANT ACTIVITIES

DPPH radical-scavenging analysis

The free radical-scavenging assay of *C. sylvestris* ribs was estimated as described by Sarr et al. (2015) method and determined according to the following equation: % DPPH radical-scavenging effect = $[(C_0-C_1)/C_0] \times 100$, where C_0 and C_1 are the concentration values of the (DPPH) solution before and after adding the extract, respectively. C_0 and C_1 were determined according to a calibration curve at 517 nm carried out with ethanolic DPPH solutions. The extract concentration able to inhibit 50% of the radical DPPH (IC_{50}) was calculated by linear

regression of the inhibition percentages. IC_{50} assay was performed in triplicate and gallic acid (GA) was used as a positive control.

ABTS cationic radical test

ABTS radical scavenging activity was evaluated according to Re et al. (1999) method, ascorbic acid was applied as a standard and absorbance was read at 734 nm using a UV-visible spectrophotometer (Jenway 7205, UK) against a control. Results of scavenging activity were calculated as % inhibition using the following equation:

$$\% \text{ Inhibition} = ((A_C - A_E)/A_C) \times 100$$

where A_C is the absorbance of control; and A_E is the absorbance of extract. IC_{50} was calculated by linear regression of the inhibition percentages and the assay was performed in triplicate.

β-carotène bleaching assay

The inhibition potential of β-carotene bleaching was estimated by the β-carotene-linoleic acid test as described by Marco et al. (1968). Absorbance was measured at 470 nm using a UV-visible spectrophotometer (Jenway 7205, UK) and BHT was applied as a reference. Inhibition percentage was calculated as the follow:

$$\% \text{ inhibition} = 1 - [AE_{0min} - AE_{120min}/AC_{0min} - AC_{120min}] \times 100$$

where AE is the absorbance of extract; and AC is the absorbance of control. IC_{50} was calculated by linear regression of the inhibition percentages and the assay was performed in triplicate.

HPLC ANALYSIS OF *C. sylvestris* AQUEOUS EXTRACT (CSAE)

The identification of phenolic compounds was determined by the high-performance liquid chromatography (Agilent 1100 series HPLC system, Germany) coupled to an UV-vis multi-wavelength detector, analysis as described in the previous study by Falleh et al. (2008). Identification was carried out by comparing the retention times and the mass spectra with those of authentic standards. In the present work, only the aqueous extract of *C. sylvestris* ribs was analysed.

ANTI-CONSTIPATION ACTIVITY *IN VIVO*

Experimental animals

Experiments were carried out on adult male Wistar rats (weighing between 200-220 g and 15 weeks old), provided by the SIPHAT breeding center (Ben Arous, Tunis). Animals experimentations were in compliance with the local ethics committee of Tunisian University for the use and care of animals and following the NIH recommendations (NRC) (Comite d'Ethique Bio-medicale (CEBM) (JORT472004)] for the Care and Use of Animals for Scientific Purposes). Wistar rats were housed in polycarbonate cages in animal house under controlled conditions of temperature (22 ± 2 °C), hygrometry (70%), with 12/12 h light-dark cycle. They were fed with standard diet composed of fat: 3%; carbohydrates: 40%; proteins: 14.5%; iron: 50 mg/kg; calcium: 1.45 mg/kg and energy density: 11.40 kj/g, provided from the industrial company (BADR, Utique, Tunisia) and water *ad libitum*.

Gastro-intestinal transit determination

Rats were distributed into five groups with six animals each. After 16 h of fasting time, the treatments were orally administered using a curved gavage needle with gauge: 16; length (inches): 3-4" and 3 mm of ball diameter. The first group (control) received 1 mL of physiological solution (NaCl 0.9%). The second group received the reference molecule yohimbine (2 mg/kg b.w.) to facilitate gastro-intestinal transit (GIT). The others were treated with *C. sylvestris* aqueous extract (CSAE) at three doses (75, 150 and 300 mg/kg b.w.). Two hours later, all groups orally received the standard charcoal meal (10% charcoal and 5% gum Arabic) according to Ali and Bashir (1993) method. Thirty minutes later, the rats were euthanized using CO_2 and their small intestines were carefully removed and gently deposed on a filter paper, then the distance traveled by the charcoal meal from the pylorus was measured. The gastro-intestinal transit was calculated as the following relation:

$$GIT = 100 \times [Dch\ (cm)/\ Lin\ (cm)]$$

where Dch is the distance moved by charcoal and Lin is the length of total intestinal.

Animal treatment and administration technique

Six groups of rats (6 animals each) were used for the long-term constipation treatment, induced by oral

administration of 1 mL of Idium (2 mg/kg b.w.in NaCl (0.9%)) for 7 days at 9 o'clock, a fixed time every day, while the control group received the NaCl (0.9%) solution only. One hour after the treatment with Idium, various doses of CSAE (75, 150 and 300 mg/kg b.w.) or yohimbine (2 mg/kg b.w.) were administered daily during the experiments.

Throughout the experiment, water and food consumption, total number of stools, stool wet and dry weight and faecal water content were recorded. Faecal water content was calculated by the following formula:

$$(\%)\ wc=100 \times [Wm\text{-}Dm/Wm]$$

where Wm and Dm the faecal wet and dry weight, respectively.

After one week, test animals were fasted 16 h prior to the experiment. For euthanasia, the rats were anesthetised by CO_2 inhalation to minimise suffering and were sacrificed by decapitation. The blood was quickly taken in heparinized tubes under ice, then placed in a centrifugal machine (UNIVERSAL 320 R, Hettich, France) at 3000 (rpm) for 15 min at 4 °C. The separated plasma was collected in Eppendorfs and stored at -20 °C for biochemical analyses.

The intestinal and colonic mucosal tissues were scraped and then homogenized in a Tris-NaCl buffer (pH= 7.6). After centrifugation (3000 rpm, 15 min, 4 °C), the supernatant was collected for oxidative stress parameters determination.

Serum analysis

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, serum glucose, triglycerides (TG), and total cholesterol were analysed using the automatic biochemical analyzer SE-LECTRA PRO XL and the corresponding commercial kit (ELI Tech Group Clinical System SAS, Tunisia).

Determination of oxidative stress indicators

Malondialdehyde (MDA) level was estimated, using thiobarbituric acid, according to Draper and Hadley (1990) method, superoxide dismutase (SOD) activity was determined referring to the method described by Misra and Fridovich (1972), glutathione peroxidase (GPx) activity was assessed according to Flohé and Günzler (1984) method, thiol groups (–SH) was analyzed according to the method described by Ellman (1959).

STATISTICAL ANALYSIS

Data were statistically analysed using one-way analysis of variance (ANOVA) and comparison of means was achieved by the Duncan's multiple range test ($p \leq 0.05$), using the SAS software (1997). IC_{50} value was determined by linear regression.

RESULTS AND DISCUSSION

DIETARY FIBER CONTENT

Insoluble dietary fibers analysis was evaluated by determining the levels of NDF, ADF and ADL. Results showed that the studied ribs of *C. sylvestris* were relatively rich in dietary fiber (Table 1). Indeed, the NDF fraction, composed of hemicellulose, cellulose and lignin, has the highest level (56.18 ± 0.91% DW). Also, the fibers extracted by the acid solution (ADF) have a relatively high percentage estimated at 44.71 ± 0.55% DW. Similarly, the ADL fraction that was formed by lignin, seems important with a rate of 12.84 ± 0.82% DW.

These findings showed higher values than those found in cultivated cardoon by Arab, Haddi and Mehennaoui (2009), which were in order of 48.46%, 30.08% and 11.19% for the NDF, ADF and ADL fractions, respectively. These results demonstrated that the *Sylvestris* variety of *C. cardunculus* can be considered as a potential source for insoluble fibers. Indeed, it is well known that fibers help in easing constipation by softening of stool and to prevent from many pathologies such as colon cancer, diabetes and cardiovascular diseases. Also, they reduce the absorption of toxins and bad fats, slow down the assimilation of carbohydrates, help to reinforce the effect barrier of the intestinal mucosa and therefore limit the risk of infection by dangerous germs (Graf et al. 2015; Sanz et al. 2018).

TABLE 1. Insoluble dietary fibers content (%) in ribs of *C. sylvestris*

Insoluble fiber fraction	Content (%)
NDF	56.18± 0.91[a]
ADF	44.71 ± 0.55[b]
ADL	12.84 ± 0.82[c]

Means followed by different letters are significantly different using Duncan's multiple range test at $p \leq 0.05$), data are means ± SD of three replicates. NDF: neutral detergent fibre, ADF: acid detergent fibre and ADL: acid detergent lignin

EXTRACTION YIELD AND CONTENTS OF PHENOLIC COMPOUNDS

In this study, three solvents (methanol, acetone, and water) were used for the extraction of bioactive compounds located in the ribs of *C. sylvestris*. As shown in Table 2, all parameters significantly ($p \leq 0.05$) varied among solvent. Indeed, methanol extract gave the highest yield (18.5 ± 0.92%), followed by the aqueous extract (12.8 ± 0.68%), then, the acetone extract (6.38 ± 0.23%). Also, the highest amounts of polyphenols and flavonoids were found in methanolic extract (97.42 ± 2.5 mg GAE/g DW and 13.56 ± 1.57 mg QE/g DW, respectively). So, contents varied according to phenolic solubility and solvent polarity, which essentially depend on the nature, structure, degree of polymerization and interaction of compounds (Bettaieb et al. 2016; Naczk & Shahidi 2006). However, no significant difference between all extract solvents in condensed tannins contents (Table 2).

Yields of bioactive molecules extraction reported in the present work were similar to those found by Mahmoudi, Khali and Mahmoudi (2013) in artichoke (*C. scolymus*) which are around 16.75% and 14% using methanol and water extraction solvents, respectively. Also, high total polyphenols and flavonoids contents in acetonic,and methanolic extracts were found compared to values reported by Falleh et al. (2008) in the artichoke, (total polyphenols content around 14.79 mg GAE/g DW and flavonoids content varies between 6 and 10 mg QE/g DW in methanolic extract.

Lower contents of condensed tannins were found from the *sylvestris* variety of *C. cardunculus* in acetonic, methanolic and aqueous extracts, compared to those detected by Mahmoudi, Khali and Mahmoudi (2013) in the artichoke (11.97, 8.86, and 7.34 mg CE/g DW with the aqueous, acetonic, and methanolic extract, respectively). These differences can be attributed to some factors such as plant parts, maturity stage and environmental factors including abiotic and biotic stimulants that regulate the biosynthesis of secondary metabolites (Djenidi, Khennouf & Bouaziz 2020).

Three *in vitro* approaches (DPPH, ABTS, and β-carotene) were used to estimate the antioxidant power of ribs extracts from *C. sylvestris* (Table 2). Significant difference between antioxidant effects among extract solvents (*$p \leq 0.05$)* was obtained and the highest activity was observed in the methanolic extract for all antioxidant properties (IC_{50} from 0.84±0.08 to 2.09±0.02 µg/mL). Furthermore, DPPH and ABTS radical-scavenging activities were high in the acetonic extract than in the aqueous, but the opposite was found for *β*-carotene test (Table 2).

These results showed that the IC_{50} were not exceeded 2.5 µg/mL, particularly in methanolic extract for all antioxidant properties, confirming their high activities according to Blois (1958) classification (extract has a strong antioxidant activity if the IC_{50}<50 µg/mL). Furthermore, the DPPH free radical scavenging activity showed a very low IC_{50} in acetonic, methanolic and aqueous extracts compared to those obtained by Khaldi, Khelifi and El Gazzah (2013), who showed a lower DPPH activity in stems of artichoke with IC_{50}=12 µg/mL in methanolic extract. Also, Falleh et al. (2008) found that the IC_{50} in the organs of the artichoke plant ranged from 23 and 118 µg/mL for hydrosols.

On the other hand, all extracts, in this study, have the ability with different potentials to inhibit the *β*-carotene bleaching and stabilize the $ABTS^{\cdot+}$ cationic radical. These results demonstrated that the potent IC_{50} can be assigned to the high amounts of phenolic compounds in ribs of *C. sylvestris*. Thus, a negative significant correlation between phenolic compounds and antioxidant activities was presented in Table 3 ($-0.946 < r < -0.516$; $10^{-4} < p < 0.0466$). Indeed, extracts with richness phenolic compounds exhibit lower IC_{50} values. The DPPH assay was highly correlated with total polyphenols (r = -0.946, $p \leq 0.001$) and flavonoids content (r = -0.795, $p \leq 0.01$), ABTS activity was strongly correlated with polyphenols (r = -0.908, $p \leq 0.001$) and *β*-carotene test was highly correlated with condensed tannins amount (r = -0.809; p $p \leq 0.01$), but weakly correlated with polyphenols (r =-0.516; $p \leq 0.05$).

Previous studies have reported that there is a strong correlation between these partners and phenolic compounds content was the best indicator of antioxidant activity which exert an effect due to their ability to scavenge free radicals and reactive oxygen species (ROS) (Saboonchian, Jamai & Sarghein 2014; Zongo et al. 2023).

COMPOUNDS PROFILE IDENTIFIED IN THE AQUEOUS EXTRACT OF *C. sylvestris*

HPLC analysis of *C. sylvestris* ribs aqueous extract (Figure 1) showed 11 compounds in the identification that were divided into six phenolic acids, four flavonoids and one other phenolic compound (Table 4). Furthermore, phenolic acids exhibited 57.16% of the total extract composition among which the chlorogenic, sinapic and caffeic acids contents were found to be the major compounds with 1.69, 1.66, and 1.53 µ/g extract, respectively. Also, in the flavonoids group, catechin was the main compound with 1.71 µ/g extract. According

to Gamal et al. (2022), genstin was discovered the major phenolic compound of artichoke bracts but Al-Subhi (2020) found that 5-o-caffeoylquinic acid (chlorogenic acid) was the most element in artichoke head. Furthermore, many derivatives of phenolic compounds have been found in both cultivated and wild cardoon especially 1,5-dicaffeoylquinic acid (cynarine) and flavonoids (apigenin and luteolin), which have been reported the main elements responsible for their biologicals activities (Silva, Jacinto & Coutinho 2022).

TABLE 2. Yield, phenolic compounds and antioxydant activities of ribs from *C. sylvestris*

Assay	Acetone extract	Methanol extract	Aqueous extract
Yield(%)	6.38± 0.23^{c}	18.5±0.92^{a}	12.8± 0.68^{b}
Polyphenols (mg GAE/g DW)	61.21 ± 1.87^{b}	97.42 ± 2.50^{a}	14.31±1.52^{c}
Flavonoids (mg QE/g DW)	8.125 ± 0.53^{b}	13.56 ± 1.57^{a}	6.20 ± 0.51^{b}
Tannins (mg CE/g DW)	1.79±0.08^{a}	2.21±0.03^{a}	1.89 ±0.05^{a}
DPPHIC$_{50}$ (µg/mL)	1.12±0.004^{b}	0.84±0.08^{b}	2.44±0.007^{a}
ABTSIC$_{50}$ (µg/mL)	4.40±0.02^{b}	0.96 ±0.01^{c}	136.67±6.75^{a}
β-caroteneIC$_{50}$ (µg/mL)	51.12±1.32^{a}	2.09±0.02^{c}	11.85±0.82^{b}

Data are means ± SD of three replicates, for each parameter and in the same line, means followed by different letters are significantly different using Duncan's multiple range test at $p \leq 0.05$. DW: dry weight, GAE: gallicacid equivalents, QE: quercetin equivalents, CE: catechin equivalents

TABLE 3. Correlation (*r*) analysis between phenolic compounds and antioxidant activities (IC$_{50}$)

Assay	DPPH	ABTS	β-carotene
Polyphenols	-0.946***	-0.908***	-0.516*
Flavonoids	-0.795**	-0.707*	-0.436^{ns}
Tannins	-0.447^{ns}	-0.290^{ns}	-0.809**

ns: not signifificant(p>0.05), $^{*}p \leq 0.05$,$^{**}p \leq 0.01$, $^{***}p \leq 0.001$

TABLE 4. HPLC identification of phenolic compounds from *Cynara sylvestris* ribs aqueous extract

Peak	Compound[a]	Rt[b](min)	Content (µg/g)
1	Gallic acid	5.134	1.17
2	Chlorogenic acid	10.973	1.69
3	Catechin	12.615	1.71
4	Catechol	13.505	1.25
5	Epicatechin	13.722	0.92
6	Syringic acid	14.645	0.13
7	*Caffeic acid*	14.862	1.53
8	Sinapic acid	18.614	1.66
9	Ferulic acid	19.295	1.16
10	Resveratrol	24.251	0.48
11	Quercetin	26.749	1.13
	Total phenolic		12.84

[a]Identification was confirmed using authentic commercial standards, [b]retention time

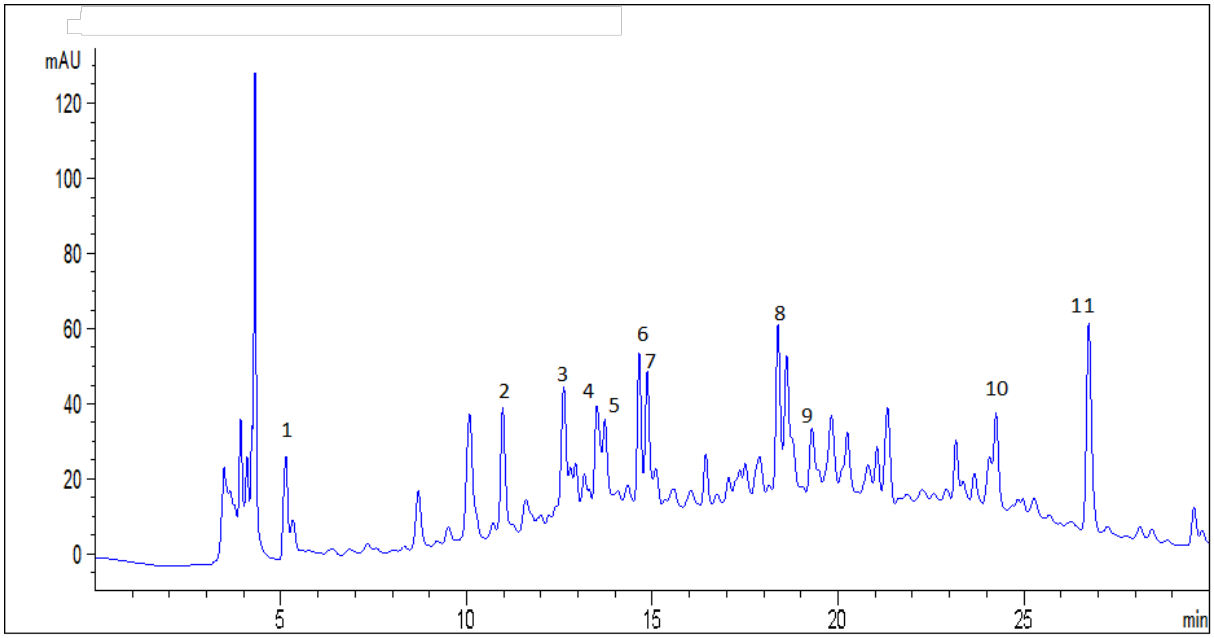

FIGURE 1. HPLC chromatograms for *C. sylvestris* ribs aqueous extract. Peaks numbers corresponding as compounds in Table 4 : 1, Gallic acid, 2, Chlorogenic acid, 3, Catechin,4, Catechol,5, Epicatechin,6, Syringic acid, 7, *Caffeic acid, 8,* Sinapic acid, 9, Ferulic acid, 10, Resveratrol, 11, Quercetin

ANTI-CONSTIPATION AND ANTIOXIDANT ACTIVITIES OF *C. sylvestris* AQUEOUS EXTRACT (CSAE) *IN VIVO*

Effect of CSAE on gastro-intestinal transit

In vivo investigation was carried out first in short-term treatment to verify the effect of the CSAE on gastrointestinal transit (GIT). Thus, GIT was determined on Wistar rats receiving increasing doses of CSAE (75, 150 and 300 mg/kg, b.w.) and compared to negative control (receiving NaCl solution).

Based on the charcoal meal assay, results showed that CSAE was associated with high values of GIT in a dose-dependant manner and a faster intestinal transit of charcoal meal was noted. A comparable GIT was noted in animal receiving yohimbine drug (2 mg/kg b.w.), used as a positive control molecule with anti-constipation effect (Figure 2). Similar results were observed with *Malva sylvestris* (Jabri et al. 2017) and *Thymus vulgaris* (Rtibi et al. 2019) in animal models.

Effect of CSAE on food, water intakes and fecal parameters among the constipated animals

As indicated in Table 5, the treatment of rats with Idium (used as a constipation inducer) caused a significant ($p \leq 0.05$) decrease in food intake (48.33±2.16 vs 101.66±4.08 g/day/rat) and in water consumption (20.4 ±2.77 vs 40.2 ±3.24 mL/day/rat), compared to the control group. Also, the defecation test showed that, in constipated rats, the number of stools was decreased (39.16±1.47 vs 52.5±5.04 n/24h/rat). This reduction was accompanied by a drop in stool wet weights (3.88±0.36 vs 8.57±0.75 g in control) and associated with a decrease in water content (20.10±0.74%). However, treatment with CSAE at various doses (75, 150 and 300 mg/kg, b.w.) or the reference molecule (yohimbine) has increased all these parameters. So, *C. sylvestris* aqueous extract improved fecal parameters and had offered a significant laxative effect of stools, the best treatment was observed with CSAE-300 mg/kg, b.w. (Table 5).

Idium is a drug used in gastroenterohepatic treatments as an antidiarrheal. Its active molecule is Loperamide which acts by slowing of the motricity of the digestive tract; binds to morphine receptors of the intestinal wall and modifies the nervous functions of the intestine which leads to a reduction in the quantity of stools, makes them more solid and slows down the frequency of stools (Rtibi et al. 2019).

These results indicate that the regulation of intestinal transit may be related to the high content of wild cardoon ribs in insoluble dietary fiber. Furthermore, it has been demonstrated that these later stimulate the intestinal transit, reduce the fermentation time of the

intestinal contents in the colon by accumulation of water and stimulation of peristalsis, prevent constipation by promoting regular functioning of the intestine and ensure health of the digestive system (Baribeau 2014; Bonithon-Kopp et al. 2001). Moreover, improvement of gastric motility after treatment with CSAE can be attributed to the effect of phenolic compounds which were largely detected in the studied extract. In fact, polyphenols have been reported to enhance intestinal transit and gastric emptying by stimulating of their contractile function (Jabri et al. 2017). The same result was observed with ferulic acid (phenolic compound) by Badary et al. (2006) in animal models. Furthermore, several tannin-containing plant extracts have been reported to have laxative effects due to their ability to increase the number and weight of stool (Kim et al. 2019). Based on previous findings, this improvement may be due to synergistic effect of phenolic compounds firstly, and in common with the insoluble dietary fiber on the other hand.

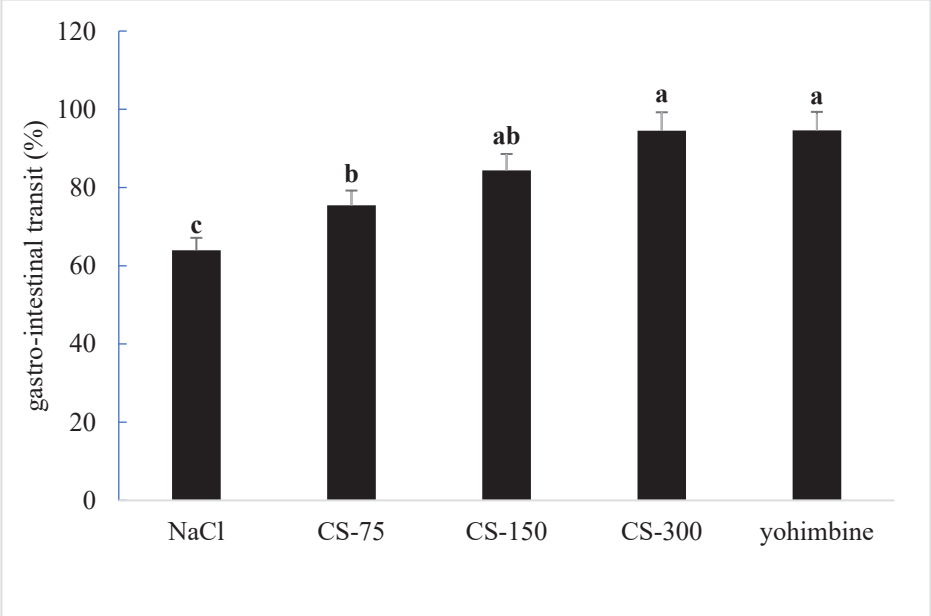

FIGURE 2. Effect of *C. sylvestris* aqueous extract (CSAE) with different doses (75, 150 and 300 mg/kg, b.w.) and yohimbine (2 mg/kg, b.w.) on gastrointestinal transit (GIT). Data are means ± SD of 6 replicates, means followed by different letters are significantly different tusing Duncan's multiple range test at $p \leq 0.05$

TABLE 5. Effect of *C. sylvestris* aqueous extract (CSAE) with different doses (75, 150 and 300 mg/kg, b.w.) and yohimbine (2 mg/kg, b.w.) on food intake, water consumption and stool parameters after Idium (2 mg/kg b.w.) treatment

Group	FI (g/day)	WI (mL/day)	NF (n/24h)	FWW (g)	FDW (g)	FWC (%)
Control	101.66±4.08^{a}	40.2 ±3.24^{a}	52.5 ±5.04^{a}	8.57±0.75^{a}	4.47± 0.31^{a}	47.84±0.52^{a}
Idium	48.33±2.16^{d}	20.4 ±2.77^{b}	39.16±1.47^{c}	3.88±0.36^{c}	3.1±0.28^{b}	20.10±0.74^{c}
Idium+ CSAE-75	73.33±4.08^{c}	25.11±3.04^{b}	47.20±3.97^{b}	4.91±0.85^{c}	3.18±0.17^{b}	35.23±0.43^{b}
Idium+CSAE-150	76.66±5.16^{c}	36.29±2.64^{a}	51.30± 1.75^{a}	6.20±0.81^{b}	3.88±0.24^{a}	37.41±0.51^{b}
Idium+ CSAE-300	87.50 ±2.78^{b}	40.64 ±3.4^{a}	50.83±4.6^{a}	6.84±0.34^{b}	3.99±0.38^{a}	41.66±0.21^{ab}
Idium + Yohimbine	90.33±4.08^{ab}	40.6 ± 2.45^{a}	51.33±4.3^{a}	8.42± 0.85^{a}	4.18 ±0.39^{a}	50.35±0.34^{a}

Data are means ± SD of 6 replicates, in each column, means followed by different letters are significantly different using Duncan's multiple range test ($p \leq 0.05$). FI: Food intake (g/day), WI: Water intake (mL/day), NF: Number of faeces, FWW: Fecal wet weight, FDW: Fecal dry weight, FWC: Fecal water content

Effect of CSAE on serum biochemical and metabolic parameters among the constipated rats

Analysis of metabolic parameters (Table 6) showed that Idium caused remarkable renal disorders. As showed by an increase ($p \leq 0.05$) in the level of creatinine (57±2.01 vs 36.46±0.9 µmol/L) and decrease ($p \leq 0.05$) in urea (4.79±0.3 vs 6.61±0.36 mmol/L) compared to the control. Also, decrease ($p \leq 0.05$) in the activities of AST (25.64±2.67 vs 37.33±4.93) and ALT (42.34±2.01 vs 46.93±2.25) were noted, as compared to the normal rats. Of particular, the co-treatment of animals with CSAE at various doses (75, 150 and 300 mg/kg, b.w.) protected the animals against these disturbances. For the other biochemical parameters (glucose, triglycerides, and total cholesterol), no significant change was recorded following the Idium-induced constipation treatment (Table 6).

Effect of CSAE on oxidative stress parameters among the constipated rats

As shown in Figure 3(A), increased ($p \leq 0.05$) MDA levels were obtained after Idium treatment in the intestinal and colonic mucosa, as compared to the control group. However, CSAE treated groups at various doses (75, 150, 300 mg/kg, b.w.) for one week showed significant ($p \leq 0.05$) and dose-dependent protection effect by reducing the MDA levels from both organs, in the CSAE treated animals.

Also, Idium-treated animals showed significant decrease ($p \leq 0.05$) in thiol groups levels in the intestinal (0.62± 0.06 nmoles/mg of proteins) and colonic mucosa (0.75±0.05 nmoles/mg of proteins), compared to the control group (0.98±0.02 and 1.03±0.05 nmoles/mg of proteins, respectively). The addition of the CSAE at increasing doses (75, 150, 300 mg/kg, b.w.) or yohimbine, has significantly ($p \leq 0.05$) corrected the reduction induced by Idium (Figure 3(B)). In colon mucosa, no significant difference ($p > 0.05$) between CSAE doses was noted, but the highest level of thiol groups (1.01±0.014 nmoles/mg of proteins) was observed in small intestine with the treatment of CSAE-300 mg/kg,b.w.

On the other hand, the intestinal and colonic activities of antioxidant enzymes (SOD and GPx) have been significantly ($p \leq 0.05$) reduced in the Idium group, compared to the normal rats (Figure 3(C) & 3(D)). Contrary, the co-treatment with CSAE at various doses (75, 150, 300 mg/kg, b.w.) or yohimbine, provided a significant and dose-dependent protection against the depletion of these enzymes caused by Idium. With CSAE-300 mg/kg, b.w., intestinal activities of SOD and GPx were improved significantly *(*$p \leq 0.05$) twice and three times respectively, compared to the Idium group. These values were similar and even better than those observed in the yohimbine treated group.

TABLE 6. Effect of *C. sylvestris* aqueous extract (CSAE) with different doses (75, 150 and 300 mg/kg, b.w.) and yohimbine (2 mg/kg, b.w.) on serum biochemical and metabolic parameters after Idium (2 mg/kg b.w.) treatment

Group	Creatinine (µmol/L)	Urea (mmol/L)	AST (UI/L)	ALT (UI/L)	Glucose (mmol/L)	Triglycerides (mmol/L)	Total Cholesterol (mmol/L)
Control	36.46±0.90^{c}	6.61±0.36^{a}	37.33±4.93^{a}	46.93±2.25^{ab}	5.48±0.35^{a}	1.14±0.04^{a}	1.14±0.06^{a}
Idium	57.00±2.01^{a}	4.79±0.30^{b}	25.64±2.67^{b}	42.34±2.01^{b}	4.84±0.10^{a}	0.98±0.01^{a}	1.36±0.10^{a}
Idium+CSAE-75	39.33±2.90^{b}	6.55±0.53^{a}	37.33±3.21^{a}	46.01±1.58^{ab}	5.82±0.76^{a}	1.32±0.17^{a}	1.32±0.17^{a}
Idium+CSAE-150	42.33±5.80^{b}	7.00±0.15^{a}	38.66±6.02^{a}	49.11±2.64^{a}	5.53±0.66^{a}	1.45±0.02^{a}	1.45±0.02^{a}
Idium+CSAE-300	42.66±4.50^{b}	6.60±0.92^{a}	36.66±3.21^{a}	49.66±5.03^{a}	4.80±0.30^{a}	1.44±0.10^{a}	1.44±1.44^{a}
Idium + Yohimbine	45.00±2.12^{b}	6.38±0.30^{a}	37.53±2.70^{a}	45.61±2.31^{ab}	5.80±0.23^{a}	0.91±0.10^{a}	1.27±0.03^{a}

Data are means ± SD of 6 replicates. For each parameter, means followed by different letters are significantly different using Duncan's multiple range test ($p \leq 0.05$)

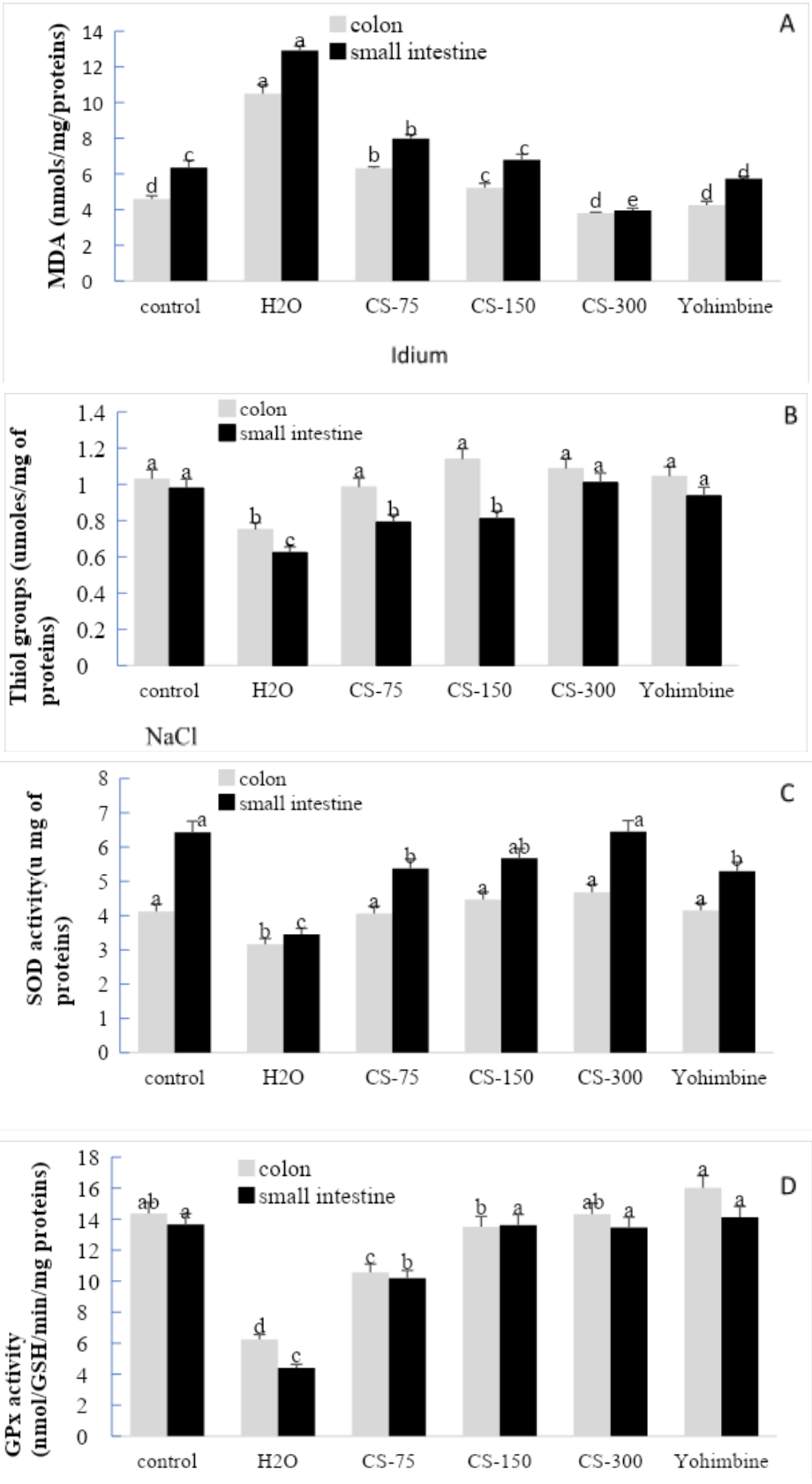

FIGURE 3. Effect of *C. sylvestris* aqueous extract (CSAE) with different doses (75, 150 and 300 mg/kg, b.w.) and yohimbine (2 mg/kg, b.w.) on colon and small intestine MDA (A), thiol groups (B) and antioxidant enzymes activities (SOD (C) and GPx (D**)**) after Idium (2 mg/kg b.w.) treatment. Data are means ± SD of 6 replicates. For each organ, means followed by different letters are significantly different using Duncan's multiple range test ($p \leq 0.05$)

The findings of this study showed that the Idium-induced constipation was accompanied by oxidative stress status established in the intestinal and colonic mucosa as shown by an increase in the MDA level, decrease in the thiol groups, as well as the reduced activities of antioxidant enzymes SOD and GPx. Similar results were found by Olofinsan, Rafiat and Ajiboye (2019) for neurotoxicity in rats treated by Loperamide. The antioxidant potential exerted by the CSAE mainly related to the abundant phenolic compounds in wild cardoon plant studied, such as flavonoids (catechin, catechol, and quercetin) and phenolic acids (chlorogenic, caffeic, and sinapic acids) (Table 4). Indeed, it has been reported that the antioxidant action of phytocompounds is not only exerted by the neutralization of free radicals, but it is also manifested by the inhibition of oxidizing enzymes and by the chelation of traces of metal ions involved in the production of ROS (Kurutas 2016). Also, Yahfoufi et al. (2018), found that the tannins are endowed with antioxidant power, hydrolysable tannins inhibit lipid peroxidation and condensed tannins inhibit the formation of superoxides. Moreover, Kim et al. (2021) found that tannin-enriched extracts of *Ecklonia cava* (brown algae) are potential therapeutic candidates for the treatment of constipation by stimulating oxidative stress modulation and muscarinic cholinergic regulation when exerting its laxative effects in chronic constipation.

In this study, wild cardoon has demonstrated an interesting antioxidant and anti-constipation activities, but some additional studies are needed to elucidate the molecular mechanisms involved in the laxative effects and antioxidant activities of this natural product.

Conclusion

Results in this study showed a significant correlation between phenolic compounds and antioxidant activities *in vitro*, also an interesting anti-constipation and inhibitory effect of oxidative stress *in vivo*. This suggests that *C. sylvestris* is a potential source of various bioactive molecules and natural antioxidants, which can appear as an alternative to synthetic antioxidants for the protection against oxidative stress. The anti-constipation activity may be related to the richness of *C. sylvestris* in part of dietary fibers and secondly of bioactive molecules, which protect with a significant and dose-dependent manner against the alterations induced by Idium. These findings and results affirmed the interesting phytochemical composition with laxative action and antioxidant protection from this variety of cardoon and could encourage its use in several applications such as food and pharmaceutical fields.

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