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Polyphenols Content and Antioxidant Activities of Selected Algerian Plants Used for Gastro-duodenal Ulcers

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ABSTRACT

This study aimed to evaluate the antioxidant potentials of aqueous extracts of four plants: Aloe ferox Miller, Zizyphus vulgaris lamk, Lawsonia inermis L. and Centaurium erythraea L. which are widely used in Algerian folk medicine for the treatment of gastroduodenal ulcers. Total phenolic, total flavonoid and tannins contents in the aqueous extracts were estimated. In vitro antioxidant activities were assessed by DPPH• radical scavenging assay, ferric reducing power activity, ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. The in vivo antioxidant activities of these plants extracts were assessed using plasma antioxidant capacity, total proteins level, catalase (CAT), glutathione (GSH) and malondialdehyde (MDA) levels. Our results suggested that these plants aqueous extracts could be good sources of phenolic compounds and an ingredient with high antioxidant potential, and explain the traditional use of these plants in phytotheray.

Keywords: *Aloe ferox* Miller, *Zizyphus vulgaris* lamk**,** *Lawsonia inermis* L., *Centaurium erythrae* L., polyphenols, antioxidant, gastric ulcer.

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INTRODUCTION

Oxidative stress is an imbalance between protective systems and the production of free radicals [1]. The excess of reactive species can damage cell lipids, proteins and DNA by oxidative action, which might result in loss of function and even cellular death [2] leading to many diseases [3] such as neurodegenerative disorders, inflammation viral infections, autoimmune pathologies, and digestive system disorders such gastrointestinal inflammation and gastric ulcer [4].

There is evidence concerning the participation of reactive oxygen species (ROS) in the etiology and physiopathologie of gastric and duodenal ulcers. The generation of ROS that seem to play an important role, namely due to generation of lipid peroxides, accompanied by impairment of antioxidative enzyme activity of cells. Several data show that exist an increased of malondialdehyde (MDA), accompanied by a decrease of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) levels [5-6-7].

The organisms use endogenous and exogenous antioxidant defenses to protect against harms of oxygen and nitrogen reactive species. They are classified in enzymatic: glutathione peroxidase (GPx), CAT and SOD; and non-enzymatic systems: reduced thiol (GSH), vitamins, minerals and polyphenols [8], which could also attenuate oxidative damage of a tissue indirectly by enhancing natural defenses of cell and/or directly by scavenging the free radical species [9]. The role of phytochemicals in the oxidative damage combat it has been very well studied [2]. Phenolic compounds are the principal class of dietary phytochemical, naturally present in plants, especially fruits, and have important

antioxidant potential [11-12], wich make them a good candidates to prevent or to treat oxidative stress related diseases as gastrodudenal ulcers.

Aloe ferox Miller (Aloaceae); *Zizyphus vulgaris* lamk (Rhamnaceae**);** *Lawsonia inermis* L. (Lythraceae**);** *Centaurium erythraea* L. (Gentianaceae**)** are widely used as medicinal plants in Algerian folk medicine for treatment of gastroduodenal ulcers.

To the best of our known, there are no reports about the phenolic content and antioxidant activity of these plants. So, the present study was carried out to estimate the total polyphenols, flavonoids and tannins contents; as well to evaluate the *in vitro* and *in vivo* antioxidant activities of the aqueous extracts of these plants.

MATERIALS AND METHODS

Plant material and preparation of aqueous extracts

The leaves of *Aloe ferox* Miller; *Zizyphus vulgaris* lamk; *Lawsonia inermis* L.and the aerial parts (leaves and flowers) of *Centaurium erythraea* L. were supplied by traditional medicine vendors. A voucher specimen was identified by Pr. Hocine Laouar from the laboratory of Botany, Faculty of Natural and Life Sciences, University Setif 1, Algeria. The plant samples were air dried at room temperature and then finely ground to a fine homogeneous powder

For preparation of the decoction, 100 g of plant powder were added into 1 L of distilled water. The suspension was boiled at 100 °C under constant shaking with magnetic stirrer for 10 min. At the end of the extraction, it was filtered through nylon mesh followed by Whatman filter paper No. 1 (Whatman Ltd., Germany). The filtrate was then concentrated under reduced pressure on rotary evaporator at 40 °C and the extract obtained was kept frozen (-18 °C) until further use.

Eexperimental design

Healthy male adult albino rats, weighing 150 –200 g were used. Animals were housed in cages with free access to food and water in an air-conditioned animal room, with 12 h/12 h light/dark photoperiod) for 1 week prior to experiment. After adaptation, the rats were randomly divided into 10 groups (six animals per group) and the treatment was given everyday via orogastric tube for 14 days as the following:

Group 1: control group was gavaged with normal saline (0.9%).
Group 2: received vitamin C (150 mg/kg) and served as the standard drug.
Groups 3 and 4: received aqueous extract of *A. ferox* at the dose of 150 and 500 mg/kg, respectively.
Groups 5 and 6: were treated with aqueous extract of *Z. vulgaris* at the dose of 150 and 500 mg/kg, respectively.
Groups 7 and 8: ware treated with aqueous extract of *L. inermis a*t the dose of 150 and 500 mg/kg, respectively.
Groups 9 and 10: ware given aqueous extract of *C. erythraea* at the dose of 150 and 500 mg/kg, respectively.

All administration solutions were given at the dose of 5 ml/kg. At the end of 15 days, 24 h of the last treatment, all animals were sacrificed. Blood freshly collected in heparinized tubes was centrifuged (3000 rpm/min for 15 min), and the supernatant (plasma) was used for determination of antioxidant capacity. The liver was removed and washed with ice-cold saline, blotted with filter paper and kept in plastic vials at -20°C until use.

Liver tissue was cut down into small pieces, placed in KCl buffer, and homogenized using dounce homogenizer in ice-cold condition to obtain 10 % homogenate. The homogenate thus obtained was centrifuged at 4000 rpm for 15 min and the supernatant collected was used for the determination of biomarkers of oxidative stress.

Determination of total polyphenols content

The total phenolic content of the aqueous extracts was determined according to Folin–Ciocalteu method [13], with some modifications. In a vial, 0.1ml of each extract was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 10 times) and incubated at room temperature for 4 min. Then, 0.4 ml of 7.5% sodium carbonate solution was added and further incubated for 90 min at room temperature. The absorbance of all samples was measured at 760 nm and the results are expressed in milligrams of gallic acid equivalents per gram dried weight (mg GAE/g DW).

Determination of total flavonoids content

Measurement of flavonoid concentration in different aqueous extracts was based on the method described by Bahorun et *al.*[14]. Each sample (1 ml) was added to1 ml of aluminum chloride ($AlCl_3$) solution (2%) and allowed to stand for 10 at room temperature. The absorbance of the mixture was determined at 430 nm against the same

mixture without the sample as a blank. Total flavonoid content was expressed as quercetin equivalent per gram dried weight (mg QE /g DW).

Determination of tannins content

The capacity to precipitate hemoglobin was determined by using bovine fresh blood according to the method described by Bate smith [15]. Briefly, a volume of each plant extract was mixed with an equal volume of hemolysed bovine blood (absorbance = 1.6). After 20 min, the mixture was centrifuged at 4000 rpm for 10 min, and the absorbance of the supernatant was measured at 756 nm. Results were expressed as mg equivalent tannic acid per gram dried weight (mg TAE/g DW).

Antioxidant activity of plant extracts: *in vitro* assays

DPPH radical scavenging assay

The DPPH radical scavenging activity was determined according to the method described by Burits and Bucar [16]. 50 µl of different dilutions of the extracts were added to 5 ml of a 0.004 % methanolic solution of DPPH. The mixture was kept at room temperature for 30 min before measuring its absorbance at 517 nm. BHT was used as a reference standard. Radical scavenging activity was calculated in percent (I %) as followed:

$$I\% = 100 (A \text{ control} – A \text{ sample}) / A \text{ control}$$

Where A control is the absorbance of the blank solution (containing all reagents except the test compound), and A sample is the absorbance in the presence of the test compound. Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration.

Reducing power

The reducing power was measured using Chung et *al*. method [17]. An aliquot of each sample or BHT (0.1ml) was mixed with 0.1ml of Phosphate buffer (200mM, pH6.6) followed 0.1ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. After incubation in water bath at 50°C for 20 min, 0.25ml of 10% trichloroacetic acid was added into the mixture, and then followed by centrifugation at 3000 rpm for 10 min. Then the resultant supernatant (0.25 ml) was mixed with 0.25 ml of distilled water and 0.5 ml of 0.1 % ferric chloride ($FeCl_3$), and the absorbance was measured at 700 nm against a blank. Increased absorbance of the reaction mixture indicated increased reducing power.

Ferric thiocyanate (FTC) assay

The antioxidant activity of the plant extracts on inhibition of lipid peroxidation was determined according to the ferric thiocyanate method as reported by Yen et *al.* [18]. A sample solution (0.5 ml) of plant fractions was mixed with 2.5 ml of 0.02M linoleic acid emulsion at pH 7.0 and 2 ml of 0.2 M phosphate buffer at pH 7.0. The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 ml of phosphate buffer. The reaction mixture was incubated at 37 °C for 5 days. To 0.1 ml of the reaction mixture at 24 h intervals was added 75% EtOH (4.7 ml), 30% ammonium thiocyanate (0.1 ml), 0.02 M ferrous chloride in 3.5% HCl (0.1 ml). Three minutes after the addition of ferrous chloride to the reaction mixture, the absorbance of red color was measured at 500 nm. Each 24 h until one day after absorbance of the control (without sample) reached maximum. BHT was used as standard. % Inhibition of lipid peroxidation is calculated by equation: Inhibition (%) = Ac – As / Ac × 100

Where, As is the absorbance of the sample on the day when the absorbance of the control is maximum and Ac is the absorbance of the control on the day when the absorbance of the control is maximum.

Thiobarbituric Acid (TBA) assay

The TBA test was conducted on the final day of FTC according to the method described by Kikuzaki and Nakatani [19] to determine the malonaldehyde (MDA) formation from linoleic acid peroxidation. The same sample preparation method as described in the FTC method was used. To 1 ml of sample solution, 20% trichloroacetic acid (2 ml) and thiobarbituric acid solution (2 ml) were added. The mixture was placed in a boiling water bath for 10 minutes. After cooling, it was then centrifuged at 3000 rpm for 20 minutes. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on the absorbance of the final day of the FTC assay. Percent inhibition was calculated according to the same equation as that in FTC method.

Antioxidant activity of plant extracts: *in vivo* assays

Effect of extracts on plasma antioxidant capacity using DPPH radical

In this test, the capacity of plasma to trap the DPPH radical was illustrated by to the method of Burits and Bucar [16] with some modifications, while being based on the same principle as that of the test of DPPH carried out previously *in vitro*. Briefly, a volume of plasma was added to DPPH methanolic solution (0.004%). After 30 min of incubation

in the darkness followed by a centrifugation, the absorbance at 517 nm is measured and the plasmatic antioxidant capacity was then calculated.

Effect of extracts on plasma reducing power

The reducing power was determined according to the method of Chung *et al.* [17]. In brief, plasma (0.1 ml) was mixed with 0.1 ml of 0.2 M sodium phosphate buffer (pH 6.6 and 0.1 ml of 1 % potassium ferricyanide. The mixture was then incubated for 20 min at 50°C. After that, 0.250 ml of 1% trichloroacetic were added, the mixture was then centrifuged for 10 min at 3000 rpm. The aliquot (0.250 ml) of the upper layer was mixed with 0.250 ml of distilled water and 0.5 ml of ferric chloride (0.1%), and the absorbance at 700 nm was measured. A higher absorbance indicated a higher reducing power.

Assessment of total protein level

Tissue protein concentration was assayed according to Gornall *et al*. [20] using the Biuret reagent and bovine serum albumin as a standard. In brief, 1 ml biuret reagent was mixed with 25 µl sample or standard (albumin), and the absorbance (540 nm) was measured after 10 min of incubation at 37 °C. Total protein level was calculated through formula:

Total protein (mg/ml) = (Abs of sample / Abs of standard) × n
Where n is standard concentration.

Assessment of Catalase activity

Catalase activity was determined using the method of Clairborne [21]. 50 µl of each tissue supernatant was added to 2950 µl of 19 mM H_2O_2 prepared in 0.1M phosphate buffer (pH 7.4). Catalase activity was measured at 240 nm for 2 min using UV-visible spectrophotometer. One unit of catalase activity is equal to 1 mmol of H_2O_2 degraded per minute at 25°. Then, the catalase activity was expressed as units per gram of tissue according to this formula:

U/g tissue = (2.3033/ T) × (log A1/A2) /g tissue
A1: Absorbance at t0
A2: Absorbance at t1.
T: Interval of time (minute).

Assessment of reduced glutathione concentration

Reduced glutathione (GSH) concentration was determined according the method illustrated by Ellman [22]. Briefly, 50 µl of supernatant were diluted in 10 ml phosphate buffer (0.1 M, pH 8). Then, to 3 ml of the mixture of dilution, 20µl of DTNB (0.01 M) were added. Absorbance is measured at 412 nm against blank after 5 min. Absorbance values were calculted using a standard curve of GSH. Reduced glutathione was expressed as µmol/ g tissue.

Assessment of lipid peroxidation

This method was described by Okhawa et *al.*[23]. Tissue homogenate (0.5 mL), 0.5 ml of TCA (20 % w/v) and 1ml of TBA (0.67 % w/v) were mixed. The tubes were boiled in water bath for 15 min and then they were cooled. After that, 4 ml of n-butanol were added to each sample and centrifuged at 3000 rpm for 15 min. The absorbance was read at 532 nm against an appropriate blank without the sample. The concentration of Malondialdehyde (MDA) was determined from a standard curve of 1, 1, 3, 3 tetraethoxypropane in the same conditions and it was expressed as n mol/ g tissue.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism (version 5.01 for Windows). *In vitro* results were expressed as mean ± standard deviation (SD) and were analyzed by one way analysis of variance (ANOVA) followed by Dunnet's test. The pharmacological results were presented as mean ± standard error of mean (S.E.M.) of six experiments. In all cases, The *P*-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Total polyphenols, flavonoids and tannins contents in plants extracts

Polyphenolic compounds clearly improves the status of different oxidative stress biomarkers [24].The biological mechanisms of these possible effects have been attributed to their antioxidant properties through several possible mechanisms, such as their ability to scavenge free radicals, break radical chain reactions, directly reducing peroxides, and stimulating the antioxidative defense enzyme activities [25]. In this study, total phenolic content was estimated by using Folin- Ciocalteu reagent and the results were summarized in Table 1. Total phenolic compounds in the aqueous extracts varied widely and ranged from 49.629 ± 0.279 to 169.407 ± 0.339 mg GAE/g extract. Also,

the *A. ferox* extract exhibited the highest total phenolic content. The contents of flavonoids were quantified using $AlCl_3$ method and results showed that *Z. vulgaris* extract had the highest amount of flavonoid content followed by *C. erythraea* extract, *L. inermis* extract and *A. ferox* extract (table 1).

The quantification of tannins contents showed that *L. inermis* extract contained the highest tannins concentration with the value of 168.4 ± 0.692 mg TAE /g extract. The lowest tannins content was noticed for *Z. vulgaris* extract with value of 71.933 ± 0.808 mg TAE /g extract.

Table 1 : Total polyphenols, flavonoids and tannins contents in plants extracts

Extracts	Polyphenols[(a)]	Flavonoïds [(b)]	Tanins[(c)]
A. ferox	169.407 ± 0.339	0.055 ± 0.0007	98.933 ± 0.115
Z. vulgaris	96.962 ± 0.169	0.409 ± 0.002	71.933 ± 0.808
L. inermis	79.222 ± 0.333	0.073 ± 0.002	168.4 ± 0.692
C. erythraea	49.629 ± 0.279	0.159 ± 0.001	82.933 ± 0.503

(a) mg Gallic acid Equivalent /g of dry extract ; (b) mg Quercetin Equivalent / g of dry extract ; (c) mg Tannic acid Equivalent /g of dry extract ; Results are expressed as means ±SD (n = 3).

Antioxidant activity of extracts: *in vitro* assays

Various methods are used to investigate the antioxidant property of samples *in vitro*. These methods included DPPH• radical scavenging assay, ferric reducing power activity, ferric thiocyanate method and thiobarbituric acid method [26].

DPPH radical scavenging activity

The DPPH radical scavenging method has been widely used as an easy and accurate test for evaluating reducing substances in biological systems [27-28-29]. DPPH radical is considered to be a model of lipophilic radical, and chains of lipophilic radicals are initiated by lipid autoxidation [30-31]. These radicals react with hydrogen donors such as phenolic components to form stable diamagnetic molecules and induce color fading of assay solutions [30-32]. The antioxidant activities obtained by the DPPH method for the aqueous extracts are displayed in Figure1. This activity was compared with BHT as a synthetic antioxidant. Figure 1 revealed that *A. ferox* extract exhibited a strongest antioxidant activity (IC_{50} = 0.0334 ± 0.0001mg/ml), which is comparable to the standard BHT. The other extracts also showed good DPPH radical-scavenging activities in the order of *A. ferox* extract > *L. inermis* extract > *Z. vulgaris* extract > *C. erythraea* extract. It was found that no significant difference ($p > 0.05$) in the antioxidant activity between *A. ferox* extract and BHT as standard, while *L. inermis* extract, *Z. vulgaris* extract and *C. erythraea* extract showed a significant difference ($P < 0.001$). Indeed, our results are in agreement with those obtained by Bouaziz et *al.* [24], who reported a clear synergistic effect among different phenolic compounds in scavenging free radicals.

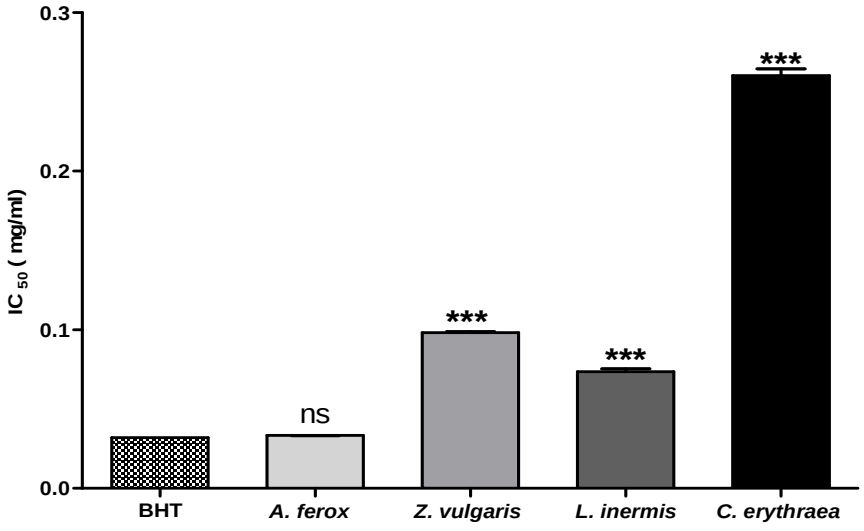

Figure 1: A comparison between different plants extracts in DPPH free radical scavenging activity. Data were presented as IC50 means ± SD (n = 3). (ns: no significant difference; *** p < 0.001) compared to BHT as standard

Reducing power capacity

The reducing capacity of the extracts, another significant indicator of antioxidant activity. It is attributed to reductones (enols), which have hydrogen-donating ability, resulting in potent antioxidant activities in test samples

[30-33]. In addition, antioxidant activities have been reported to be directly correlated with reducing power abilities in certain plant-based compounds [34]. As shown in Figure 2, there was significant difference ($p < 0.001$) among the different extracts in reducing power compared to BHT as positive control (IC_{50} = 0.0466 ± 0.0006 mg/ml). The *A. ferox* extract (0.1007 ± 0.0006 mg/ml) showed the better reducing power than other extracts followed by *L. inermis* extract (0.2087 ± 0.0025 mg/ml), *Z. vulgaris* extract (0.2632 ± 0.0012 mg/ml) and *C. erythraea* (0.4918 ±0.0017 mg/ml). Many stadies have found highly antioxidant activity of phenolic compounds is mainly attributed to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [35].

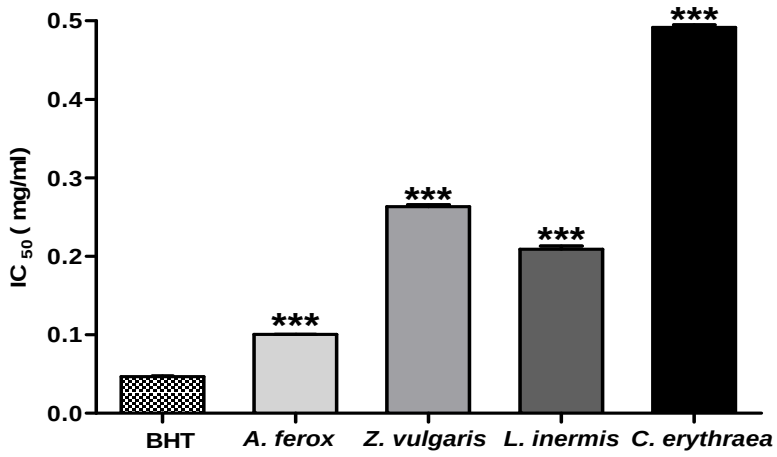

Figure 2: A comparison between different plants extracts in reducing power assay. Data were presented as IC_{50} means ± SD (n = 3). (* p < 0.001) compared to vitamin C as standard**

Ferric thiocyanate (FTC) assay

In this study, the FTC method was used to measure the peroxide levels during the initial stage of linoleic acid oxidation. Peroxide reacts with ferrous chloride to form reddish ferrous chloride and this reduction is due to increased level of unstable melonaldehyde compound from the linoleic acid oxidation. The concentration of peroxide decreases as the antioxidant activity increases [36]. As illustrarated in Figure 3, the percent inhibitions of plants extracts decreased in the order of *C. erythraea* extract (59.41± 0.341%), *Z. vulgaris* extract (36.16 ± 0.784%), *L. inermis* extract (13.30 ± 0.304%) and *A. ferox* extract (8.577 ± 0.351%), respectively. There was significant difference ($p < 0.001$) among the different extracts compared to BHT as positive control (80.07 ± 0.097%).

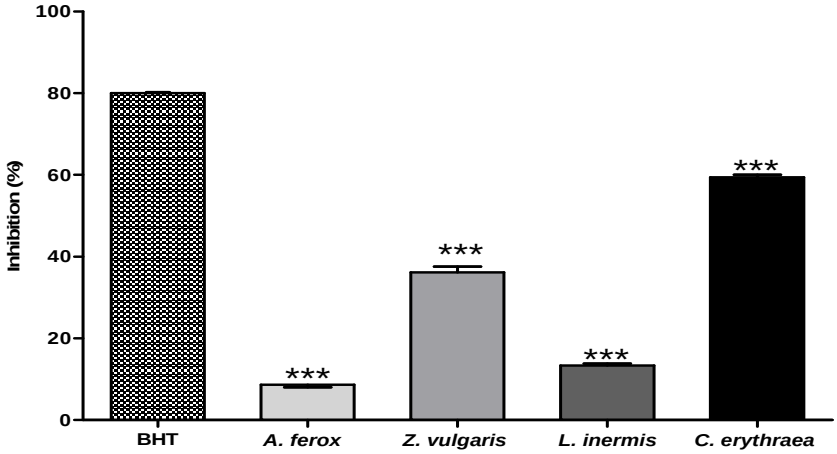

Figure.3: Antioxidant activities of different plants extracts (2 mg/ml at 96 h of incubation) measured by FTC method. BHT was used as reference antioxidant. Values are % means ± SD (n = 3). (* p < 0.001) compared to BHT as standard**

Thiobarbituric acid (TBA) assay

At a later stage of lipid oxidation, peroxide decomposes to form carbonyl compound that are measured by the TBA method. During the oxidation process, peroxides are gradually decomposed to lower molecular weight compounds, such as malonaldehyde, which can be measured by TBA method on the final day of the incubation period [24-36]. Using the thiobarbituric acid (TBA) method, all extracts also showed good antioxidant activities (Figure 4). The percent inhibition of MDA formation of plants exstracts and BHT decreased in the following order: BHT (83.84 ± 0.5770 %) > *C. erythraea* extract (70.40 ± 0.8910 %) > *Z.vulgaris* extract (63.67 ± 0.8910 %) > *L. inermis* extract

(35.67± 2.464%) >*A. ferox* (35.19 ± 0.2772%). These findings justify the increasing interest in phenolics due to their ability to inhibit oxidative degradation of lipids [37].

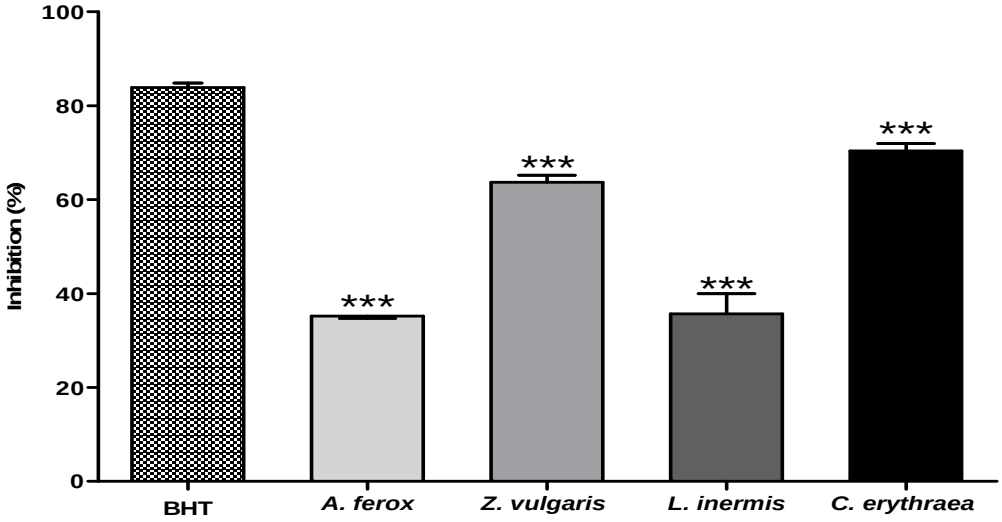

Figure 4: Antioxidant activities of different plants extracts (2 mg/ml) measured by TBA method. BHT was used as reference antioxidant. Values are % means ± SD (n = 3). (* p < 0.001) compared to BHT as standard**

Antioxidant activity of extracts: *in vivo* assays

Animal studies offer a unique opportunity to assess the contribution of the antioxidant properties of plants extract and plants polyphenols to the physiological effects of plants extracts administration in different models of oxidative stress.

Plasma antioxidant capacity

Plasma antioxidant capacity using DPPH radical

The result shown in Figure 5 indicated that all extracts doses increased the plasma antioxidant capacity in the range of 12.56 ± 1.004 % to 46.13 ± 2.389 % compared to control group (8.962 ± 1.339 %). However, only the two doses 150 and 500 mg/kg of *L. inermis* extract, 500 mg/kg dose of *C. erythraea* extract and 500 mg/kg dose of *Z. vulgaris* extract were as stronger (p < 0.001) as vitamin C (28.92 ± 2.418 %) to increasing the total plasma antioxidant capacity.

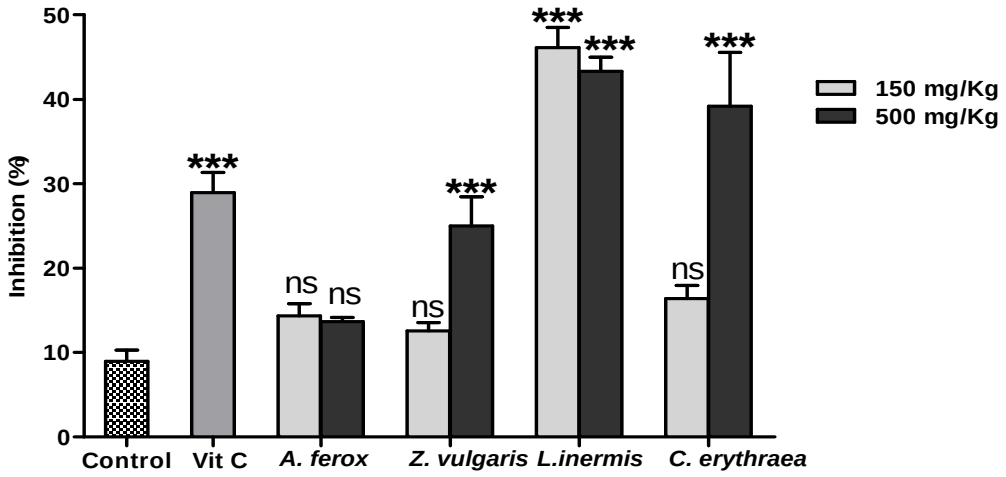

Figure 5: A comparison between different plants extracts in plasma antioxidant capacity using DPPH radical. Data were presented as % means ± SEM (n = 6). (ns: no significant difference; *** p < 0.001) compared to control group

Plasma reducing power

The effects of the extracts on plasma reducing power are shown in Figure 6. It can be observed that all the extracts showed electron donation capacity, but only the reducing power of the dose 500 mg/kg of *C. erythraea* extract (1.164 ± 0.177) was significantly higher than that of control group. These obtained results showed a positive association between plants extracts administration and plasma antioxidant capacity using DPPH scavenging assay and reducing power and these effects may be primarily attributed to its high phenolic and tannins contents which

were estimated previously. Hence, some experimental studies have been performed to confirm the high significant correlation between the measured plasma antioxidant capacity and the total phenol content of plant-derived extracts and beverages [38-39].

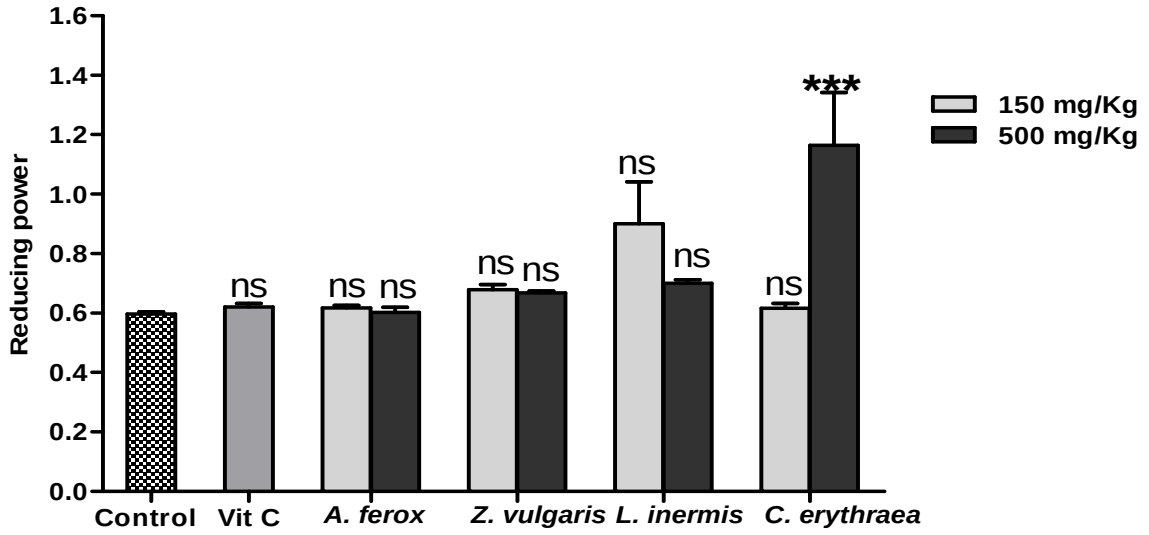

Figure 6: A comparison between different plants extracts in plasma antioxidant capacity using reducing power. Data were presented as means ± SEM (n = 6). (ns: no significant difference; *** p < 0.001) compared to control group

Effect of extracts on hepatic antioxidant status

Aerobic organs such as the liver generate reactive oxygen species that induce oxidative tissue damage. These radicals, which react with cell membranes and thus induce lipid peroxidation or cause inflammation, have been implicated as important pathological mediators in many clinical disorders [40-41].

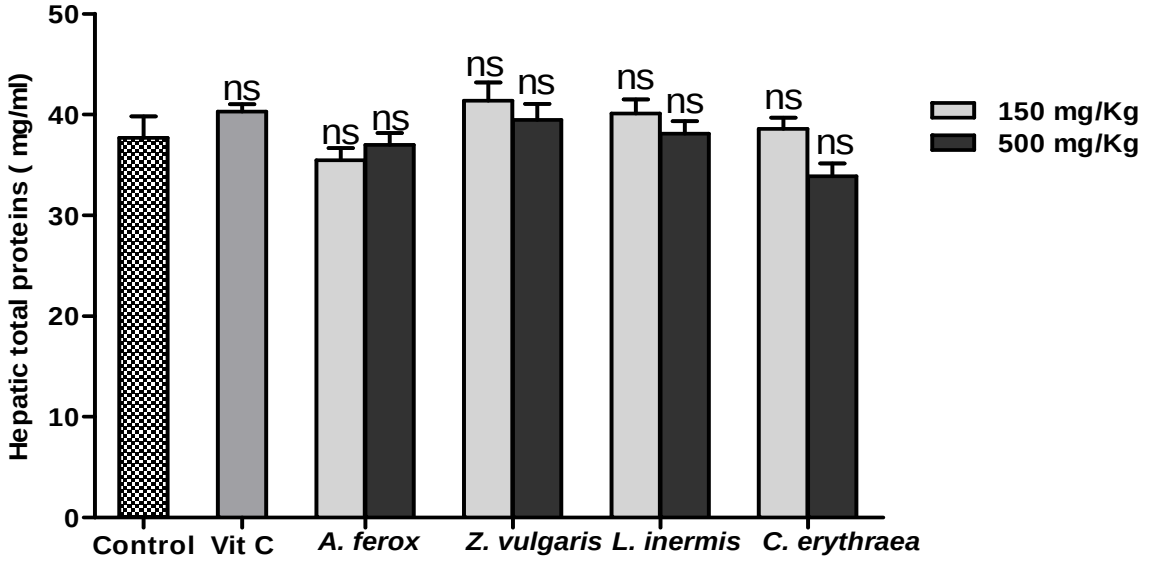

Figure 7: Effect of different plants extracts and vitamin C on total proteins level in liver of rats. Values are given as mean ± SEM (n=6). (ns: no significant difference) compared to control group

Total proteins level

Reactive oxygen species (ROS), such as the superoxide anion (O_2•-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (•OH), are produced as by-products of aerobic metabolism in mitochondria and can cause damage to DNA, lipids and proteins [42]. Oxidative damage to proteins may result in chemical modification of amino acids, aggregation or cross linking of proteins or protein fragmentation. As seen in Figure7, the administration of the four studied plants extracts and vitamin C had no effect (no significant difference) on proteins level in liver compared to control (37.7 ± 2.122 mg/ml) and vitamin C groups (40.29 ± 0.743mg/ml).

Very limited data suggest that plant extracts administration may protect proteins from oxidative damage [43-44] and also has a stimulatory effect on protein synthesis which accelerates regeneration of cells [38-42].

Catalase activity

SOD, CAT and GPX constitute a mutually supportive team of defence against ROS. Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and in the liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [41]. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. In this study, it was observed that the treatment of rats with all extracts doses increased activity of catalase in liver (Figure 8), but this increase didn't reach statistically significant difference; except for the dose 500 mg/kg of *Z. vulgaris* extract and *L. inermis* extract ($p < 0.01$) when compared to control group (54.28 ± 6.232 U/g tissue). Thus, an increase in activity of catalase evaluated in this study suggests the good antioxidant proprieties of different plants extracts treatment and this supports other reports [37]. This high antioxidant enzyme activity is probably attributed to the presence of polyphenolic compounds which may have many benefits in treating oxidative stress related diseases as peptic ulcer.

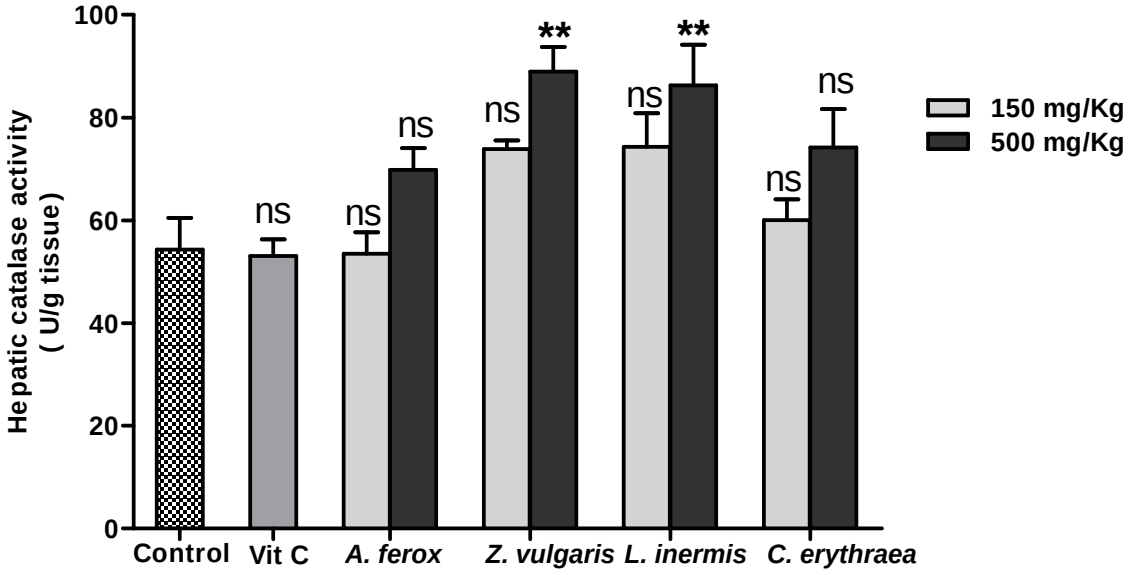

Figure 8: Effect of different plants extracts and vitamin C on catalase activity in liver of rats. Values are given as means ± SEM (n=6). (ns: no significant difference; ** p < 0.01) compared to control group

GSH level

The non-enzymatic antioxidant, glutathione is one of the most abundant tripeptides present in the liver. Its functions are mainly concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and maintenance of membrane protein thiols and as a substrate for glutathione peroxidase and GST [41]. Glutathione peroxidase is a selenium containing enzyme which catalyses the reduction of H_2O_2 and lipid hydroperoxides, generated during lipid peroxidation, to water and oxygen [45]. As seen in Figure 9, the aqueous extracts of *A. ferox* and *Z. vulgaris* did not alter the level of GSH in liver compared to control (32.24 ± 1.036 µmol/g tissue) and vitamin C groups (37.91 ± 2.840 µmol/g tissue). The treatment with *C. erythraea* extract at two different doses (150 and 500 mg/kg) showed higher effect on GSH level (68.80 ± 6.257µmol/g tissue and 90.79 ± 2.314µmol/g tissue, respectively; $p < 0.001$). Also, the administration of *L. inermis* extract at doses of 150 and 500 mg/kg in rats increased significantly the level of GSH with values of 50.35 ± 3.560 µmol/g tissues and 53.24 ± 1.246 µmol/g tissues, respectively ($p < 0.05$) compared to control group. Recent studies on various plants and herbal formulations also having the similar effect [37-46-45-44-41]. The phytochemical molecules as polyphenols that are present in the plants aqueous extracts might be the reason for bringing up GSH level in the treatment groups.

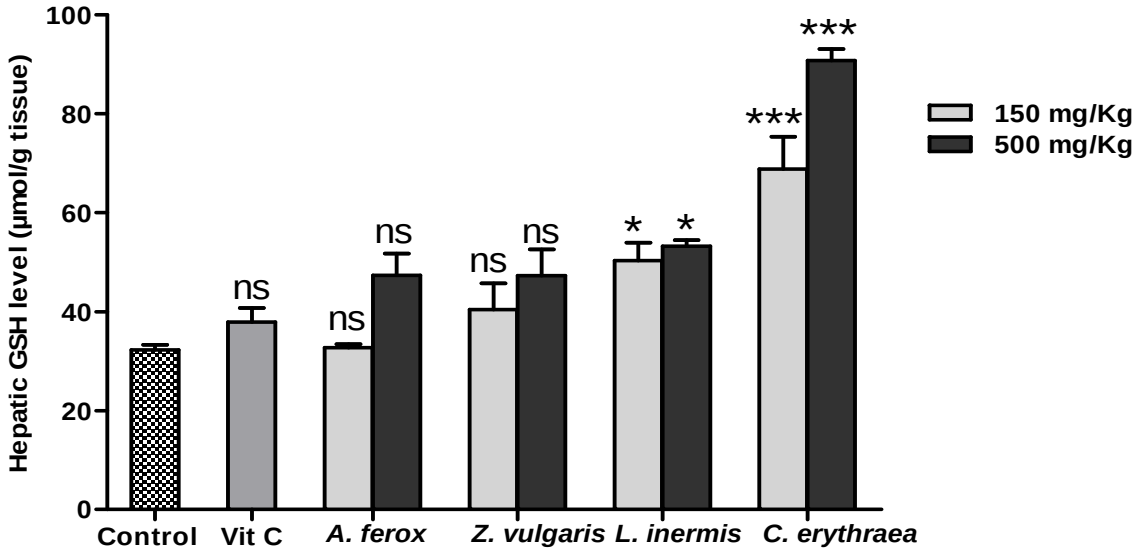

Figure 9: Effect of different plants extracts and vitamin C on reduced glutathione level in liver of rats. Values are given as means ± SEM (n=6). (ns: no significance difference; * p < 0.05 ; *** p < 0.001) compared to control group

Lipid peroxidation

Lipid peroxidation is a chain reaction normally occurring at low levels in all cells and tissues. It is enhanced in many physiologic and pathologic conditions. There are various factors that stimulate lipid peroxidation [47]. MDA is one of the end products in the lipid peroxidation process.It is formed during oxidative degeneration as a product of free oxygen radicals, which is accepted as an indicator of lipid peroxidation. It was observed that treatment (Figure 10) with *Z. vulgaris* extract at dose 150 mg/kg and all plants extracts at dose of 500 mg/kg significantly reduced ($p < 0.001$) the level of MDA in liver compared to control group (72.480 ± 2.227 nmol/ g tissue). However, other extracts doses decreased the MDA level but this decrease was statistically not significant when compared to control group. As mentioned above, this distinct drop in MDA levels may be due to the high levels of exogenous antioxidants as polyphenols in these extracts. MDA levels are an important marker of lipid peroxidation and can be modulated by phenolic compound intake and the present finding confirmed most published results in many studies [37-48-49]. Moreover, the reduction of the lipid peroxidation level in groups treated with plants extracts could be due to the increase in the activity of CAT and concentration of the GSH compared to the control group **(**Figure 8 and 9). Recently, Bouaziz *et al.*[37], reported that if the CAT and GSH activities are not sufficiently enhanced to metabolize hydrogen peroxide, this can lead to increased hydrogen peroxide and TBARS levels.

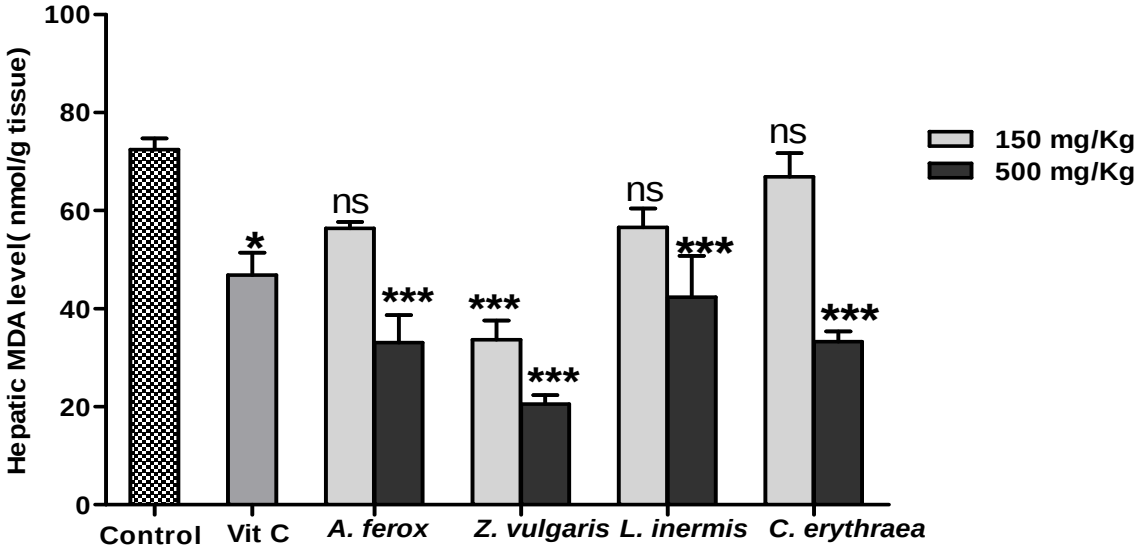

Figure 10: Effect of different plants extracts and vitamin C on MDA level in liver of rats. Values are given as means ± SEM (n=6). (ns: no significant difference; * p < 0.05; *** p < 0.001) compared to control group

CONCLUSION

In conclusion, the studied antiulcerative plants contained high amounts of phenolics and exhibited a good antioxidant activity. The results obtained may be useful in gastroduodenal ulcer therapy. However, further investigations are needed to determine the active compound(s) and to elucidate the mechanisms involved in gastroprotection.

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