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Antioxidant activities of *p*-coumaroyl maslinic acid extracted from Fruit of *Zizuphus mauritiana Lam*

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Abstract: Fruit of *Zizuphus mauritiana* have a high nutritional value and are rich in various active ingredients. This fruit has been commonly used in Traditional Medicine for its various pharmacological activities, such as its anticancer, antiepileptic, anti-inflammatory, anti-insomnia, neuroprotective effects and antioxidants by slowing down the process of free radical oxidation. The study aimed to identify *p*-coumaroyl maslinic acid compound present in fruit of *Z. mauritiana* extract and assess the antioxidant potential both in *In-vitro* and *In-vivo* effects. *In-vitro* measurement was assessed by its free radical scavenging activity such as 2, 2-diphenyl -1- picrylhydrazyl (DPPH) and ABTS as well as Total reducing capability (FRAP). The *In-vivo* antioxidant effects of *p*-coumaroyl maslinic acid extract (CMAE) were evaluated in rats for 14 days, where different concentrations of extracts (50, 100, 150, and 200 mg/kg/day) were orally administrated daily. Antioxidant profiles were performed by measuring malondialdehyde (MDA), blood proteins, reduced glutathione (GSH), glutathione peroxydase (GPX), catalase (CAT) and superoxide dismutase (SOD) activities in red blood cells. CMAE revealed excellent free radical scavenging activity (IC50) with DPPH (6.54±0.60 µg/ml), ABTS (35.19±0.55 µg/ml) and FRAP (3.61±0.10 µg/ml) assay. Furthermore, CMAE decreased the level of MDA and SOD, and increased levels of GSH, GPX and CAT in blood. Taken together, these results signify the excellent antioxidant properties of CMAE of *Z. mauritiana* fruit, which might be useful for the treatment of oxidative-stress related diseases such as chronic diseases.

1. Introduction

The African continent holds an enormous resource of floral biodiversity and its medicinal plants have remained a main reservoir of phytochemicals for pharmaceutical drug development (Earth Trends, 2003; Mbatchou 2004). The local populations, especially South of the Sahara, have depended on medicinal plants as the main source of remedy for the treatment of several medical disorders over the past centuries. It has been reported that only 3 out of 20 patients are able to buy prescribed drugs in hospitals and only 1 out of every 1000 patients is able to consult a specialist (Kuete & Efferth 2010). Because of this, there is a rich tradition in the use of herbal medicines for the treatment of several ailments and plans are on the way to integrate traditional medicine in the health care system even though the plans have not been put into action yet (Nkongmeneck *et al*. 2007). Cameroon however has a rich biodiversity, with ~8,620 plant species (Earth Trends, 2003; Mbatchou 2004), some of which are commonly used in the treatment of several chronic diseases (Kuete & Efferth,

2010). As an alternative to these difficulties, Cameroonians are using nutraceutical foods, which are ordinary foods that have components or ingredients incorporated in them to give a specific medicinal or physiological benefit other than a purely nutritional effect (Effoe et al. 2020). Now, the nutraceuticals related research for improving its quality and quantity is an important area for ongoing biotechnological investigations (Bickford *et al.* 2012). Moreover, the Covid 19 pandemic has proven that in Africa and especially in Cameroon, due to the strong ethnobotanical potential, it is possible to overcome many diseases such as chronic diseases by using nutraceutical plants. Among those African medicinal plants, *Ziziphus mauritiana* Lam. is one of the traditional medicinal plants which have many nutritional and therapeutic potentials. Fruit of *Zizuphus mauritiana* have a high nutritional value and are rich in various active ingredients, including vitamin C, phenols, flavonoids, polysaccharides, triterpenes, saponins, vitamins, and other active ingredients (Guo *et al.* 2021 ; lu *et al.* 2021). This fruit has been commonly used in Traditional Medicine for its various pharmacological activities, such as its anticancer, antiepileptic, anti-inflammatory, anti-insomnia, neuroprotective effects (Kou *et al.* 2015) and antioxidants by slowing down the process of free radical oxidation (Murray *et al.* 2009) as well as anticorrosion (Oukhrib *et al.*, 2017). Antioxidant activity is often correlated with the presence of phenolic compounds, such as triterpenes, alkaloids, flavonoids, and polysaccharides (Bozalan *et al.* 2011; Gujral *et al.* 2011). In recent years, many studies on the chemistry and biological activity of *Zizuphus sp.* have been carried out. Phytochemical studies revealed that *Zizuphus sp* contains various chemical constituents, including triterpenic acids, flavonoids, saponins, alkaloids, amino acids, phenolic acids and polysaccharides (Xie *et al.* 2017; Song *et al.* 2019). Therefore, the beneficial effects of health are derived from this variety of bioactive compounds. Triterpenoids are one of the most characteristic and major bioactive compounds of the jujube fruit mainly in the form of triterpenes and saponins (Guo *et al.* 2015).

Several review articles have shown that triterpenes and triterpenic acids, derivatives of pentacyclic triterpenes, have strong pharmacological activities, such as antioxidative, anti-inflammatory, anticancer, hepatoprotective, anti-diabetic and anti-microbial activities, combined with low toxicity (Mallavadhani *et al.* 2019; Madasu *et al.* 2020). Which makes the triterpenoids of *Z. mauritiana* potential targets for the development of new drugs. These pharmacological activities have also been ascribed to Maslinic acid (MA) which is a pentacyclic triterpene and is widely distributed throughout the plant kingdom. The antitumoural activity of maslinic acid was reported to occur in several types of cancer (Rufino-Palomares *et al.* 2013). The discovery of new natural and safe health products in the form of plant extracts represents a real challenge today (Diass *et al.* 2023). Thus, efficient extraction and further utilization of bioactive triterpenes of jujube and its products have been attracting attention in recent years (Zhang *et al.* 2019).

Although the antioxidant activity of *Z. mauritiana* has been reported, the active compounds and their mechanism of action is largely unknown. This research is intended to isolate the antioxidant compounds (*p*-coumaroyl maslinic acid) from the kernel of *Z. mauritiana* and to determine its antioxidant activity.

The present studies were performed to assess *In-vitro and In-vivo* antioxidant activities of *p*-coumaroyl maslinic acid (CMA) by using classic methods.

2. Methodology

2.1. Plant material

This study was carried out on the fruit of *Ziziphus mauritiana*, because of its high nutritional and therapeutic value and the diversity of its uses. Dried jujube samples were purchased from farmers

in Mokolo in the department of Mayo Tsanaga (Far North Region), an area where jujube is predominant.

Photo : fruit of *Ziziphus mauritiana*

2.2. Extraction and isolation of p-coumaroyl maslinic acid

Triterpenoic acids were extracted using the method described by Yagi *et al.* (1978). After extraction with 95% ethanol (1.5 liters/500g sample), the alcohol is removed by vacuum evaporation. The suspension of the ethanolic extract in water was then extracted with butanol. This was separated by Chromatography on a silica gel 60 column using benzene-acetone (7/3) to obtain a resinous substance. The resinous material was partitioned with 2% Na_2CO_3 and ethyl acetate (EtOAc). The EtOAc phase was recovered and the acetate evaporated in vacuo. The residue was fractionated successively on a silica gel 60 column using the following solvents: - Chloroform - Ethyl acetate (85/5) - Benzene - acetone (5/1). The fractions collected were eluted on a TLC plate (solvent: Ethyl acetate - Methanol - Water; 10/1.35/1/) and the active fractions were identified by vaporisation of a methanolic solution of DPPH 0.2%. The active fractions were combined and quantified against a standard (Ursolic acid: 0-16.5 µg) by UV-Visible spectrophotometry at 220 nm. Figure 1 shows the structure of p-coumaroyl maslinic acid (Lee *et al.* 2004).

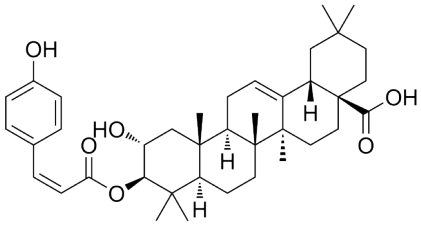

Figure 1: *p*-coumaroyl maslinic acid (Lee *et al.* 2004).

2.3. In vitro Measurement of Antioxidant Properties

2.3.1. 2, 2-Diphenyl -1- Picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

The free radical scavenging activity of the samples was determined according to the method of Kumari *et al.* (2016). A freshly prepared solution of DPPH in methanol (6×10^{-5}M) was used for the UV measurements. The samples of different concentrations (4–64 µg/mL) were added to DPPH solution in 1:1 ratio followed by vortexing. Then, it was allowed to take place in the dark at room

temperature. Ursolic acid is taken as a standard. The inhibition percentage of DPPH radical scavenging activity was calculated using the following equation **Eqn. 1**:

Inhibition (%) = [(A0 − A)/A0] × 100 **Eqn. 1**

where, A0 is the absorbance of DPPH in the absence of the sample and A is the absorbance of DPPH in the presence of the sample.

The IC50 values (the concentration required to scavenge 50% of the free radical) were estimated from a plot of % inhibition against the concentration of the sample solutions.

2.3.2. Ferric Reducing Antioxidant Power assay (FRAP)

The total reduction capability of samples was determined according to the method of Kumari *et al.* (2016). 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferric cyanide were added to 1 mL of samples in different concentrations (4–64 µg/mL), followed by gentle mixing. The mixture was incubated at 50◦C in a water bath for 20 min. The reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid and the mixture was centrifuged at 4000 rpm for 10 min. From the top layer, 2.5 mL was transferred into the tube containing 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3.6H_2O$), mixed thoroughly. After 5 min, the absorbance was measured at 700 nm against blank. Ursolic acid is taken as a standard.

2.3.3. ABTS+ Radical Cation Decolorization Assay

The antioxidant activities of the extracts were determined by the improved ABTS+ radical cation scavenging ability with the slight modification (Sarma *et al.* 2016). ABTS+radical cation was produced by mixing 7 mM 2, 2′- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM potassium persulfate ($K_2S_2O_8$), incubated at room temperature in the dark. To determine the ABTS radical scavenging activity, 3 mL of ABTS+ solution was mixed thoroughly with 0.2 mL of different concentration (4–64 µg/mL) of extracts. Ursolic acid is taken as a standard. The reaction mixture was allowed to stand at room temperature for 6 min. The percentage inhibition was calculated by the following formula **Eqn. 2**:

%Inhibition = (OD of control − OD of sample) × 100/OD of control **Eqn. 2**

2.4. In vivo Measurement of Antioxidant Properties

2.4.1. Animals and ethics

Male albinos Sprague Dawley rats (160-180 g) were housed in polycarbonate cages in a controlled environment with a temperature of 25 ± 2°C, relative humidity (40–60%), with a 12-h light–dark cycle (12h/12h: 7 – 19 h light and 19 – 7h dark) (Gaíva *et al.* 2003). The investigation conforms to the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). During an acclimatization period of 1 week, the rats received tap water and a commercial rat diet *ad libitu* (Baba *et al.* 2000). At the end of this period, the rats were weighed and randomly assigned to one of the different groups (n = 6 / group) according to the experimental studies. Experimental studies received approval of the local ethical committee for animal handling and experimental procedure. Each animal was used only once.

2.4.2. Experimental Procedure

The triterpenoids fraction from *Ziziphus mauritiana*, CMA (50, 100, 150 and 200 mg/kg; b.w), were used in this study. Thirty-six male rats were randomly divided into five groups, each consisting of 6 animals, which included:

- Group 1: Untreated control rats which received distilled water for 14 days.
- Group 2: Positive control received 50mg/kg bw of vitamin C for 14 days.
- Group 3: Received 50 mg/kg bw of CMA extract for 14 days.
- Group 4: Received 100 mg/kg bw of CMA extract for 14 days.
- Group 5: Received 150 mg/kg bw of CMA extract for 14 days.
- Group 6: Received 200 mg/kg bw of CMA extract for 14 days.

Administrations were done orally, the extract dissolved in 1 mL of distilled water per 100 g of body weight. 24 h after the last dose, all animals were anesthetized with chloroform and blood samples were immediately collected from the heart by cardiac puncture in two tubes to obtain serum and plasma (heparin tubes). Serum was separated by centrifugation at 3000 rpm for 5 min (4°C) and plasma was separated by centrifugation at 1500 rpm for 10 min (4°C). Serum was used for protein assessment and plasma was used for MDA determinations and Oxidative stress which was performed by measuring GSH; GPX; CAT and SOD activities in red blood cells.

2.4.3. Estimation of lipid peroxidation (MDA)

Lipid peroxidation was estimated colorimetrically by thio-barbituric acid reactive substances (TBARS) using the modification method of Niehius and Samuelson (1968). In brief, 0.1 ml of sample (10% w/v) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 15% trichloroacetic acid and 0.25 N HCl). After vortex mixing, samples were maintained at 95°C for 20 min. Afterwards samples were centrifuged at 3000 rpm for 10 min and supernatants were read at 532 nm against reference blank. A calibration curve was constructed using MDA as standard and the results were expressed as nmol/mg protein. Percentage inhibition was calculated using the equation **Eqn. 3**:

$$\% \text{ lipids Inhibition} = \{Ao - A1\}/Ao \times 100 \qquad \textbf{Eqn. 3}$$

Where; Ao is the absorbance of the control and A1 is the absorbance of the sample extract.

2.4.4. Estimation of protein concentration

The protein content was estimated by Biuret method (Gornall *et al.*, 1949) using bovine serum albumin as a standard.

2.4.5. Determination of reduced glutathione (GSH)

Reduced glutathione was quantified by the GSH assay, as reported in Ellman (1959). The assay is based on the oxidation of GSH by 5,5-dithiobis (2-nitrobenzoic acid) (DTNB). DTNB and GSH react together, producing 2-nitro-5- thiobenzoic acid (TNB) having a yellow color. Briefly, the sample was prepared by mixing DTNB (2.4 mL), buffer (0.5 mL), and respective dilution of the supernatant (0.1 mL). The GSH concentration was determined by measuring absorbance at 412 nm using a UV-Vis spectrophotometer using phosphate buffer as a blank.

2.4.6. Determination of glutathione peroxidase (GPX)

Glutathione peroxidase (GPX) activity was analyzed by the method of Rotruck *et al.* (1973). To 0.2 ml of buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of tissue homogenate were added. To that mixture, 0.2 ml of glutathione solution and 0.1 ml of hydrogen peroxide were added. The contents were mixed well and incubated at 37°C for 10 min along with the control tubes containing all the reagents but no enzyme. After 10 minutes, the reaction was arrested by the addition

of 0.4 ml of 10% TCA. 0.2 ml of tissue homogenate was added to the control tubes. The tubes were centrifuged and supernatant was assayed for glutathione content by adding Ellman's reagent.

2.4.7. Determination of catalase (CAT)

Catalase activity was assessed by the method of previously reported by Naz *et al.* (2022). Phosphate buffer (0.1 M, pH 7.4) was taken as a blank. The samples were analyzed against a control. A measure of 40 µL of the plant supernatant was added to the H_2O_2 solution (40 mM; 0.6 mL), and the total volume was made up to 3 mL and mixed thoroughly. The final reading was noted at 240 nm in triplicate. The results were expressed as micromoles of hydrogen peroxide decomposed/min/mg of protein.

2.4.8. Determination of Superoxide dismutase (SOD)

The activity of superoxide dismutase was assayed by monitoring its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT). Superoxide dismutase was assayed as described by Misra and Fridovich (1972). The assay mixture contained 0.5 ml of hepatic PMS, 1 ml of 50 mM sodium carbonate, 0.4 ml of 25 µm nitroblue tetrazolium and 0.2 ml of freshly prepared 0.1 mM hydroxylamine-hydrochloride. The reaction mixture was mixed quickly by inversion followed by the addition of clear supernatant of 0.1 ml of sample (10% w/v). The change in absorbance was recorded at 560 nm. Percentage inhibition was calculated using this equation **Eqn. 4**:
% superoxide dismutase inhibition = [(normal activity – inhibited activity)/(normal activity)] × 100%.

2.5. Statistical analysis

The experimental results were expressed as means ± standard deviation. For each group, the result obtained was the mean for 6 rats. All results were analyzed using a one-way analysis of variance. Duncan's Multiple Range test was performed to evaluate differences between groups. Differences between means were considered to be significant at $p < 0.05$.

3. Results and Discussion

In this work, the antioxidant activity of the various samples is assessed by in-vitro tests (DPPH, ABTS, FRAP) in the extract and in vivo tests (Protein; MDA; SOD; GPX and CAT) in plasma and blood serum as target fluids specialising in the transport of toxification products.

3.1. In vitro Measurement of Antioxidant Properties

In vitro measurement of antioxidant properties was assessed by DPPH, ABTS, FRAP tests. Results are presented in table 1.

Table 1: *In vitro* antioxidant activities of **p-coumaroyl maslinic acid extract** (CMAE)

Elements	**IC_{50} Value**		
Triterpenoic acids	ABTS (µg/ml)	DPPH (µg/ml)	FRAP (µg/ml)
p-coumaroyl maslinic acid (CMA)	3.61±0.10	6.54±0.60	35.19±0.55
Ursolic acid (standard)	47.92±0.83	47.92±0.83	47.92±0.83

3.1.1. DPPH Radical Scavenging Activity

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by extracts having antioxidant potential. It produced hydrazine by converting the

unpaired electrons to paired electron due to the hydrogen donating ability of the extract (Ozsoy *et al.* 2008)**.** As shown in **table 1**, the IC50 values of p-coumaroyl maslinic acid extracts of *Z. mauritiana* fruit and ursolic acid were 6.54 ± 0.60 and 47.92±0.83 µg/mL respectively. In this present study, the IC50 value of the standard, ursolic acid was significantly higher free radical scavenging activity compared to *p*-coumaroyl maslinic acid extract while the lowering IC50 value indicates a higher free radical scavenging activity. The observation demonstrates the antioxidant ability of extracts of CMA by several *in vitro* and *in vivo* methods. The experimental results showed that extract of CMA showed strong DPPH scavenging activity. The results obtained in this study show that, the IC50 value of CMA (6.54 ± 0.60 µg/mL) in DPPH radical scavenging assay was far lower in comparison with those reported by Abalaka *et al.* (2011) (18-124 µg/ml) ; Ramar *et al.* (2017) (38.07µg/ml) on extract of *Z. mauritiana* leaves and Mahamat *et al.* (2021) (0.139 - 0.998 mg/ml) on extracts from Leaves and Bark of *Bauhinia rufescens* Lam. According to Kumari *et al.* (2016), there is strong correlation between DPPH and FRAP methods. In this study, CMA showed significantly reduced the ferric cyanide complex to the ferrous form (reducing capabilities). This agree the previous reports of Ramar *et al.* (2017) who believe that the antioxidants in the extract were determined by assessing by their ability of extract to reduce the ferric cyanide complex to the ferrous form. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (Pal *et al.*, 2011). Higher reducing power means better abilities to donate the electron and the free radical form stable substances by accepting the donated electrons, resulting in the termination of radical chain reaction (Deori *et al.* 2014).

3.1.2. Total Reduction Capability

Table 1 shows total reduction capacity of p-coumaroyl maslinic acid extract. From this table 1, the IC50 of *p*-coumaroyl maslinic acid was 35.19±0.55. Ursolic acid were used as positive control and its reducing power was 47.92±0.83 µg/mL. These results demonstrated that *p*-coumaroyl maslinic acid extract had marked the difference in ferric ions (Fe^{3+}) reducing ability as compared to the ursolic acid. In the present study, the reducing ability of the extracts of CMA (35.19±0.55 µg/ml) was lesser comparable with previous studies (90.70 µg/ml) (Ramar *et al.*, 2017). The previous report confirmed that the hydroxyl radicals are extremely reactive free radicals formed in the biological system and there are no any specific enzymes to defend against them in human (Liu *et al.* 2005). The presence of hydroxyl radical in the body may lead to the oxidative DNA damage. Therefore, it is very important to find the solution using natural products with good scavenging activity against this ROS. Earlier report also demonstrates that the scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical (Wang *et al.*, 2008). In this study, extract of *p*-coumaroyl maslinic acid showed an important effect in hydroxyl radical scavenging activity.

3.1.3. ABTS Radical Cation Decolourization Assay

The ABTS radical scavenging ability is an important method for determining the antioxidant ability. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm, which decreases with the scavenging capacity (Wang *et al.* 2020). The results from the ABTS radical scavenging ability was found to be high in ursolic acid (IC50: 47.92±0.83 µg/mL) than in CMA (IC50**:** 3.61±0.10**).** This result is lower the finding result of Ramar *et al.* (2017) (19.8µg/ml).

Overproduction of NO causes cancer, inflammation, neurodegenerative, chronic inflammatory diseases, ischemia reperfusion and other pathological conditions (Bajpai *et al.* 2014). Some researches

reports demonstrate that extract of *Z. mauritiana* plays an important role in NO suppression, this can explain the differential inhibitory effect obtained with various methods used in this study (Kumari *et al.* 2016). In the present study, the extracts scavenging the NO which results in the ABTS activity, confirm that the extract of CMA showed a good radical scavenging capacity with low IC50 value (3.61±0.10 µg/ml). According to Kumari *et al.* (2016) there is a strongly correlation between FRAP and ABTS. For this reason, it is possible to confirm that the extract of *p*-coumaroyl maslinic acid has a good radical scavenging capacity and can be used against oxydative stress and chronic deseases. For Chidambaram *et al.* (2013), free radical and hydroxyl radical are the major active oxygen species causing enormous biological damage. The results had clearly corroborated the efficacy of CMA as a promising source of inhibiting LPO. In order to deal with damaging activities of free radicals, aerobic organisms process antioxidant defense systems. The enzymatic antioxidant defenses include SOD, GPX and CAT (Irshad *et al.* 2002). These activities play an important role in the progress of the chronic diseases, therefore in the treatment of chronic diseases, food antioxidant treatment may useful and should be added to combined therapy for these patients. It is thus important to check the impact of CMA consumption on enzymatic antioxidants

3.2. In vivo Measurement of Antioxidant Properties

The biochemical assessment in this study is conducted to show the benefic effects of CMA on management of chronic diseases which can be linked to the oxidative stresses (Betteridge *et al.* 2000). *In vivo* Measurement of Antioxidant Properties was evaluated by determination of Protein; MDA; SOD; GPX and CAT in blood

3.2.1. Evaluation of Proteins

Protein oxidation is another marker of oxidative stress (Witko-Sarsat *et al.* 2003). This gives an indication of the denaturation of the body's proteins. Figure 2 shows the evolution of protein levels in plasma.

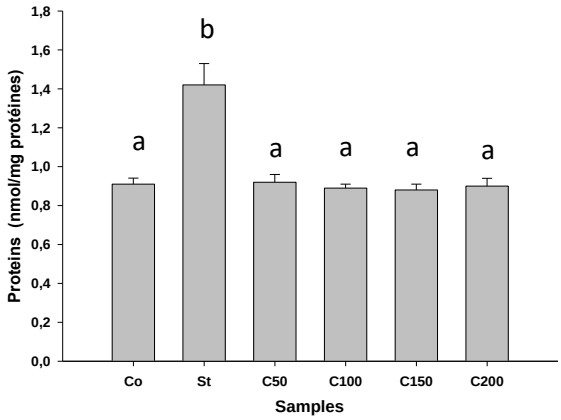

Figure 2: Effect of CMA consumed on proteins

Figure 2 shows changes in plasma protein levels. It can be seen that CO protein levels are not significantly different from the experimental values of C50 C100 C150 and C200. The concentration values obtained for C50 (0.92±0.04); C100 (0.89±0.02); C150 (0.88±0.03); C200 (0.90±0.04) were not significantly different ($p<0.05$) from the control (0.91±0.04). On the other hand, the experimental values obtained were below the standard (1.42±0.11). This result suggests that the protein-protective action of CMA is greater than the standard. These values are close to those obtained by Ben Saad *et*

al. (2017) (0.65-1.8 nmol/mg proteins). The increase with the standard could be explained by stimulation of the cells to secrete antioxidant enzymes following consumption of CMA extracts. This confirms the protective action of CMA against protein oxidation by free radicals. Indeed, the imbalance between the defense systems and the production of free radicals leads to biochemical alterations in the body's cells, such as the appearance of DNA breaks or damage to the integrity of the cell membrane by the induction of lipid peroxidation and alterations to proteins (Moffarts *et al.* 2005). These modifications to peptides by the addition of products of lipid peroxidation generally lead to a loss of catalytic or structural function in the proteins affected **(**Levine 2002). These cells are generally more sensitive to the action of proteases, which causes them to be eliminated. This could explain the low levels obtained with the control and treated samples.

3.2.2. Evaluation of Malondialdehyde (MDA)

Oxidative stress can be correlated to the lipid molecular peroxydation and can be assessed by determination of inherent products yielded. This can be done by evaluation of MDA which is one of the best indexes of cell destruction due to the fact that this product is rapidly formed in serum than in normal cell. MDA concentration can be then reflected the oxidative stress degree of rats (Irshad *et al.* 2002) and constitutes a biological marker of lipid peroxidation. Figure 3 shows the effect of CMA consumed on Malondialdehyde (MDA).

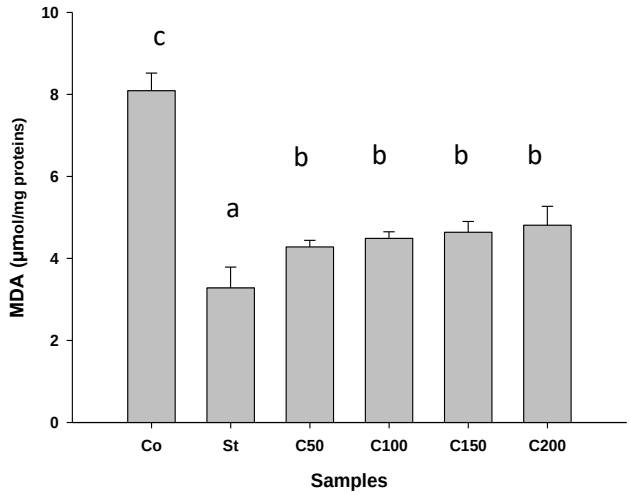

Figure 3: Effect of CMA consumed on Malondialdehyde (MDA).

The MDA assay is the most widely used parameter for assessing the importance of oxidative stress in numerous pathologies. In contrast to proteins, the MDA results show that whatever the dose administered to the rats, a decrease in MDA levels was observed compared with the control (CO). The values obtained were 8.09±1.30 (CO); 4.28±0.16 (C50); 4.49±0.16 (C100); 4.64±0.26 (C150); 4.81±1.46 (C200) and 3.28±0.99 (St) respectively. According to the statistical analyses, there was no significant difference between the C50, C100, C150 and C200 values. These values are higher than those obtained by (Merghem *et al.* 2019) (11-19 nmol/g tissue) on *Ruta Montana* L. extracts and (Tajudeen *et al.* 2022) (7.67 - 10.83 nmol.10^{-7}/ml) on jujube leaf extracts. The values found are similar to those observed by Saeed *et al.* (2014) on the effect of long-term morphine treatment on oxidative stress enzymes in male rats. The elevated values of MDA were observed in Control animals, which lead to the cell damage indicating over production of free radicals and/or the inability of the antioxidant defense system. On the other hand, the drop in levels observed with the other samples

could be explained by the inhibition of reactive oxygen species following the ingestion of CMA extracts. Compared with the standard (St), there was no significant difference ($p<0.05$) between the C100, C150 and C200 doses. We can therefore say that for MDA, vitamin C and CMA have the same antioxidant power for doses 100, 150 and 200. Lipid hydroperoxides formed by free radical attack on polyunsaturated fatty acid residues in phospholipids can react with redox metals (such as Fe^{2+}, Fe^{3+}), ultimately producing mutagenic and carcinogenic malondialdehyde (MDA) (Scibior *et al.* 2006). It is also well known that the peroxidation of polyunsaturated fatty acids in membrane phospholipids leads to a deficit in membrane functions, notably through a reduction in fluidity and the inactivation of membrane receptors and enzymes (Michel 2008). This can lead to changes in membrane permeability. Free radicals, acting as pro-oxidants, are involved in the generation of oxidative stress and the imbalance in the oxidant-antioxidant balance. Oxidative stress induces a high production of Reactive Oxygen Species (ROS), which are highly toxic to cells, particularly cell membranes. These ROS interact with the lipid bilayer to produce lipoperoxides (Sivajothi *et al.* 2008).

3.2.3. Evaluation of GSH

The cell's first antioxidant defence system is glutathione (GSH), which has a sulphydryl (-SH) function. Thanks to this free -SH function, it binds to toxic metabolites. Glutathione (GSH) performs its role in synergy with antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GSH-R), glutathione S-transferase (GSH-ST) and catalase (CAT), which are effective in eliminating toxic free radicals generated by oxidative stress (Ahanger *et al.* 2014; Wu *et al.* 2014; Abd_Allah *et al.* 2015). Figure 4 shows the effect of CMA consumed on glutathione reductase (GSH-R)

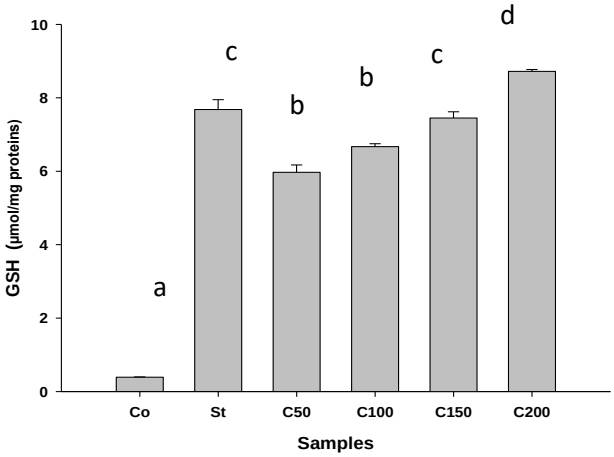

Figure 4: Effect of CMA consumed on glutathion reductase (GSH-R)

Analysis of GSH-R values shows a significant increase ($p<0.05$) from 0.39±0.01 (CO) to 8.72±0.05 (C200). The values for C50 (5.97±0.20) and C100 (6.67±0.08) were lower than the standard (7.68±0.27) ($p<0.05$). These results are similar to those determined by Kalidoss and Krishnamoorthy (2011), but higher than those assessed by Merghem *et al.* (2019) (0.44-1.02 µmol / g tissue) on *Ruta montana* extracts. According to Taleb-Senouci *et al.* (2009), the changes in GSH levels observed with the different doses can be considered a particularly sensitive indicator of oxidative stress. This suggests that the increase in GSH levels reflects the cell's defensive action against ROS. However, endogenous enzymatic and non-enzymatic antioxidants are responsible for detoxifying the body by combating the deleterious effects of free radicals **(**Cho *et al.* 2002). When ROS generation exceeds

cellular antioxidant capacity, oxidative stress develops, leading to cellular dysfunction and tissue damage. Consuming jujube could therefore counteract all these harmful effects on health.

3.2.4. Evaluation of Glutathion peroxidase (GPX)

Glutathion peroxydase is an enzyme which destroys the free radicals, takes free radical from cell and protects the cell membrane against oxidation. GPX allows the conversion of oxygenate water into water. Figure 5 shows the effect of CMA consumed on Glutathion peroxidase (GPX)

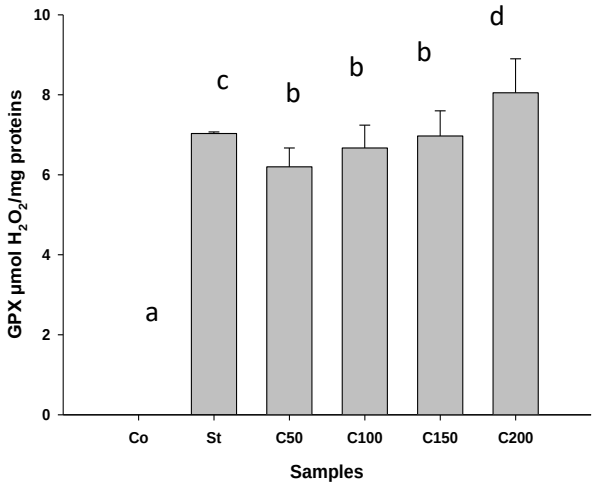

Figure 5: Effect of CMA consumed on Glutathion peroxidase (GPX)

The results of this study showed a clear increase in GPX activity in plasma following treatment of rats with CMA extracts. The values obtained with C50 (6.20±0.47); C100 (6.67±0.57); C150 (6.97±0.63); C200 (8.05±0.85) all showed a significant difference ($p<0.05$) compared with the standard (7.03±0.52). These results indicate that only the content of C200 is higher than that of the standard. Similar results were obtained by Ben Saad *et al.* (2017) (4-8 µmol H_2O_2/min/mg protein). However, the values found by Shtukmaster *et al.* (2010) (40-80 µmol H_2O_2/min/mg protein) are higher. It is thought that the increase in GPX levels could be explained by stimulation of the cells to secrete antioxidant enzymes following consumption of CMA extracts.

GPX acts as a direct scavenger of free radicals (Wu *et al.* 2014) and is involved in the regeneration of oxidised vitamin E (Ravi *et al.* 2004). GPX can also react with H_2O_2 and lipid peroxides to eliminate reactive intermediates by reducing hydroperoxides (Ahanger *et al.* 2014; Wu *et al.* 2014; Abd_Allah *et al.* 2015). It therefore acts as a protective physiological antioxidant in biological systems (Fatima & Mahmood 2007). Consumption of CMA would therefore have helped to protect biological systems, which explains the increase in GPX levels in the blood. Consumption of jujube would therefore be indicated against oxidative stress. GPX works in synergy with antioxidant enzymes, including catalase (CAT).

3.2.5. Evaluation of catalase (CAT)

Catalase (CAT) is an antioxidant enzyme involved in defending the cell against the toxic effects of hydrogen peroxide (H_2O_2) by catalysing its breakdown into water (H_2O) and molecular oxygen (O_2), and is a biomarker of oxidative stress (Jebali *et al.* 2007). Figure 6 shows the results of the effect of CMA extract consumption on CAT. Like the observations made on GSH, an increase in CAT levels was noted. These levels vary from 6.57±0.04 (CO) to 15.13±0.78 (C150). The values obtained were St (7.92±0.04); C50 (11.90±1.69); C100 (13.65±1.31); C150 (15.13±0.78) and C200

(14.65±1.20). A significant difference ($p<0.05$) was observed between the value of the standard and the experimental concentrations. They are close to those estimated by Ben Saad *et al*. (2017) (8-16 µmol H_2O_2/min/mg protein) and lower than those of Si *et al*. (2002) (12-20 µmol H_2O_2/min/mg protein) and Kalidoss and Krishnamoorthy (2011) (16-21 µmol H_2O_2/min/mg protein). The variation observed with the different doses could be explained by a stimulation of the cells to secrete antioxidant enzymes following consumption of CMA extracts. In fact, the increase in CAT activity could be the result of activation of the enzyme by superoxide anion.

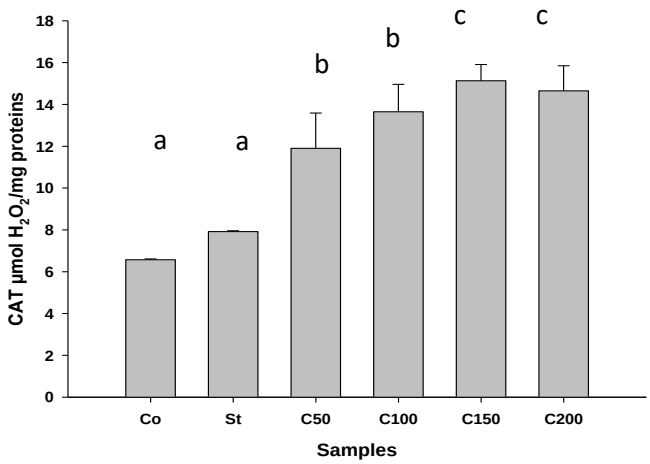

Figure 6: Effect of CMA consumed on catalase (CAT)

This also explains SOD's ability to protect CAT. Some authors believe that the induction of specific CAT activity in correlation with SOD constitutes the cell's first line of defense against oxidative stress under the pro-oxidant effect of free radicals **(**Liu *et al.* 2010; Uzun *et al.* 2010). However, increased production of hydrogen peroxide can exceed the antioxidant capacities of this enzyme, resulting in inhibition of their activity (Atli *et al.* 2006). In this context, supplementing foods with CMA could help solve this problem. The low activity of the standard compared with the doses studied suggests that jujube CMA extract has a higher antioxidant activity than the vitamin C used as standard.

3.2.6. Evaluation of Superoxide dismutase (SOD)

SOD is one of the most important antioxidant enzymes in the body's defence system. The major function of SOD is to catalyse the dismutation of superoxide anion (O2--) into hydrogen peroxide (H_2O_2), thereby reducing the toxic effects of this free radical (Ben Amara *et al.* 2011). This test permits to evaluate the effect of CMA consumption on the dismutation of free radicals. Figure 7 shows the effect of CMA consumed on Superoxide dismutase (SOD).

Contrary to the observations made with the CAT, the SOD assay showed decreasing values compared with the control (Figure 7). The Plasma SOD of the control group (104.10±5.06 µmol H_2O_2/mg proteins) was higher than that of the standard (26.42±3.66 µmol H_2O_2/mg proteins) and experimental groups. The values obtained for the different doses were 46.96±3.59 µmol H_2O_2/mg proteins (C50); 45.05±2.53 (C100); 43.69±1.84 (C150) and 38.31±3.40 (C200) respectively. According to the Duncan test there is significant difference ($p < 0.05$) between the value of plasma SOD obtained with standard and experimental groups studied. The results obtained with CMA extracts (38-47 µmol H_2O_2/min/mg protein) are similar to those of Kalidoss and Krishnamoorthy (2011) (36-43 µmol H_2O_2/min/mg protein) and are higher than those obtained by Si *et al.* (2002) (23-35 µmol H_2O_2/min/mg protein). This decrease is thought to be due to the inactivation of catalase, an enzyme

involved in the detoxification of hydrogen peroxide by superoxide anion. Thus, the reduction in SOD activity is thought to play an important role in protecting tissues from highly reactive hydroxyl radicals via catalase (Sathishsekar & Subramanian, 2005). In view of the results of this analysis, we can say that CMA extract is more antioxidant than vitamin C. Given that lipid peroxidation is associated with several types of biological damage, jujube consumption could therefore help combat numerous human diseases, including cancer, ageing and atherosclerosis (Gulcin *et al.* 2010).

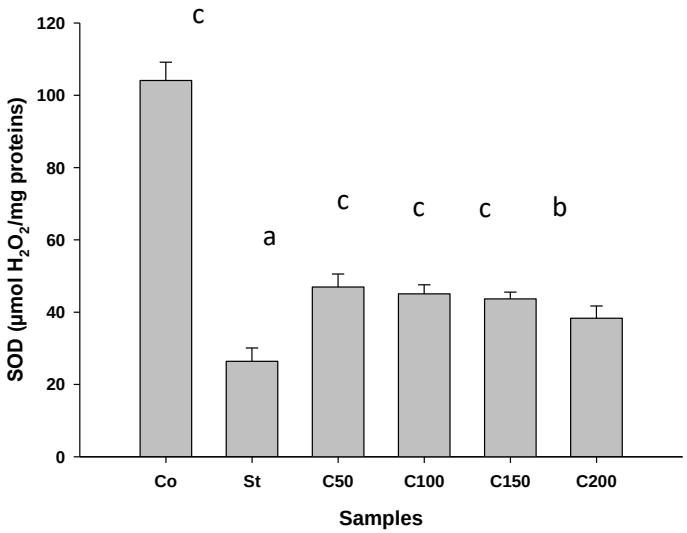

Figure 7: Effect of CMA consumed on Superoxide dismutase (SOD).

Conclusion

The present study was carried out to evaluate the Antioxidant activity of CMA of *Zizuphus*. *mauritiana*, this activity was tested *in vitro* and *in vivo* by using classic methods. The results showed that extracts lead to increased antioxidant capacity toward DPPH, ABTS and FRAP radicals, and caused amelioration in the bloob antioxidant status, by decreasing the MDA concentration, Superoxide dismutase (SOD) and increasing the rate of reduced glutathione (GSH), Glutathion peroxidase (GPX); catalase activity (CAT) and protein in blood. In view of the above, we can say that eating jujube could help fight against many chronic diseases, including cancer, ageing and atherosclerosis.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.

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