Elion Itou RDG
A) Animal Physiology Laboratory, Faculty of Science and Technology, Marien University Ngouabi, BP69, Brazzaville, Congo
B) National Institute for Research in Health Sciences, Scientific City of Brazzaville (formerly ORSTOM), Route de l'Auberge de Gascogne (chateau d'eau), Congo
C) Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, University Marien Ngouabi, BP 69, Brazzaville, Congo

Mambeke HM
Animal Physiology Laboratory, Faculty of Science and Technology, Marien University Ngouabi, BP69, Brazzaville, Congo

Boukongo RP
Animal Physiology Laboratory, Faculty of Science and Technology, Marien University Ngouabi, BP69, Brazzaville, Congo

Etou Ossibi AW
A) Animal Physiology Laboratory, Faculty of Science and Technology, Marien University Ngouabi, BP69, Brazzaville, Congo
B) National Institute for Research in Health Sciences, Scientific City of Brazzaville (formerly ORSTOM), Route de l'Auberge de Gascogne (chateau d'eau), Congo
C) Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, University Marien Ngouabi, BP 69, Brazzaville, Congo

Morabandza CJ
Department of Natural Sciences, Ecole Normale Supérieure, Marien University, Ngouabi Congo, B.P 69, Brazzaville, Congo

Abena AA
Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, University Marien Ngouabi, BP 69, Brazzaville, Congo

Corresponding Author:
Elion Itou RDG
A) Animal Physiology Laboratory, Faculty of Science and Technology, Marien University Ngouabi, BP69, Brazzaville, Congo
B) National Institute for Research in Health Sciences, Scientific City of Brazzaville (formerly ORSTOM), Route de l'Auberge de Gascogne (chateau d'eau), Congo
C) Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, University Marien Ngouabi, BP 69, Brazzaville, Congo

Phytochemical study and evaluation of the antiulcer effect of aqueous and hydroethanolic extracts of the recipe for the leaves of *Eriosema erici-rosenii* re fr. and *Neoboutonia melleri* müll. arg. prain in mice

Elion Itou RDG, Mambeke HM, Boukongo RP, Etou Ossibi AW, Morabandza CJ and Abena AA

Abstract
This work aimed to justify the traditional use of the recipe based on *Eriosema erici* and *Neoboutonia mülleri* in the treatment of gastric ulcers. The ulcer was induced by the HCl/ethanol mixture. To elucidate the antiulcer mechanism; the effect of the aqueous (300 and 600 mg/kg) and hydroethanolic (300 and 600 mg/kg) extracts was evaluated on lipid peroxidation, the production of antioxidant enzymes, the release of nitric oxide. At the doses used, the extracts of the recipe significantly reduced gastric lesions. In addition, they showed an antioxidant effect not only by inhibiting the ferric reducing power but also by opposing lipid peroxidation and improving the expression of antioxidant factors by increasing the production of catalase (CAT), glutathiones (GSH; GPx), super oxide dismutase (SOD). This coupled with a nitrogen effect. The phytochemical screening revealed the presence of flavonoids, tannins, terpernoids which could justify the antiulcer and antioxidative effect observed.

Keywords: Gastric ulcer, antioxidant, recipe, *Eriosema erici* and *Neoboutonia melleri*

1. Introduction
The gastrointestinal tract is one of the most important organs of the human body, vulnerable to different diseases including gastric diseases in particular, peptic ulcer disease (PUD). The latter is defined as a rupture in the continuity of the gastric mucosa [1]. It is a multifactorial condition whose pathophysiology results from an imbalance between deleterious aggressive factors (pepsin, HCl) and protective mucosal defensive factors (prostaglandins, mucus and bicarbonate barrier and adequate blood circulation) [2]. It is well known that alcohol consumption, use of non-steroidal anti-inflammatory drugs, stress and Helicobacter pylori infection increase the chances of developing gastric ulcer [3]. This pathology is diagnosed by endoscopy on signs of oedema, redness and swelling of the mucosa [4]. UGD is characterized by necrosis, neutrophil infiltration, reduced blood flow, increased oxidative stress and inflammation [5]. Extravasation of neutrophils at the site of injury increases the concentration of reactive oxygen species (ROS) and other inflammatory mediators, causing oxidative damage with deleterious effects on cells. Peptic ulcer disease is very common in the African tropics with a higher frequency in urban areas where its incidence is increasing [6]. It remains the primary cause of upper gastrointestinal bleeding in 36% of cases [7]. In Congo-Brazzaville, studies have shown that gastroduodenal ulcers represent the first upper digestive lesion proven by endoscopy [8]. Conventional treatments, although numerous and effective, are not well followed by patients because of their long duration of treatment, their exorbitant cost and the resulting side effects. Therefore, a safe and effective alternative therapeutic approach to current treatments could be medicinal plants, recognized as a source of new drugs and a valid alternative for primary health care [9]. In order to contribute to the enhancement of the Congolese flora and the improvement of the health status of the population, in the present work we are interested in the study of the anti-ulcer effect of the recipe of leaves of Eriosema erici and Neoboutonia melleri in Swiss mice.

2. Material and methods
2.1. Plant material: The plant material consisted of the leaves of *Eriosema erici* and *Neoboutonia mülleri* collected in Mossendjo in the Department of Niari (Republic of Congo)

in September 2020. Botanical identification of the plant material was done by Mousamboté, botanist systematistof Higher Normal School of Agronomy and Forestry (HNSAF) and confirmed at the Herbarium of the National Institute for Research in Exact and Natural Sciences (NIRENS) witch a collected sample was compared to a reference sample number 5640 and 1900 respectively for *E. erici* and *N. melleri*. After harvest, these leaves were dried separately at the Laboratory of Pharmacodynamics and Experimental Physiopathology (L2PE) for two weeks at room temperature (26 ± 1 °C). After drying, they were pulverized separately using a mortar. The powders obtained were mixed to prepare the aqueous extract and the hydroethanolic extract of the recipe. The aqueous extract of the recipe was prepared by decoction. 12.5 g of *Eriosema erici* powder and 12.5 g of *Neoboutonia melleri* powder were mixed with 500 mL of distilled water in a heating balloon and boiled for 15 minutes. After cooling and filtration, the aqueous decoction obtained was evaporated at a temperature of 55 °C using an evaporator (Thermosi SR 1000). The hydroethanolic extract of the recipe was prepared by maceration. 12.5 g of *Eriosema erici* powder from and 12.5 g of powder from *Neoboutonia Melleri* were mixed with 500 ml of the 50% hydroethanolic solution. The mixture was left for maceration with magnetic agitator for 48 hours. After filtration, the hydroethanolic maceration was evaporated at a temperature of 55 °C using an evaporator (Thermosi SR 1000). The aqueous and hydroethanolic extracts of the recipe obtained was kept to assess acute and subacute toxicity.

2.2. Animals materiel
Albino mices (25 - 30 g) of either sex these three-month-old obtained from the Faculty of Science and Technical of Marien NGOUABI-University were used. They were fed with a standard feed and water ad libitum. They were acclimatized during one week before experimentation and were housed under standard conditions (12 hours light and 12 hours dark) and at the temperature of 27 °C ± 1°C. The rules of ethics published by the International Association for the Study of Pain [10] have been considered.

2.3. Evaluation of the gastroprotective effect of extracts from the recipe in mice
The method reported by Elion Itou *et al*., (2018) [6] was used. The mice fasted 18 hours before the experiment were divided into 7 groups of 5 mice each and treated per-os with different doses of distilled water (negative control, 5 mL/Kg), distilled water (positive control, 5 mL/Kg), sucralfate (reference molecule, 100 mg/kg), aqueous extract from the recipe (300 and 600 mg/kg) and hydroethanolic extract from the recipe (300 and 600 mg/kg) fifty (50) min before oral administration of the hydrochloric acid mixture (0.3M) - 60% ethanol (0.2 mL/mouse). 1 hour after the administration of the ulcerogenic agent, except for the negative control group, the mice were sacrificed by dry blow. The stomach of each mouse was removed, then opened along the greater curvature using a scissor. Each stomach was washed out with physiological solution. Then each stomach was spread on a white sheet to better observe the ulcers formed with the naked eye and using an electric binocular magnifying glass (Leica, 2000). Ulcerative lesions appear in the gastric mucosa as black and red lines that run parallel along the axis of the stomach. The number and severity of ulcers are determined according to an arbitrary scale of 0 to 6 reported by Germano *et al*., (1996) [11]. The ulcer index corresponds to the average of the lesions. In order to appreciate the interference of the extracts of the recipe with the mode of induction of ulcers by ethanol and on

gastric microcirculation, we evaluated the effect of the extracts on lipid peroxidation, the production of some antioxidants and the concentration in nitric oxide (NO).

2.4. Preparation of stomach homogenates
Each stomach taken was weighed and then ground in 150 mM Tris-KCl buffer (pH 7.4) in a porcelain mortar so as to have 10% homogenates. These homogenates were then centrifuged at 4000 rpm for 30 min. The collected supernatant was aliquoted and stored in the freezer (-32°C) for the determination of the oxidative stress parameters (MDA, SOD, CAT, GSH, GPx), the assay of proteins, NO and the frap test.

2.5. Total protein assay
The colorimetric method of Bradford, (1976) [12] which uses Coomassie blue as a reagent was carried out to determine the protein concentration in the homogenate prepared from the stomach of each mouse.

2.6. Tissue assay of oxidative stress parameters
The determination of oxidative stress biomarkers was carried out using a plate reader (PHOMO Autobio) at specific wavelengths using gastric tissue homogenates. The tissue levels of malondialdehyde (MDA), reduced glutathione (GSH) were determined respectively according to the methods of Ohkahawa *et al*., (1979) [13]; Weckbercker and Cory, (1988) [14]. The enzymatic activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) respectively according to Aebi, (1984) [15]; Patel and Katyare (2006) [16] and Flohé and Günzler, (1984) [17].

2.7. Dosage of nitric oxide (NO)
The evaluation of the quantity of NO in various samples of gastric tissues was made according to the method of (Boudard *et al*., 1994) [18].

2.8. Assay of ferric reducing power (Frap)
The reducing power reducing power of the gastric tissue after treatment with the extracts of the recipe is determined by spectrophotometry using the method reported by Bassene, (2012) [19]. The absorbance was measured at 700 nm on a spectrophotometer. The antioxidant activity linked to the reducing power of the extracts is expressed in Reducing Power (RP) using the following formula:
$PR = 100(Aa-Ab)/Aa$; with Aa = absorbance of the extract; Ab = white absorbance

2.9. Phytochemical screening
Phytochemical screening based on color and/or precipitation tests was carried out on the aqueous and hydro-ethanolic extracts of the powder of the leaves of the recipe by *E erici* and *N. melleri* according to the method reported by Clarke, (1975) [20]

2.10. Statistical Analysis
The Excel 2016 software was used to process the data. All values were expressed as mean ± standard error of mean (SEM). Analysis of variance followed by Student-Fischer t test "t" was performed. The significance level was set at $p < 0.05$.

3. Results
3.1. Effect of aqueous and hydroethanolic extracts from the recipe of *Eriosema erici* and *Neoboutonia melleri* leaves on ulcers induced by 0.3 M Hcl / 60% Ethanol in mice
Macroscopic observation of the gastric mucosa of the animals shows that oral administration of the HCl/Ethanol

(HCl/Ethanol) solution produced characteristic gastric lesions in the glandular portion depending on the treatments received (Figure 1). Indeed, strong lesions are visible to the naked eye in the form of haemorrhagic furrows in the gastric mucosa of mice having received the HCl/Ethanol mixture (positive control group, figure 1b)alone compared with negative control group (figure 1a). On the other hand, the mice treated with sucralfate (reference molecule) and the extracts of the recipe at doses of 300 and 600mg/kg presented moderate lesions with significantly reduced lengths (figure 1 c, d, e, f and g). Furthermore, the aqueous (300 and 600 mg/kg) and

hydroethanolic (300 and 600 mg/kg) extracts of the recipe significantly reduced (p < 0.001) the ulcer index (Table 1). These indices are 12.64± 0.263; 3.568 ± 0.25; 4.57± 0.241; 2.62± 0.27; 5.24 ± 0.34; 4.15 ± 0.2762 respectively for the positive control, the sucralfate, the aqueous extract (300 and 600 mg/kg) and the hydroethanolic extract (300 and 600 mg/kg). The highest percentage of mucosal protection is obtained with the aqueous extract at a dose of 600 mg/kg, i.e. 88.92%, followed by sucralfate and the hydroethanolic extract 600 mg/kg, i.e. 75.62% and 71.67% respectively.
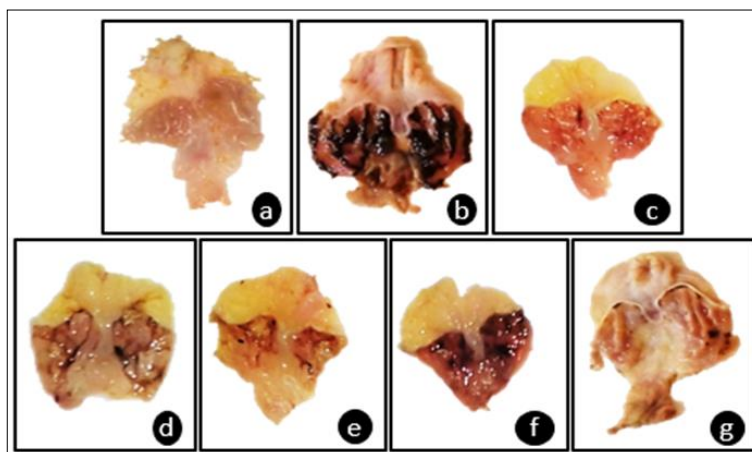
Fig 1: Macroscopic observation of gastric mucosal lesions induced by 0.3M Hcl/60% ethanol in mice. a: negative control group, b: positive control group (distilled water + Hcl/ethanol); c: sucralfate+ HCl/ethanol; d and e: aqueous extracts (300 and 600 mg/kg) + Hcl/ethanol; f and g: hydroethanolic extract (300 and 600 mg/kg) + Hcl/ethanol

Table 1: Effect of aqueous and hydroethanolic extracts of the recipe on ulcer index in mice

| Treatment	Doses	Ulcers index (mm)	Protection (%)
Negative control group (distilled water)	5 mL/Kg	00± 00	100
Positive control group (distilled water + HCl/ethanol)	5 mL/Kg	12.64± 0.263[###]	/
Sucralfate + HCl/ethanol	100	3.568 ± 0.25[***]	75.62
Aqueous extract+ HCl/ethanol	300	4.57± 0.24[***]	68.64
	600	2.62± 0.27[***]	88.92
Hydroethanolic extract + HCl/ethanol	300	5.24 ± 0.34[***]	64.19
	600	4.15 ± 0.27[***]	71.67
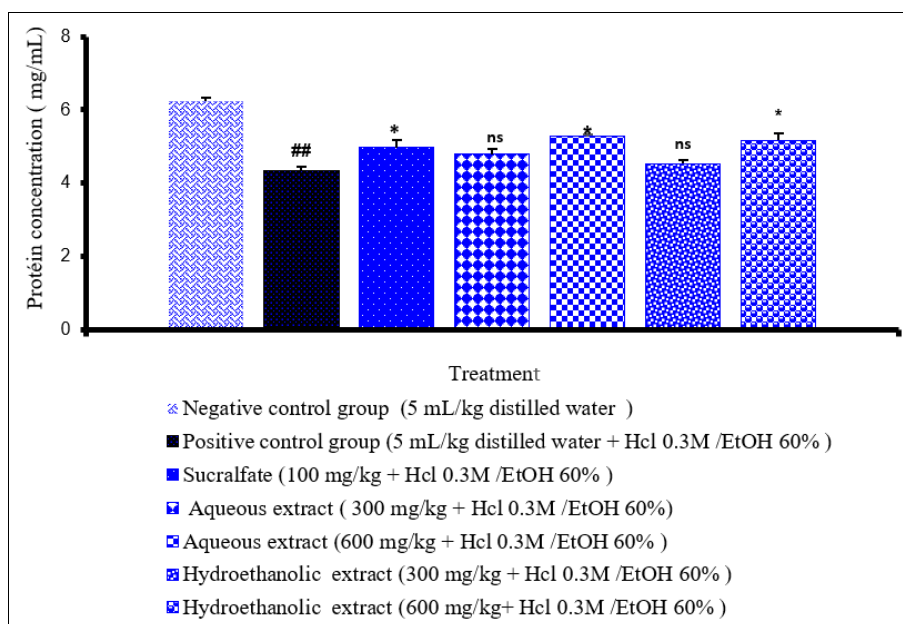
Fig 2: Effect of the aqueous and hydroethanolic extracts of the recipe on the protein concentration after Hcl 0.3M / Ethanol 60% induced ulcers in mice. Each value represents the mean ± ESM. [##]$p< 0.01$: Significant different (Student t-test) versus negative control group; [*]$p< 0.001$: Significant different (Student t-test) versus positive control group; ns ($p>0.05$: no signicant different (Student t-test) versus positive control group. EtOH= ethanol

3.2. Effect of aqueous and hydroethanolic extracts from the recipe of *Eriosema erici* and *Neoboutonia melleri* leaves on lipid peroxidation with malondialdehyde (MDA)

The results of the extracts on the concentration of MDA are presented in Figure 3. The acute administration of the 0.3 M HCl / 60% Ethanol mixture in mice leads to an increase in the concentration of MDA compared to the control batch with values which pass from 1.808 to 3.311 nmol/mprotein. Conversely, treatment with extracts from the recipe, such as suralfate, led to a significant reduction ($p<0.01$; 0.001) in the MDA content as a result of lipid peroxidation at the gastric level. Strong reductions are reported for the aqueous and hydroethanolic extract at the dose of 600 mg/kg with respective concentrations of 1.865 and 1.818 nmol/mprotein.
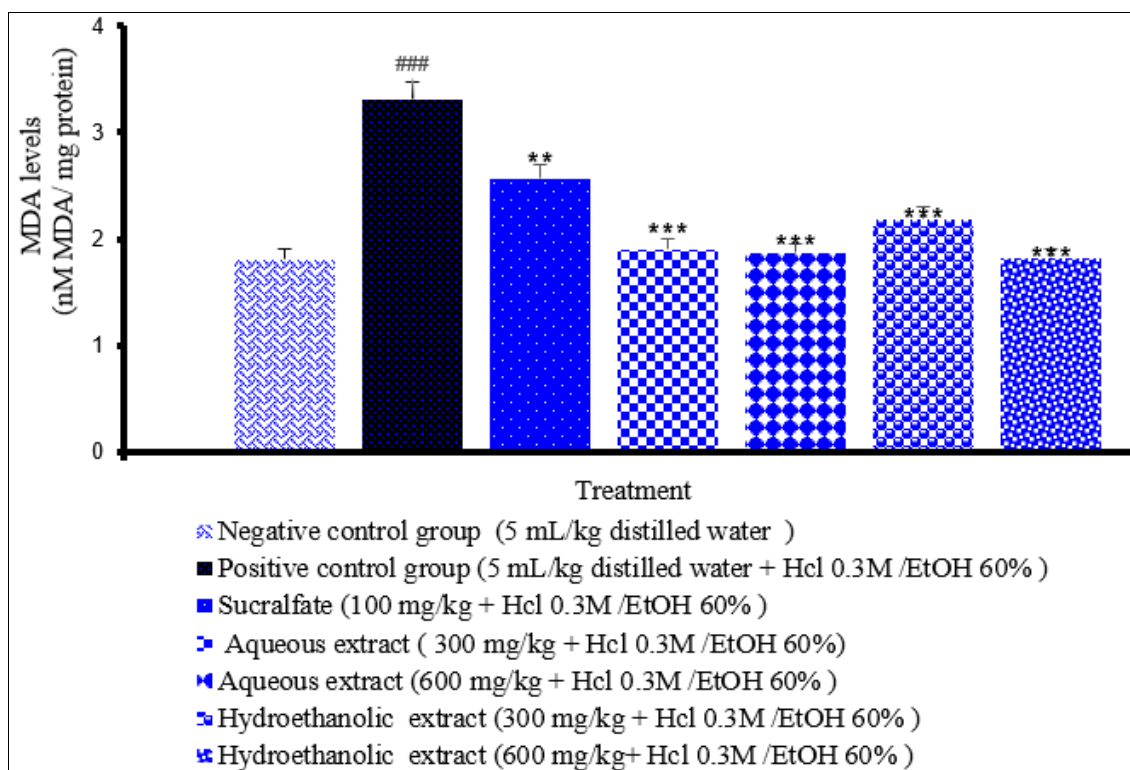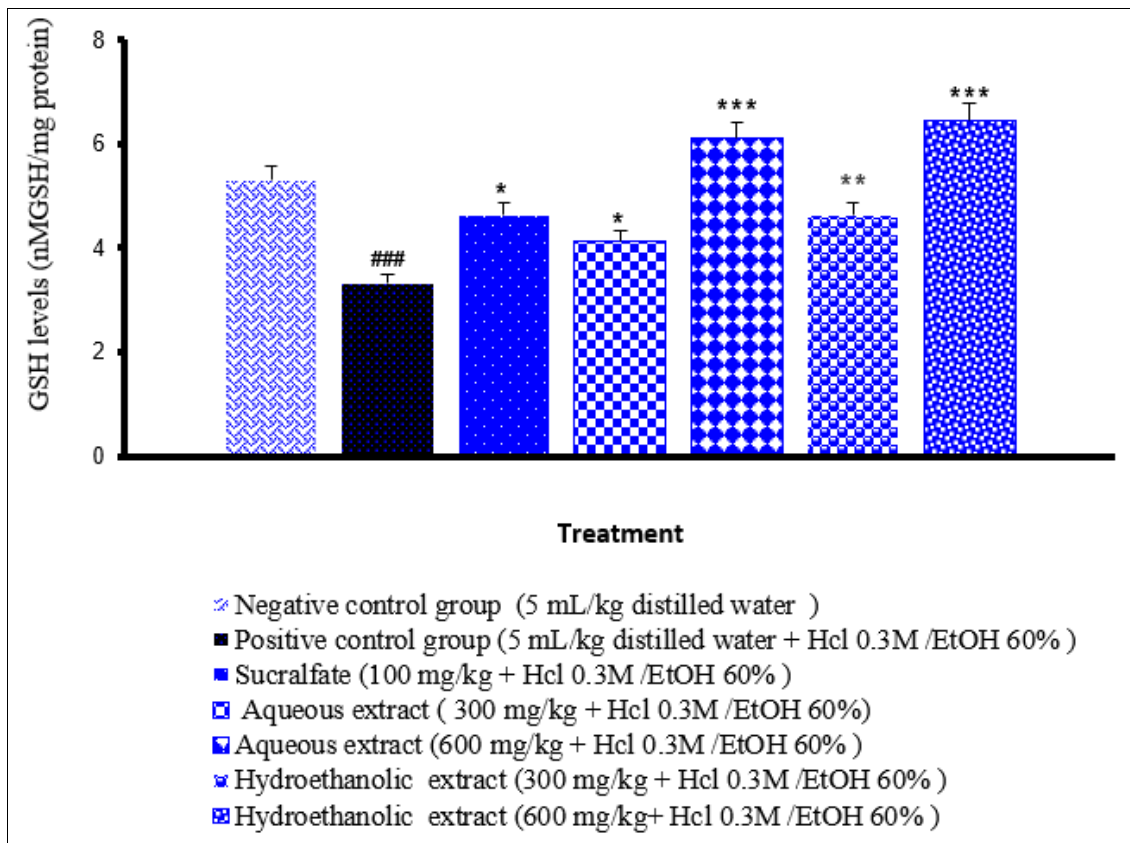

Fig 3: Effect of the aqueous and hydroethanolic extracts of the recipe on MDA activity after Hcl 0.3M / Ethanol 60% induced ulcers in mice. Each value represents the mean ± ESM. ###$p< 0.001$: Significant different (Student t-test) versus negative control group; **$p< 0.01$; **$p<0.001$: Significant different (Student t-test) versus positive control group. EtOH= ethanol
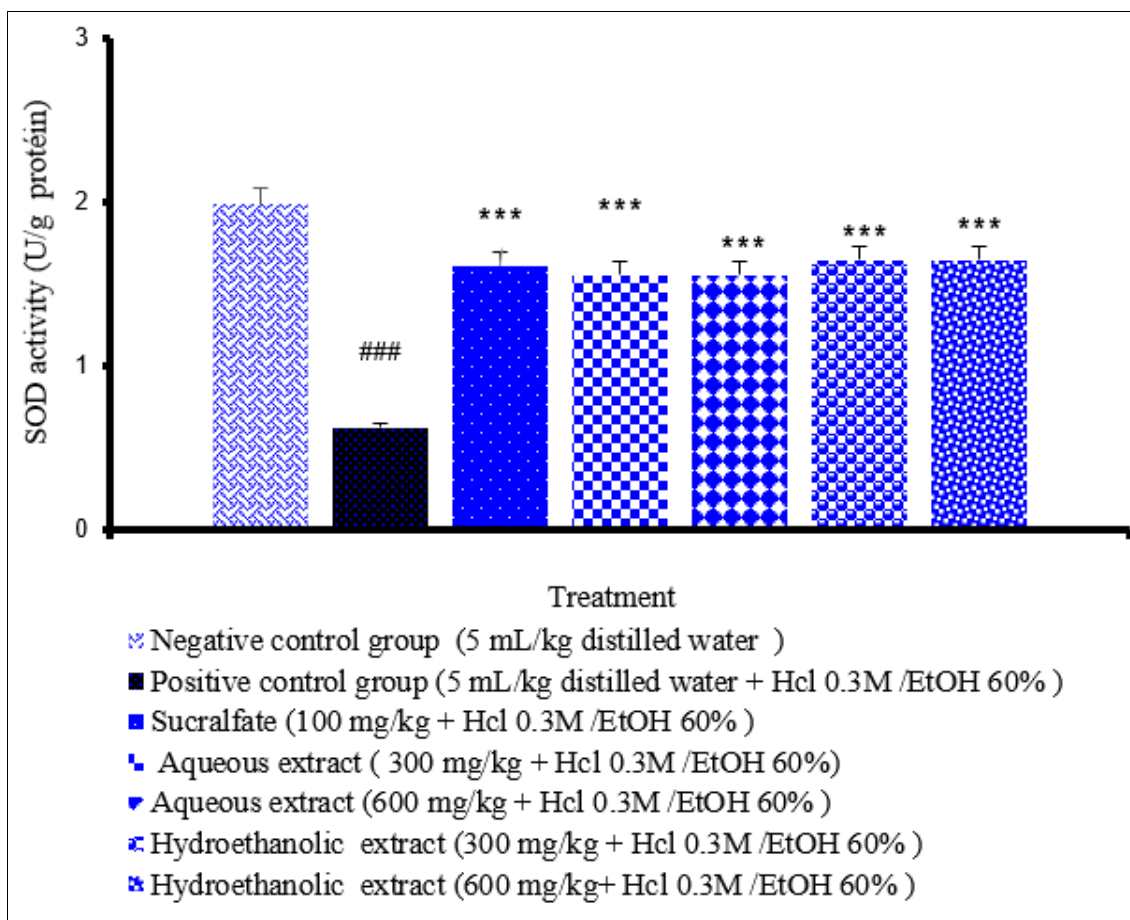
3.3. Effect of aqueous and hydroethanolic extracts from the recipe of *Eriosema erici* and *Neoboutonia melleri* leaves on the activity of non-enzymatic and enzymatic antioxidative

The results of the variation in the level of the non-enzymatic antioxidant (GSH) after induction of gastric ulcer by HCl/EthOH are represented by figure 4. A significant decrease ($p<0.001$) in the level of GSH in the gastric tissue of the mice treated with the inducer alone (positive control) compared with those having received only distilled water (negative control). On the other hand, a significant increase ($p<0.001$) of GSH is noted in the mice treated with the extracts of the recipe as well as with sucralfate compared with the positive controls. The two extracts (aqueous and hydroethanolic) at a dose of 600 mg/kg showed a higher GSH level than those of healthy animals (distilled water) with respective concentrations of 6.11; 6.46 and 5.30 nMGSH/mg protein. With regard to enzymatic antioxidants (SOD, CAT and GPx), the results of the assay are presented in figures (5,

6 and 7). A significant decrease ($p<0.01$; $p<0.001$) in the activities of all the enzymes is observed in the positive control animals (HCl/Ethanol) compared to the negative control (distilled water). On the other hand, treatment with extracts from the recipe as well as with the reference molecule leads to a significant increase ($p<0.05$; $p<0.01$; $p<0.00$) in the activity of SOD, CAT and GPX compared to the positive control batch. The SOD values (figure 5) are around 0.625; 1.614; 1.552; 1.557; 1,640 and 1,644 U/g of proteins respectively for positive control group, sucralfate, aqueous extract (300 and 600 mg/kg) and hydroethanolic extract (300 and 600 mg/kg). For the CAT (figure 6), they are 0.035; 0.055; 0.054; 0.056; 0.0510 and 0.055 μM H2O2/min/mg of protein respectively for positive control group, sucralfate, aqueous extract (300 and 600 mg/kg) and hydroethanolic extract (300 and 600 mg/kg). For GPx (figure 7), these values are 0.036; 0.059; 0.064; 0.099; 0.089 and 0.094 respectively for the positive control group, sucralfate, the aqueous extract (300 and 600 mg/kg) and the hydroethanolic extract (300 and 600 mg/kg).
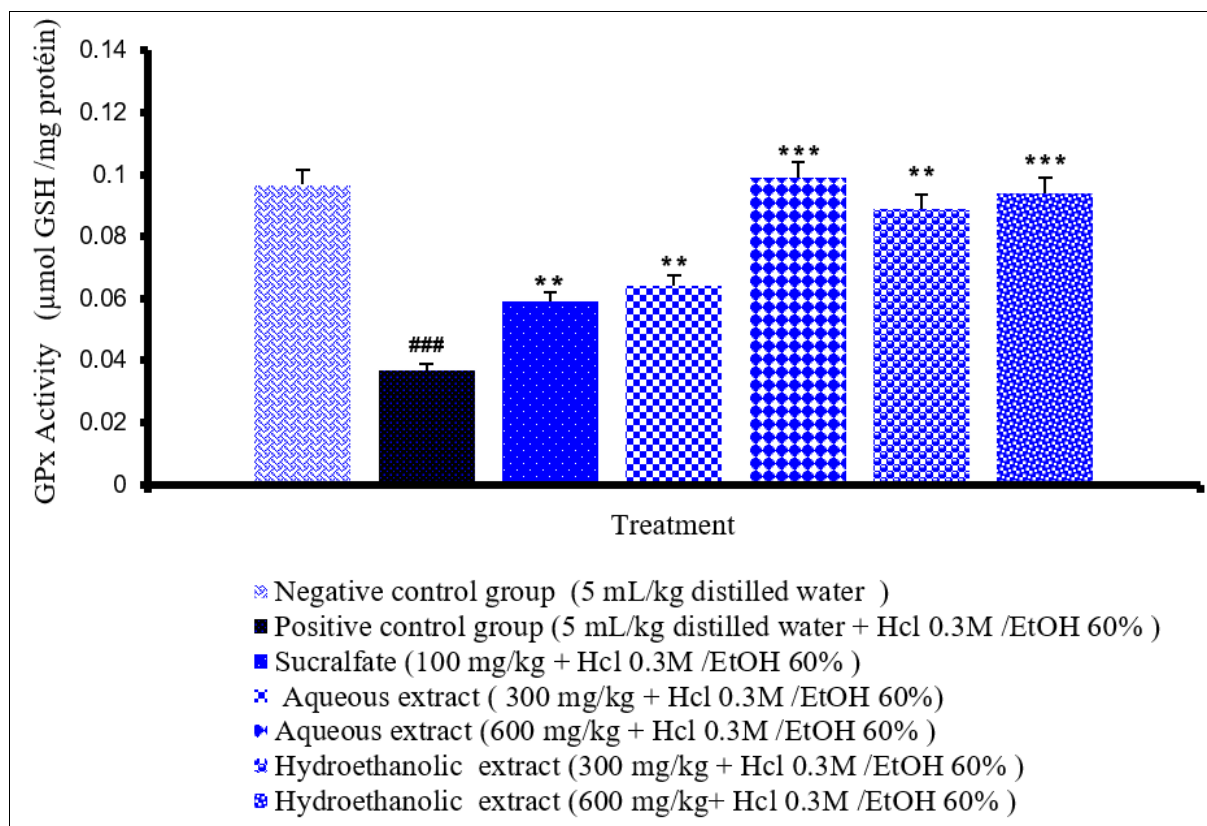
Fig 4: Effect of the aqueous and hydroethanolic extracts of the recipe on no- enzymatic activity glutathione concentration after Hcl 0.3M / Ethanol 60% induced ulcers in mice. Each value represents the mean ± ESM. ###$p < 0.001$: Significant different (Student t-test) versus negative control group; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$: Significant different (Student t-test) versus positive control group. EtOH= ethanol

Fig 5: Effect of the aqueous and hydroethanolic extracts of the recipe on the enzymatic activity of superoxide dismutase (SOD) after HCl 0.3M / Ethanol 60% induced-ulcers in mice. Each value represents the mean ± ESM.. ###$p < 0.001$: Significant different (Student t-test) versus negative control group;***$p < 0.001$: Significant different (Student t-test) versus positive control group. EtOH= ethanol
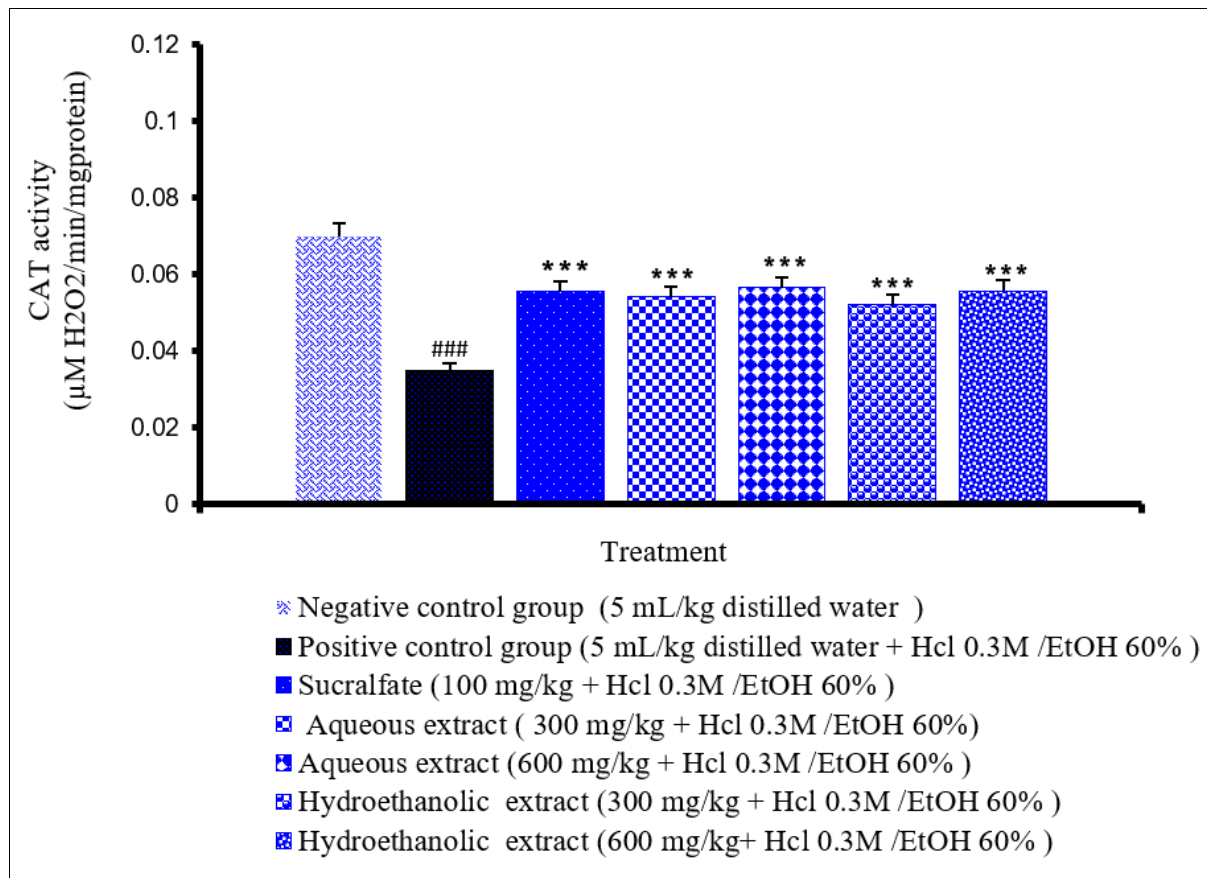
Fig 6: Effect of the aqueous and hydroethanolic extracts of the recipe on the enzymatic activity of catalase (CAT) after HCl 0.3M / Ethanol 60% induced-ulcers in mice. Each value represents the mean ± ESM. ###$p < 0.001$: Significant different (Student t-test) versus negative control group; ***$p < 0.001$: Significant different (Student t-test) versus positive control group.

Fig 7: Effect of the aqueous and hydroethanolic extracts of the recipe on the enzymatic activity of glutathione peroxidase (GPx) after HCl 0.3M / Ethanol 60% induced-ulcers in mice. Each value represents the mean ± ESM. ###$p < 0.001$: Significant different (Student t-test) versus negative control group; **$p < 0.001$; ***$p < 0.001$: Significant different (Student t-test) versus positive control group. EtOH= ethanol

3.4. Effect of aqueous and hydroethanolic extracts from the recipe of *Eriosema erici* and *Neoboutonia melleri* leaves on gastric cytosolic nitric oxide (NO) concentration

The variation in NO concentration following the various treatments received is illustrated in Figure 8. A significant decrease ($P<0.001$) in NO is noted in mice treated with the 0.3 M Hcl / 60% Ethanol mixture alone (positive control) compared to negative control group. However, those treated with different extracts as well as with the reference molecule (sucralfate) the NO concentration increased significantly (;$**p< 0.01$; $P<0.001$) compared to the positive control.
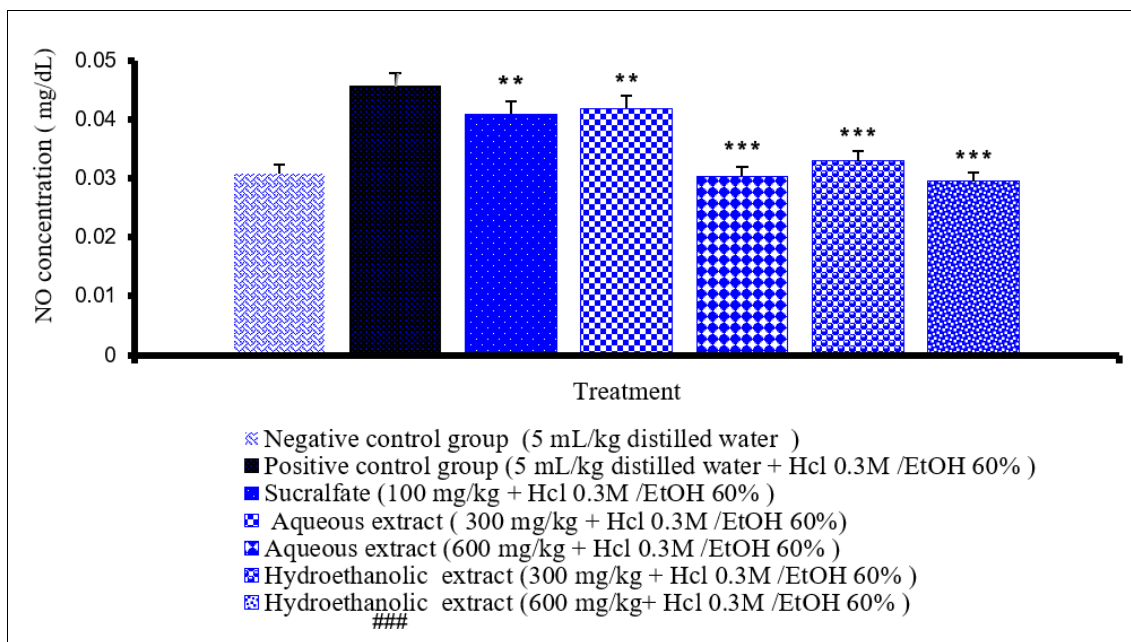
Fig 8: Effect of the aqueous and hydroethanolic extracts of the recipe on NO concentration after HCl 0.3M / Ethanol 60% induced-ulcers in mice. Each value represents the mean ± ESM. ###$p< 0.001$: Significant different (Student t-test) versus negative control group;$**p< 0.01$; $***p< 0.001$: Significant different (Student t-test) versus positive control group. EtOH= ethanol

3.5. Effect of aqueous and hydroethanolic extracts from the recipe of *Eriosema erici* and *Neoboutonia melleri* leaves on the ferric reducing power (Frap)

Figure 9 reports the effect of recipe extracts on ferric reducing power. The results obtained show that the extracts of the recipe have the capacity to reduce iron. This results in a significant increase ($p<0.001$) in the percentage inhibitions compared to the positive control group. The aqueous extract at a dose of 600 mg/kg as well as sucralfate gave strong inhibitions approaching that of healthy animals (negative control) with respective values of 20.30; 19.55 and 24.53%.

Figure 9: Variation of the Frap reducing power according to the treatments after induction of ulcers by the mixture Hcl 0.3M / Ethanol 60% in mice. Each value represents the mean ± ESM. ###$p< 0.001$: Significant different (Student t-test) versus negative control group;$***p< 0.001$: Significant different (Student t-test) versus positive control group. EtOH= ethanol
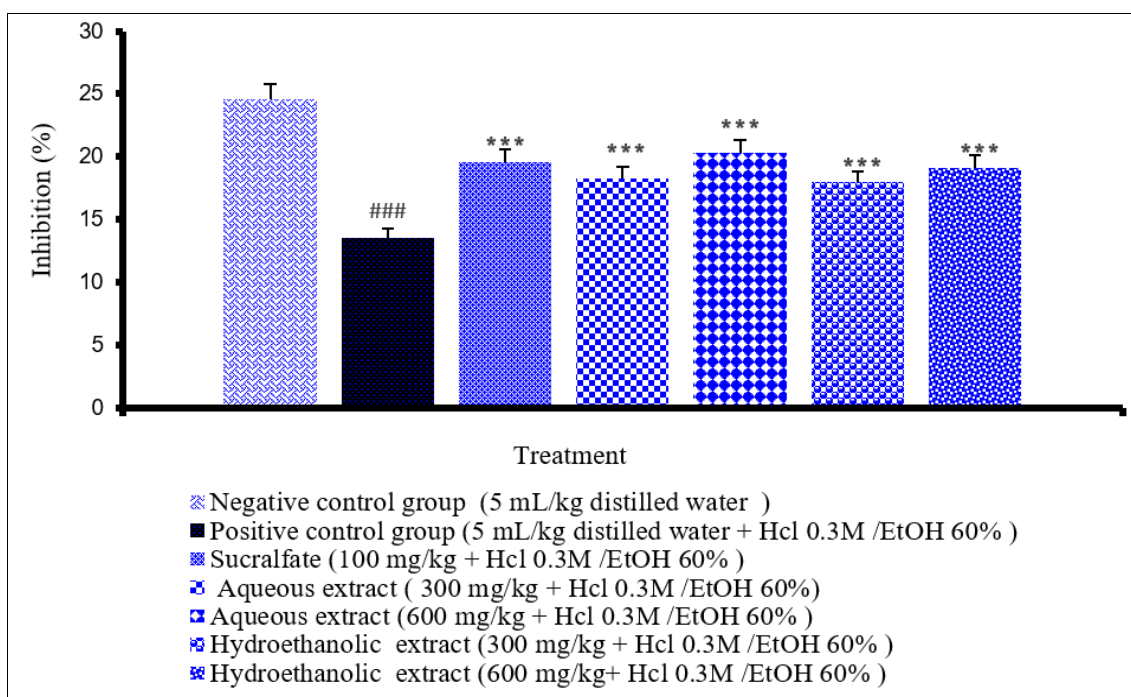
3.6. Phytochemical tests of the recipe extracts

The phytochemical screening of the two extracts revealed the presence of several secondary metabolites among other flavonoids, alkaloids, terpenoids, tannins, saponosides, steroids and tri-terpenoids, anthraquinones and Oses-holosides. On the other hand, there is an absence of mucilages in the hydroethanolic extract.

4. Discussion

This work aimed to justify the traditional use of the recipe based on *Eriosema erici* and *Neoboutonia mülleri* in the treatment of gastric ulcers. The gastric ulcers were induced by using HCl/ethanol model. This model was used because it closely resembles ulcers in humans. The results obtained show that the two extracts of the recipe from the leaves of *E. erici* and *N. mulleri* at the doses used (300 and 600 mg/kg) such as sucralfate significantly reduced the ulcer index. Indeed, Sucralfate, used as a reference molecule in this study, is a polysulfated disaccharide polymerizing by electrostatic bonding in an acid medium and forming a viscous and adhesive gel. It thus creates a protective barrier and stimulates the synthesis of endogenous gastric prostaglandins [21]. Some authors have shown that polysaccharides prevent the formation of gastric ulcers binding to the mucosal surface to provide a protective coating, decreasing gastric acid and pepsin secretions, protecting the mucus barrier, reducing oxidative stress and /or inflammatory response of the gastric mucosa [22]. This is the case of the polysaccharides found in *Lachnum sp.* [24], *Potato* [24], *Curcuma longa* [25], and seaweed *Solieria filiformis* [26]. From these observations, the effect observed with the two extracts could be attributed to the polysaccharides probably present in these extracts of the recipe; but also by one or more interferences with the mediators involved in the induction of gastric ulcers by the ethanol/HCl mixture. Indeed, hydrochloric acid and ethanol are among the few elements that the stomach absorbs. Their absorption causes ulcers which result in the appearance of large dark red lesions. Hydrochloric acid could induce gastric lesions by increasing the acidity of the stomach and by activating the NFkB pathway [27] thus resulting in the triggering of the inflammatory reaction which leads to the release of pro-inflammatory mediators (histamine), thus aggravating the lesions. Loss of normal mucus color with the presence of signs of necrosis and hemorrhages in the gastric mucosa [28]. These lesions are most likely related to the depletion of mucus and a constrictive effect on veins and arteries in the gastric mucosa, decreasing blood flow and thus producing congestion and inflammation [29]. Furthermore, hydrochloric acid can be self-harming, as it can disrupt gastric epithelial integrity to cause bleeding and/or perforation and likely trigger a local inflammatory response [30]. The H+ ions released by hydrochloric acid pass through the mucus-producing cells and reach the deeper layers [31]. Ethanol, on the other hand, has a triple action inducing ulcers: it erodes the mucous membrane, causes its congestion and cell necrosis [32], this leads to disturbances in gastric secretion, in particular by reducing the production of mucus (Ode and Asuzu, 2011) [33], alterations in permeability, depletion of the gastric mucosa and production of free radicals [34]. Ethanol is able to diffuse into the gastric mucosa and damage both epithelial and endothelial cells. Thus, the inflammatory cells accumulated around the damaged mucosa release a large number of inflammatory cytokines such as TNF- and IL-6 in a high concentration [35]. It causes an overproduction of reactive oxygen species, such as superoxide anion (O2-), hydrogen peroxide (H2O2) and hydroxyl radical (OH-) which promote lipid peroxidation and the formation of bleeding ulcers. The fact that the two extracts of the recipe very significantly reduce the ulcer index like sucralfate, suggests a cytoprotective effect which would be exerted by opposing the induction mechanisms of ulcers by the 0.3 M HCL/ 60% ethanol. These results corroborate those of Ifeanyi *et al.* (2020) [36] and Coelho *et al.* (2020) [37]. Respectively on the antiulcer effect of the methanolic extract of *Euphorbia hirta* and honey combination in rats and the chemical profile, and gastroprotective effect of the roots of *Jatropha elliptica*.

On the other hand, ethanol-induced gastric lesions are mainly related to intense infiltration in the submucosa which promotes the formation of reactive oxygen species (ROS), a decrease in mucus, a depletion of sulfhydryl groups and a decrease in blood flow, leading to damage to the gastric mucosa and leading to oxidative stress which could cause potential damage to cell metabolism [38]. Also, ROS contribute to the pathogenesis of lesions by attacking structural and enzymatic proteins, damaging DNA and playing a main role in lipid oxidation [39]. Likewise, especially the hydroxyl radical, play a major role in causing oxidative damage to the mucosa in all types of ulcers.

The production of reactive oxygen species (ROS) and oxidative damage therefore remains a crucial step in the pathogenesis of gastric ulcer [40]. To this end, elucidating the effect of the extracts of the recipe on the level of proteins, lipid peroxidation and production of antioxidants after induction of ulcers by the HCL/ethanol mixture seemed to us judicious. The results obtained show that the protein level increased significantly ($p<0.05$) in the animals treated with the two extracts of the recipe at a dose of 300 and 600 mg/kg compared to those which received the 0.3 M Hcl/ethanol 60 mixture. % (positive control). This suggests that these extracts are opposed to the protein denaturation caused by the ethanol/HCl mixture. Indeed, proteins are present in almost all animal cells. They contain thiol groups (-CH2-SH) which have a defense role in the body [41]. Protein denaturation is a process in which proteins lose their tertiary and secondary structures as well as most of their biological functions [42]; which can lead to the production of autoantigens [43]. Denaturation of tissue proteins is one of the causes of inflammatory diseases such as UGD, as well as autoimmune diseases such as arthritis [44]. Autoantigen production in some inflammatory diseases may be due to protein denaturation, membrane lysis and the action of proteinases. It can therefore be said that the denaturation of tissue proteins is a marker of inflammatory diseases [45]. Therefore, the ability of a substance to inhibit protein denaturation signifies an apparent potential for anti-inflammatory activity [46]. In addition, our results indicate a significant decrease ($p<0.001$) in cytosolic MDA in mice treated with aqueous and hydroethanolic extracts of the recipe at the doses used. This clearly reveals the cytoprotective effect of these extracts against the cytotoxicity of the 0.3 M HCl/60% ethanol solution *in vivo* as an inducer of gastric stress. It is widely documented that lipid peroxidation is a process that participates in the formation of lipid radicals and the rearrangement of unsaturated radicals such as Alkanes, malondialdehyde (MDA) and hydroperoxides that cause significant cell damage. MDA is a mutagenic and genotoxic agent that can contribute to the development of cancer in humans [47]. Lipid peroxides can directly induce DNA chain breakage and oxidation of cells [48]. The cytoprotective effect of the extracts would be due to the uptake of MDA molecules by the active principles or else

to the inhibition of the chain reactions of lipid peroxidation [49]. This ability to capture MDA molecules implies that the extracts of the recipe would participate intensely in the production of antioxidants in order to restore the balance of the oxidative system. Similar results are obtained by Amang *et al.* (2014) [50] on the cytoprotective and antioxidant effect of the methanolic extract of *Eremomasta*x leaves in rats and Prazeres *et al.* (2019) [51] on antioxidant and antiulcerogenic activity of the dry extract pods of Libidibia ferrea (Fabaceae). To better understand the possible mechanism by which extracts from the recipe could act to promote gastroprotection, several antioxidants were assayed. Our results reveal that the levels of non-enzymatic (GSH) and enzymatic (CAT, SOD and GPx) antioxidants were significantly increased ($p<0.05$; $P<0.01$; $P<0.001$) in the gastric homogenates of mice treated with aqueous extracts and hydroethanolic acid of the recipe at the two doses used compared to the positive controls. This would attest that the extracts oppose lipid peroxidation by increasing the production of antioxidants. GSH, which is the first link in the chain of antioxidant defense in mammalian cells, is involved in many cellular functions, including the transport of amino acids [52]. It has been reported that its decrease causes oxidative stress, which plays a key role in the pathogenesis of several diseases [53]. Indeed, the administration of ethanol in humans reduces gastric glutathione and induces damage to this organ [54] and its depletion induces increased ulceration in the gastric mucosa [55]. The ability of extracts to significantly increase GSH could be explained by their participation in the induction of GSH synthesis or its regeneration or even both effects. Jiao-Kun *et al.* (2016) [56] showed that natural antioxidants can strengthen endogenous antioxidant defense by restoring the optimal balance by neutralizing ROS. All these observations on the antioxidant power *in vivo* are confirmed in this study by the fact that the gastric homogenates of the mice treated with the extracts of the recipe also showed an inhibition of the ferric reducing power. These results suggest that the antiulcer effect of the recipe extracts is due, at least in part, to its antioxidant activity against xenobiotic-induced oxidative damage. These extracts would act directly in the enzymatic reaction by increasing the production of antioxidants and reducing the production of ROS but also, by strengthening the blood flow to the mucosa via the nitrergic (NO) pathway. Also, compounds that act as antioxidants or activate the redox system are important in restoring gastric tissue [57]. Similarly, the best-known antiulcerogenic effects of polyphenols today are through their antioxidant properties through various methods of mucosal protection [58]. Similar results were published by Zainul *et al.* (2016) [59] in their study on Gastroprotective activity of chloroform extract of sMuntingia calaburaand Melastoma malabathricumleaves.

The phytochemical screening of the two extracts revealed the presence of several secondary metabolites among other flavonoids, terpenoids, tannins, saponosides, steroids and anthraquinones and Oses-holosides. The presence of these secondary metabolites could justify the antiulcer and antioxidant effects of two extracts of the recipe [60, 61, 57, 59].

6. Conclusion
Our study showed that at 300 and 600 mg/kg the aqueous and hydroethanolic extracts of the recipe exert a gastroprotective effect which would be due to their antioxidant capacity. The biological effects observed in this study would be attributable to the secondary metabolites revealed (flavonoids, tannins, terpenoids, etc.). Thus, the aqueous and hydroethanolic

extracts of the leaves of *E. erici* and *N. melleri* would be an interesting source for the development of a new phytotherapeutic formula for the treatment of peptic ulcer disease.

Conflict of interest
The authors declare that they have no conflict of interest.

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